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ANALYSIS OF THE EFFECTS OF DISINFECTION OF THE SURFACES AND EQUIPMENT IN DIFFERENT PRODUCTION UNITS ON A PHEASANT FARM BY THE APPLICATION OF PARACETIC ACID AND FORMALDEHYDE BASED SOLUTIONS

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Abstract: Pheasant farms represent semi-closed production facilities with several production units that are cyclically connected and are used for growing pheasants of different age categories. Production units consist of an aviary for parent stock, egg storage room, incubation station, facilities for raising young pheasants and open aviaries where pheasants are raised till their release on hunting areas. Continuous implementation of biosafety measures at all stages of the technological process of production is necesarry in order to achieve good production results ; that is raising healthy pheasants. Disinfection is one of the most important biosigurative measures which must be continually implemented in farm facilities. In this paper, the effect of disinfection with peracetic acid and paraformaldehyde based solutions has been monitored at different stages of production, and on different surfaces. Monitoring of the microbiological status of the area within the production facillity resulted in reduction of the total number of bacteria, fungi and mold in a smaller or greater extent depending on the place of sampling and type of a production unit.
Key words: pheasant farm, disinfection, peracetic acid, formaldehyde, microbiological status

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INTRODUCTION

Preservation of the number of pheasants in nature and attractiveness of pheasant hunting necessitated the need to increase production volumes on pheasant farms. Uspešnost farmske proizvodnje fazana uslovljena je poštovanjem određenih tehnoloških normativa i kontinuiranim sprovodjenjem neophodnih biosigurnosnih mere u skladu sa biosigurnosnim protokolom, u cilju dobijanja vitalnih jedinki dobrog zdravstvenog statusa. (Anon, 2012; Đorđević, 2009) Pheasant farms represent specific farm facilities, which consist of several production units, where a simultaneous breeding of pheasants of different age group is carried out. When it comes to health risk, this type of farms represent a great health risk because there are several age classes within a single commercial yard, which requires the need for continuous implementation of biosafety measures defined by the biosafety protocol, with constant monitoring of the health status of individuals at different stages of production (Pavlović, Floristean, 2004). Testing of the effect of disinfection was carried out in the following production units: aviary for parental stock, egg storage room, incubation station, facilities for raising young pheasants , open aviaries for keeping 2-6 weeks old pheasants as well as open aviaries for keeping pheasant from 6 weeks of age to the moment of releasing them into the hunting area.

In addition to the regular maintenance of hygiene of facilities and equipment, disinfection is of great importance because it represents the best way of reducing the microorganisms in these facilities (Ilić et al., 2008; Matković, Matković, 2006)

In Table 1 we show the most commonly used disinfectants in facilities in poultry farming as well as their disinfection properties. Most disinfectants listed in Table 1 exhibit bactericidal, fungicidal and virulicidal effects, which makes them effective for application in specific conditions of pheasants farms. In literature, there is no much information about use of disinfectants on farms.

The efficacy of these disinfectants is most easily and effectively checked by taking swabs from treated surfaces before and after disinfection. The effectiveness of the disinfection is significantly influenced by the selection of an adequate disinfectant and preparation that removes impurities of organic and inorganic origin, which, if not removed, result in the reduction of the effects of the applied disinfectants.

If disinfectants are applied uncontrollably or in inadequate doses microorganisms over time become resistant to applied disinfectant agents.

The paper analyzes the effects of two disinfectants (persacetic acid based and formaldehyde based ones) on different surfaces in different stages of production.

MATERIAL AND METHODS OF WORK

Upon completion and before the start of a new production cycle, all facilities-production units and equipment were mechanically cleaned from visible impurities and washed with warm water (up to 56oC) using standard equipment (brooms, brushes, etc.) in accordance with the defined biosafety protocol. After the mechanical cleaning and sanitary washing of all facilities had been completed, all production units were painted, while the floors were treated with 2% aqueous disinfectant solution based on peracetic acid using motor sprayer, while equipment (feeders, water troughs, batteries, egg crates)was disinfected by immersion in 2 % aqueous solution of peracetic acid disinfectant.

