BIO Web of Conferences **149**, 01013 (2024) https://doi.org/10.1051/bioconf/202414901013
Genetic Resources 2024

Ecological and mycological assessment of the air environment of poultry houses and methods of its improvement

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Abstract. The sanitary state of the air environment in the conditions of industrial poultry farming with the concentration of a large number of poultry in a limited area increases the possibility of aerogenic spread of pathogens of various diseases. A significant amount of opportunistic microflora and fungal spores also accumulate in the air of poultry houses, which can cause mass disease in poultry. Constantly keeping poultry indoors in poultry farms leads to a decrease in the resistance of the bird's body. Moreover, unfavorable phenomena are aggravated by deionization of air, the complete absence of ultraviolet insolation, and an increased level of mycological contamination, which reduces the immunobiological reactivity of the body and contributes to the emergence of a number of diseases. Methods. Bacteriological studies were carried out on materials from poultry farms in the Karabudakhkent and Khasavyurt regions of the Republic of Dagestan. Temperature and air humidity parameters were determined according to generally accepted methods. The dust content in the air was assessed by the gravimetric method. Air analysis for mycological contamination was carried out using Czapek agar medium. After the growth of colonies on the surface of Czapek's medium, the species composition of the fungal cultures was determined and their toxicity was determined in laboratory animals. Results. In the air samples studied, fungi of the genus Aspergillus, Mucor, and Penicillium were isolated. Our studies of poultry house air found that 41.5 % of the total spores belonged to molds of the genus Penicillium, 3.5 % to Aspergillus and 55 % to the genus Mucor. Conclusions. The indicators of the temperature and humidity conditions of the air environment of the poultry

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house were within the limits of zoohygienic standards and met the requirements. Mycological and dust pollution of the poultry house air environment is increased during the daytime, especially during the period of feed distribution and feeding of poultry with the release of fungi from the genus Penicillium, Mucor and Aspergillus.

1 Introduction

One of the factors of atmospheric pollution is poultry farms, which serve as a source of spreading pathogenic and non-pathogenic microorganisms over considerable distances. The air in the poultry house and in the environment is subjected to dust and mycological pollution. The presence of dust particles and fungal spores in feed negatively affects not only the organism of poultry, but also the organism of service personnel. An important aspect of poultry farming enterprise activity is environmental protection, for this purpose barrier technologies are created, making it possible to prevent microorganisms release not only into the production environment, but also beyond the production limits [1].

Ecology at the poultry farm should take into account the requirements of environmental protection. The main problems existing in poultry farming affect the preservation of poultry health, increase in productivity, ecology, especially, in climatic zones with hot climate. Global environmental pollution, non-compliance with veterinary and sanitary rules and norms of technological design of poultry farms, feeding, active use of drugs, the presence of stress factors leads to disturbance of homeo-stasis of the organism [2-10].

Poultry farming is one of the most important branches of the agro-industrial complex of the Republic of Dagestan, which plays an important role in ensuring food security. The measures on state support of poultry farming taken in recent years make it possible to raise this industry by attracting investments. In recent years, large poultry farms have been built in Buynaksky, Khasavyurtovsky, Babayurtovsky and Magaramkentsky districts. At present, a lot of work is being done in the republic to create optimal conditions for keeping poultry and search for new solutions aimed at increasing natural resistance, preserving health and obtaining high productivity [10].

Violation of poultry housing conditions creates prerequisites for the emergence of diseases. Analysis of the epizootic situation in Dagestan, on infectious diseases of birds, showed the spread of diseases: colibacillosis, Newcastle disease, salmonellosis, pulchlorosis, tuberculosis and many others. Bacterial infections account for more than 80 %, viral infections - about 12 %, mycoses and mycotoxicoses - 8 % of the total number of unfavourable points. The reason for such a wide spread of infectious diseases is the violation of veterinary and sanitary rules. Low sanitary culture in keeping poultry in individual yards and peasant farms is of particular concern in the republic [2].

For better preservation of livestock and obtaining from its maximum production in poultry houses it is required to maintain not only heat, humidity, air velocity, but also reduction of mycological contamination and dustiness of air [4].

It is important to reduce mycological contamination and dustiness of the air in poultry houses, to create an optimal microclimate as a factor contributing to increased productivity and well-being of the flock [9].

Microscopic fungi are widespread in nature and cause diseases of various organisms of plant and animal origin. These diseases have been known for a long time, but their nature, forms and features of course in recent years have become more diverse and malignant. Such phenomena are associated with ecological shifts in the biosphere. The use of modern antibacterial agents and biogenic preparations contributes to the increase in the number of fungal diseases in animals [11-13].

Nowadays, it is difficult to find a branch of agriculture in which fungi would not play a negative role to a greater or lesser extent. They are characterised by high stability and great adaptability to external environmental factors [5].

Sanitary and mycological condition of poultry house air environment and prevention of mycosis and mycotoxicosis of poultry remains topical and is one of the most important problems of veterinary medicine. The growth of morbidity of animals with fungal diseases is connected, first of all, with immunosuppressive effects of modern technogenic civilisation on animal organism. Environmental pollution, use in veterinary medicine of immunosuppressive drugs, cytostatics, corticosteroids and broad-spectrum antibiotics - this is not a complete list of factors that weaken the natural defence mechanisms of the animal organism and contribute to the development of primary and secondary fungal diseases [4].

Currently, the topical issue is to obtain biologically complete and ecologically clean livestock products. For this purpose the following are promising: directional influence on the processes of metabolism, metabolism and elimination of xenobiotics at oral use of mineral-sorbents; development of biologically justified and economically favourable technologies of natural aluminosilicates use in animal and poultry feeding to increase natural resistance, productivity, prevention of alimentary diseases and obtaining high quality products [6].

The main factors affecting the growth intensity of mould fungi and the development of fungi of a particular species are humidity, temperature, nutrient supply and acidity of the medium. When environmental conditions are favourable for the growth of mould fungi, the growth of fungi of different species can occur. When environmental conditions deteriorate, mould fungi of the species that is best adapted to exist under the given conditions begin to dominate [8].

Sources of mould fungi can release spores, mycelial fragments, as well as cellular components and life products such as p-glucans (polysaccharides contained in the fungal cell wall), ergosterol (a steroidal compound, (a steroidal compound found in the cell wall of fungi), toxins and VOCMPs (volatile organic compounds that are products of microorganisms, such as certain aldehydes, alcohols, esters, ketones). In cultivation, colonies can grow not only from spores but also from mycelium fragments [11, 12].

The number and distribution of spores dispersed in the air depends on the genus and species of mould fungi. Thus, in order to assess the sources of mould fungal release in an enclosed space, it is necessary to distinguish specific fungal species according to their type of spore release. Experience has shown that even minor contamination of wall and floor materials with mould fungi can lead to elevated airborne levels if the fungal species present produce dry spores that are easily dispersed in the air (for instance, Penicillium and Aspergillus). In contrast, the airborne spore content will be much lower if the materials are colonised by the colonies of, for instance, mould fungi of the genera Acremonium and Fusarium or the species Stachybotrys chartarum, as they produce relatively large spores encased in a mucus sheath and thus difficult to disperse through the air [12].

In addition, it should be taken into account that spores of mould fungi are not necessarily present in air or settled dust as specific spores, but also as aggregates or adhered to particulate matter. Depending on the method of analysis, the spores are determined as specific spores or as aggregates [13, 14].

Building materials, indoor air and house dust contain not only culturable but also unculturable spores, some of which may cause the same allergic and toxic reactions as culturable spores. Therefore, techniques have been developed to ensure the detection of both culturable and non-culturable spores using a microscope [11].