Ground surfaces in aviaries were treated with a 3% aqueous solution of peracetic acid based disinfectant using a motor sprayer. After cleaning and washing, disinfection of the incubators and the hetchers was done with formalin vapor (60 ml of formaldehyde and 40 g of Potassium permanganate were used per 1 m3 of space). Carton boxes for transporting eggs (cardboards) and straw mats were disinfected with formalin vapor. Straw had been fumigated before it was put in the aviary for parent stock and the aviary for breeding 2-6 weeks old pheasants. Formal vapors were left for 30 minutes, after which rooms, incubators, hatchers, cardboard storages, and rooms in which straw was fumigated were well-ventilated.

Determination of microbiological status was carried out on surfaces in production areas , that is on walls and floors, equipment (feeders, water troughs, egg crates, batteries), egg cartons and eggs storage facilities, incubators and hatchers, interior of incubators and mats. All the samples were taken randomly and 5 swabs were taken from each surface. In order to determine the microbiological status, swabs from the surfaces were taken using commercial sterile swabs presoaked with sterile saline. Using a plastic template (10x10cm), the swabs were taken from the same surface selected randomly 30 minutes before and after the disinfection. The swabs from ground surfaces were taken by using shoe covers that were put on when entering the aviary and taken off before leaving it. Sampling of the soil from the aviary was carried with sampling depth of 5 cm to detect the presence of anaerobes. Checking the microbiological status of the production site was done before the eggs were hatched. The swabs were taken from the surfaces of feeders, water roughs, walls and floor of the aviary as well as the from the ground in the aviaries using shoe covers according to the previously defined procedure. Before and after the fumigation was carried out, 5 samples of straw were taken in order to check the bacteriological and mycological status. From the egg storage rooms, swabs were taken from the surfaces of the wall ,the floor, egg crates and egg cartons. Swabs

from the surface of the wall and floor of the facility in which incubators were placed were also taken, as well as the swabs of each incubator and egg crates. Likewise, swabs from the surface of the wall and floor of the facility in which hatchers were placed were taken, as well as the swabs of each hatcher and each egg crate in a hatcher.

In the aviaries for breeding pheasants up to 2 weeks of age, swabs were taken from the walls and the floor, as well as from the surfaces of empty batteries using shoe covers in accordance with previously defined procedure. Swabs were also taken from the walls, feeders,water troughs and soil in the aviaries for breeding pheasants that are 2-6 weeks of age, as well as in the aviaries in which 6 weeks old pheasants are kept till being released into the hunting area

After sampling, the swabs were placed in a hand-held refrigerator and transported to the laboratory. In the laboratory conditions, bacterial counts were determined, cultural characteristics were examined and the detection of enterobacteria, salmonella, anaerobic, fungi and mold was performed. Swab processing was done by homogenizing them for several minutes in a 10ml saline solution with the addition of 1% peptone from which dilutions were made.

The total number of bacteria was made in the dilution series from 1:10 to 1:10 000 000 in the medium for total number of bacteria.

Detection of enterobacteria was done by using a nutritent agar with 5% sheep blood, brilliant green bile lactose broth, endo agar and McConkey agar.

The potential presence of salmonella was carried out in pre-enrichment medium - buffered pepton water (Bio Merieux, France) and incubated for 18-24 h at 370 C. Then 0.2 ml was transferred to selective enrichment medium Rappaport Vasilliadis (HiMedia) at 41, 50 C and Selenite cysteine broth at 370 C during 18-24h. After incubation, 0.1 ml of liquid culture was seeded on XLD, McConkey agar and Brilliant green agar (HimMedia). The substrates were then incubated at 370C for 24h after which they were examined for the presence of colonies corresponding to Salmonella spp. Identification of bacteria was done by testing cultural, macro and micro-morphological characteristics and biochemical activities by standard and commercial tests. BBL Crystal System (Becton Dickinson, USA) was used to confirm identification.

Determination of the presence of anaerobic bacteria was done on the Tarozzi broth and Zeissler agar by seeding soil samples(1gr sample in 9 ml of saline) in a dilution of 1: 100 - 1: 1 000 000 on a sulphite agar (HiMedia) The final identification of anaerobic bacteria was done with the BBL Crystal Anaerobes ID Kit (Becton Dickinson, USA). The total number and determination of fungi and mold was done on Sabouraud agar in a dilution series of 1:10 to 1:10 000 000.