Detection and identification of mould fungi is performed after cultivation based on morphological criteria, biochemical reactions and/or molecular analysis techniques, or by direct research under the microscope. Identification based on morphological structure (macroscopic research, stereoscopy and microscopy) both after pre-culturing and by direct microscopy is still the most preferred method of mould fungi detection [3, 7, 10].

Purpose of research: monitoring of mycological contamination of the air environment of poultry houses, with determination of fungi composition, depending on the season of the year, in the conditions of poultry houses located in the Caspian lowlands of the Republic of Dagestan.

2 Methods and materials

The work was carried out at the Department of Epizootology of Dagestan State Agrarian University named after M.M. Dzhambulatov, the Department of Animal Diseases and Veterinary and Sanitary Expertise of Vavilov University, the Republican Veterinary Laboratory and the Rosselkhoznadzor Laboratory. Epizootic situation on infectious diseases of birds was studied by surveying poultry farms, using reports of district veterinary departments and zonal veterinary laboratories, reports of the Veterinary Committee of the Republic of Dagestan. Research material for bacteriological and mycological research was sampled in poultry farms of Karabudakhkent and Khasavyurtovsky districts of the Republic of Dagestan.

Poultry farms were selected with the same meteorological conditions. The Caspian lowland, where the farms under study are located, belongs to the warm belt with temperature fluctuations from +30 to -20 ^{0}C.

Research was conducted in the premises for keeping broiler chickens of crosses 'Kobb-500', 'Ross-308' at poultry farms located in the lowland part of the Republic of Dagestan (Agroholding 'Kakashurinskaya' of Karabudakhkent district, SPK 'Batyr' of Khasavyurtovsky district and OAO 'Khasavyurtovskaya poultry farm'). Poultry was kept in accordance with the recommendations of VNIITIP.

Poultry houses for chickens were built of reinforced concrete structures: length 72 m, width 18 m, height 3 m. The buildings are designed for growing chickens on deep bedding (straw cut 12-15 cm) up to 45 days of age.

Air exchange in the room is carried out by two supply fans KF-4 with a capacity of 16 thou. m^3/hour each. Forced exhaust ventilation includes 12 'Klimat 44' units with a capacity of 3500 m^3/hour each. Natural air extraction is carried out through five exhaust shafts with a cross-section of 100×100 cm.

Sampling methods in accordance with ISO 16000-16 (filter sampling) and ISO 16000-18 (sedimentation sampling) were used in the research. Air samples were taken by aspirator. Dust content in the air was determined by weight method.

Windows and doors in the rooms were closed 8 hours before sampling. Samples were taken in the centre of the room at a distance of at least 1 m from the bearing walls and at a height of approximately 0.25 to 1.5 m from the floor. For filter sampling, a sterile filter holder with filter media inserted in it was connected to the sampling nozzle. Sampling was carried out for 1 min using the sedimentation method. The air sample volume was at least 50 litres. Air sampling was performed twice a day: in the morning from 5 to 7 and in the afternoon from 14 to 16 hours at the level of 0.25 m and 1.5 m from the floor. For this purpose, the poultry house was conventionally divided into three parts: the northern end section of the house (NES), the room centre (RC) and the south end section (SES).

To determine the total number of spores of mould fungi, slit-impactors were used to determine the total number of spores of mould fungi in the air of enclosed rooms, from the surface of which the settled air contents were washed off with 10.0 ml of 9% NaCl solution. Then tenfold dilution of the sample wash was made and spore counting was carried out in a Goryaev chamber using phase-contrast microscopy with a light microscope magnification of 40 times.

For research on mycological contamination of the air, agarised Chapek's medium was used. After the growth of colonies on the surface of Chapek's medium, the species composition of fungal cultures was determined.

Mathematical processing of data was carried out using MS Excel software package, we calculated statistical indicators arithmetic averages (X), errors of the average value of (±m).

3 Results and discussion

The ecology of the poultry farm must take into account the requirements of environmental protection. Violation of environmental ecology also harms human health. Lack of sunlight, noise, unfavourable microclimate, presence of harmful gases that negatively affect not only poultry, but also the organism of the staff. The results of our research on the temperature regime of the air environment of the chicken house are presented in Figure 1.

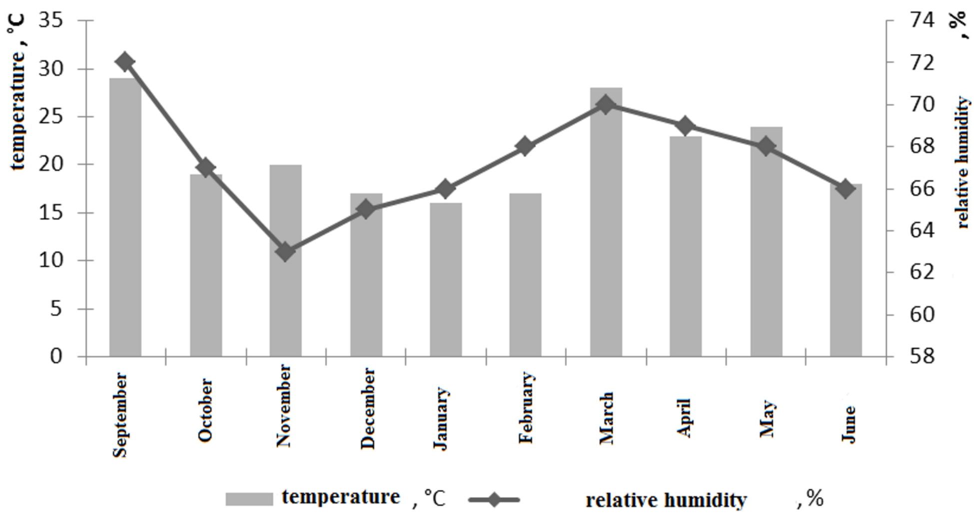

Fig. 1. Microclimate parameters in poultry houses.

The data of figure 1 show that the temperature of air environment of poultry house has some range of fluctuations, but does not exceed optimal zoohygienic norms.

Humidity of air environment of broiler chickens as a factor influencing thermoregulation and heat metabolism of organism should be considered simultaneously with temperature of air environment.

Relative humidity in autumn period had some range of fluctuations: In September relative humidity exceeded zoohygienic norms and was 71.36±0.29 %, with fluctuations of 71.03±0.10-72.29±0.72 %, in October 67, 3±0.54 %, with a variation of 65.9±0.13-69.16±0.82 %, in November 62.9±0.35 %, with a variation of 62.6±0.31-63.2±0.38 % and in december 65.24±0.82 %, with a variation of 63.71±0.60-66.1±1.14 %.

In spring-summer period relative humidity corresponded to zoohygienic norms. In March, the relative humidity index was 70.0±0.39 % with fluctuations of 69.2±0.37-70.8±0.43 %, in April 69.6±0.38 % with fluctuations of 69.3±0.31-70.0±0.43 %, in May 67.6±0.43 % with fluctuations of 67.2±0.37-67.9±0.48 % and in June 66.42±0.78 % with fluctuations of 64.58±0.74-67.4±0.94 %.

Analysing the obtained results on temperature-humidity regime for broiler chickens, it is possible to conclude that temperature and relative humidity indices of indoor air environment corresponded to optimally permissible norms of technological design of poultry farming enterprises.

Dustiness and general bacterial contamination of indoor air (Figure 2) is of great hygienic importance.

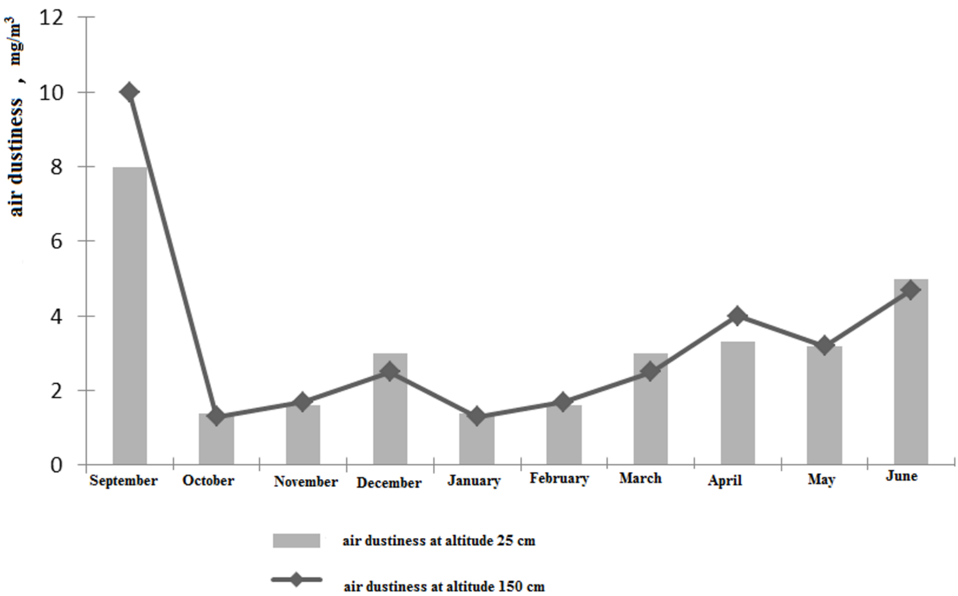

Fig. 2. Air dust content in poultry houses for keeping broiler chickens, (mg/m^3).

In the autumn period, air dustiness in the room for keeping chicks up to 45 days of age averaged 3.96±0.2 mg/m^3 at variations from 1 to 11 mg/m$^{3.}$

During the winter period, air dustiness in the room for keeping chicks up to 45 days of age averaged 1.4±0.18 mg/m^3, at fluctuations from 1 mg to 7 mg in 1 m^3 air.

Thus, in the autumn-winter period, the dustiness of the air in the chicken housing was dependent on the functioning of forced ventilation, temperature and humidity conditions in the room, as well as on the activity of poultry movement.

In spring-summer period dustiness in the chicken house averaged 3.75±0.35 mg/m^3 air, with fluctuations from 1 to 12 mg/m^3 air.

Air dustiness in the chick housing in spring-summer period depended on the operation of the supply and exhaust ventilation system and in inverse dependence on additional fresh air supply. Dust content in the air is largely determined by the activity of poultry movement, technological processes and relative air humidity.

Fluctuations in the degree of air dustiness were within a wide range, which is explained by the fact that the inlet ventilation system could not fully provide sufficient fresh air supply, and its additional supply through open windows depended on the outside air temperature. When the outside air temperature decreased, more fresh air was additionally supplied and the dustiness of the air in the chick house decreased. When the outside air temperature increased, less fresh air was supplied to the room and dustiness increased.

Increase of dust in poultry house air was observed during daytime, especially during feed distribution, feeding (depending on the age of the birds) and cleaning of the house.

Research on dustiness of the air in the chicken house showed that dustiness increases significantly in spring, summer and autumn periods compared to winter periods.

Air dustiness is largely determined by the amount of fresh air intake into the room from the age of the birds, temperature and humidity regime, bird movement activity, as well as from the dry type of feeding.

In mycological research it was found that the air in the chicken house contains a large number of mould fungi spores (Table 1-3).

Table 1. Air pollution of premises for keeping chickens up to 45 days of age with *Penicillium* spores (spores/m^3).

Month	Height from floor (m)		Average number of spores
	0.25	1.5	
September	4-8 5±1	4-12 8±1	6±1
October	57-420 138±2	60-250 120±1	129±1
X±m 1 round	74±1.5	64±1	132±1
November	-	1-62 7±1	3±1
December	11-1002 239±64	11-640 322±40	280±52
X±m 2 round	119.5±32	325±20.5	141.5±26.5
March	12-925 228±59	38-1000 238±62	233±60
April	46-360 88±20	48-610 158±36	123±28
X±m 3 round	279±39.5	198±49	178±44
May	2-20 5±1	4-10 3±1	4±1
June	100-460 207±42	120-400 270±32	238±37
X±m 4 round	106.0±21.5	136.0±16.5	121.0±19.0

Table 2. Air pollution of premises for keeping chickens up to 45 days of age with *Aspergillus* spores (spores/m^3).

Month	Height from floor (m)		Average number of spores
	0.25	1.5	
September	5-38 18±2	10-33 24±1	21±1
October	12-120 71 ±7	55-90 78±2	74±4
X±m 1 round	32±3	34±1	33±1
November	1-32 5±2	8-25 3±1	4±1
December	8-37 14±2	8-37 14±2	14±2
X±m 2 round	9±2	8±1	9±1
March	1-50 9±3	3-39 9±2	9±2
April	9-60 18±3	6-30 18±1	18±2
X±m 3 round	14±3	14±1	14±2
May	2-20 3±1	1-10 2±1	2±1
June	12-32 19±1	16-60 26±2	15±1
X±m	7±1	9±1	8±1

Table 3. Air pollution of premises for keeping chickens up to 45 days of age with *Mucor* spores (spores/m^3).

Month	Height from floor (m)		Average number of spores
	0.25	1.5	
September	6-18 9±1	8-18 8±1	8±1
October	11-350 132±31	45-300 128±16	130±18
X±m 1 round	105±28	116±19	110±23
November	50-600 175 ±61	185-535 212±39	193±50
December	4-455 109±29	14-621 120±39	114±34
X±m 2 round	142±45.0	166±39.1	153.5±4.2
March	25-480 116±30	48-823 140±50	128±40
April	7-450 92±28	6-375 76±24	84±26
X±m 3 round	235±51	206±50	221±50
May	77-600 134±58	97-350 165±28	149±43
June	140-403 308±29	188-588 638±44	473±36
X±m 4 round	350±52	648±43	499±48

In the spring period, the air of the chicken house for chicks up to 45 days of age contained on average: Penicillium 178±44 spores per 1 m^3, with variations from 12 to 1000, Aspergillus - 14±2 spores in 1 m^3, at fluctuations from 1 to 60, Mucor - 221±50 spores in 1 m^3, at fluctuations from 6 to 1300 spores in 1 m^3 of air.

Spores of mould fungi of the genus Penicillium, Aspergillus and Mucor were detected in the air of the chicken house during this period. Of the total number of spores, 41.5 % belonged to mould fungi of the genus Penicillium, 3.5 % to Aspergillus and 55 % to the genus Mucor.

An increase in air pollution by mould fungi spores was observed during the daytime, especially during feed distribution.

In the summer period, the air of the chicken house for keeping chicks up to 45 days of age contained on average: Penicillium - 46±1 spores per 1 m^3, with variations from 2 to 460, Aspergillus - 8±1 spores per 1 m^3, with variations from 2 to 60, Mucor - 499±48 spores per 1 m^3, with variations from 77 to 1490 spores per 1 m^3 of air.

Spores of mould fungi of the genus Aspergillus, Penicillium and Mucor were detected in the air of the chicken house during this period. Of the total number of spores, 8 % belonged to mould fungi of the genus Penicillium, 1 % to Aspergillus and 91 % to the genus Mucor.

In the autumn period, the average number of spores in the air of a chicken house for keeping chicks up to 45 days of age was: Penicillium - 46±1 spores per 1 m^3, with variations from 4 to 420, Aspergillus - 33±1 spores per 1 m^3, with variations from 5 to 120, Mucor - 110±23 spores per 1 m^3, with variations from 6 to 600 spores per 1 m^3 of air.

In the air of the chicken house during this period spores of mould fungi of the genus Mucor – 57.4 % of the total number of spores, of the genus Penicillium - 24.2 %, of the genus Aspergillus - 18.4 % were detected.

An increase in air pollution by mould fungi spores was observed during the period of feed distribution and addition of new straw bedding.

During the winter period, the average number of spores in the air of the chicken house was: Penicillium - 353±49 spores per 1 m^3, with variations from 11 to 870, Aspergillus - 9±1 spores per 1 m^3, with variations from 1 to 37, Mucor -121±37 spores per 1 m^3, with variations from 4 to 820 spores per 1 m3 of air.

Spores of mould fungi of the genus Penicillium 73 % of the total number of spores, of the genus Aspergillus - 2 % and of the genus Mucor - 25 % were found in the room air during this period.

Increase of air pollution by mould spores was observed during daytime, especially during feed distribution and feeding period.

Thus, the air of poultry houses and chicken houses was significantly contaminated with spores of mould fungi. The bulk of mould fungi belonged to the genera Penicillium and Mucor.

The number of mould fungi spores in the air of poultry houses largely depends on the technological processes, poultry activity, as well as feed infestation by mould fungi.

It is characteristic that the number of mould fungi spores in the air of poultry house increased during feeding period, when birds show active movements and strongly raise dust in the air. Therefore, the main source of air pollution by mould fungi spores is the affected feed and bedding material.

It has been noted by various researchers that mycosis of poultry is characterised by the fact that fungi entering the organism with feed germinate and multiply in certain organs and tissues, having both local and general effects on the organism

In our research 15 sick chickens were studied. After clinical examination, the sick chickens were slaughtered. In addition, 124 chicken cadavers were subjected to pathological and anatomical research. At autopsy, in 90% of the cases, grey nodules, the size of a pinhead, were found on the surface of the lungs.

Similar changes were found on the surface of the liver in 10% of cases. From the affected internal organs, cultures of microorganisms and mould fungi spores were sown on nutrient media. As a result of research it was found that in 32 cases Escherichia coli cultures were isolated from the heart, lungs and brain and 107 cases of Aspergillus fungi.

Measures to protect the air basin of the territory of poultry farms can be subdivided into general and private. General measures of air pollution control include: high sanitary culture of the industry, uninterrupted operation of micro-climate systems (first of all ventilation), removal of manure, thorough cleaning and disinfection of premises, organisation of sanitary-protection zone, etc. The measures are aimed at cleaning and disinfection of poultry farms' premises. Private measures are aimed at air purification, disinfection and deodorisation. To prevent external recirculation of polluted air the following is recommended. Polluted air emission from the premises should be carried out upwards by a flare with removal above the roof ridge by means of exhaust pipes to the height calculated for creation of ‘aerodynamic shadow’. Clean air intake should be carried out from below in end parts or in specific chambers outside the room, taking into account the direction of prevailing winds, and the concentration of harmful gases and microflora at the intake points should not exceed 20% of the maximum permissible concentrations in the room. It is recommended to locate the point of polluted air emission vertically at least 5 m and horizontally at least 20 m from the place of intake of incoming air. A centralised supply and exhaust ventilation system should be provided, making it possible to make extensive use of filters to clean the air of contaminants.

Installation of diffusers with bactericidal lamps or air purification filters on the exhaust and supply ventilation systems will make it possible to reduce air pollution by 88-99% due to purification of the removed air. In case of absence of special filters, it is possible to use coarse filters made of local materials: sawdust, glass fibre, lavsan fabric, etc.

4 Conclusions

The conducted research in conditions of poultry farms showed that indicators of temperature-humidity regime of poultry house air environment were within zoohygienic norm and met the requirements of zonal-climatic conditions of the Republic of Dagestan.

The greatest mycological and dust contamination in the air environment of poultry house was observed in the daytime, especially in the period of feed distribution and feeding of poultry due to the use of dry type of feeding.

During mycological research, fungi of the genus Penicillium, Mucor and Aspergillus were identified in the air environment of the poultry house. The greatest increase in the number of spores of mould fungi in the air was observed during the feeding period of the birds.

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