RESULTS AND DISCUSSION

Tables 1 and 2 show the sampling points within the production units and equipment from which swabs were taken according to the previously defined procedure, as well as the average number and type of bacteria, fungi and mold before and after disinfection.

Table 1. Results of bacteriological tests

Place of sampling	Average number of bacteria before disinfection	Average number of bacteria after disinfection
Facility for parent stock		
Wall	$3x10^6$ saprophytes	$4x10^3$ saprophytes
Floor	$2x10^8$ saprophytes	$3x10^4$ saprophytes
Feeders	$5x10^7$ saprophytes	$4x10^3$ saprophytes
Water troughs	$3x10^6$ saprophytes	0
Mat	small number,, saprophytes	0
Ground of the aviary	$3x10^6$ coliform, saprophytes	$3x10^3$ saprophytes
Room for egg storage:		
Wall	$7x10^5$ saprophytes	$2x10^1$ saprophytes
Floor	$4x10^6$ saprophytes	$3x10^2$ saprophytes
Egg crate	$3x10^5$ coliform, saprophytes	$1x10^2$ saprophytes
Egg cartons	$7x10^5$ coliform, saprophytes	$3x10^3$ coliform, saprophytes
Eggs	$1x10^3$ saprophytes	0
Incubation station:		
Wall	$2x10^5$ saprophytes	$1x10^2$ saprophytes
Floor	$3x10^6$ saprophytes	$2x10^2$ saprophytes
Interior of the incubator	$4x10^3$ saprophytes	0
Egg crates	$3x10^5$ coliform, saprophytes	0
Hatchers		
Wall	$3x10^5$ saprophytes	$4x10^1$ saprophytes
Floor	$2x10^6$ saprophytes	$4x10^2$ saprophytes
Interior of hatchers	$5x10^2$ saprophytes	0
Egg crates	$2x10^4$ saprophytes	0
Facility for 2 weeks old pheasants		

Wall	$6x10^6$ saprophytes	$3x10^3$ saprophytes
Floor	$4x10^7$ saprophytes	$4x10^3$ saprophytes
Surfaces of empty batteries	$2x10^4$ saprophytes	$1x10^2$ saprophytes
Facility for 2 - 6 weeks old pheasants		
Wall	$2x10^6$ saprophytes	$4x10^3$ saprophytes
Floor	$3x10^7$ saprophytes	$2x10^4$ saprophytes
Feeders	$5x10^7$ coliform, saprophytes	$2x10^4$ saprophytes
Water troughs	$3x10^6$ saprophytes	0
Earthen floor in the aviary	$3x10^5$ coliform , saprophytes	$4x10^3$ saprophytes
Facility for pheasants older than 6 weeks		
Wall	$7x10^5$ saprophytes	$3x10^2$ saprophytes
Floor	$2x10^6$ saprophytes	$3x10^4$ saprophytes
Feeders	$3x10^7$ saprophytes	$6x10^4$ saprophytes
Water troughs	$3x10^4$ saprophytes	$2x10^2$ saprophytes
Earthen floor in the aviary	$4x10^6$ coliform, saprophytes	$3x10^3$ saprophytes

During the experiment , when it comes to the species of coliform bacteria Escherichia coli, Klebsiella spp. and Enterococcus spp., were isolated, while most of the saprophytes were different types of Bacillus spp., Micrococcus spp. and -hemolytic Streptococcus spp.

On the basis of the results obtained in Table 1, it is noted that there has been a decrease in the number of bacteria on all swabs after disinfection with persistent acid and formaldehyde based preparations. After the disinfection carried out on swabs, the presence of coliform bacteria has not been established, except for swabs taken from egg cartons, where the total number of bacteria remained high after disinfection. This is explained by poor practice in the previous period, where reusable use of egg cartons was applied.

Saprophytic bacteria that were isolated from the swabs after the disinfection were mainly members of the genus Bacillus. These results are explained by the fact that members of the genus Bacillus, in adverse environmental conditions, form spores that are significantly more resistant to the activity of the applied disinfectant than vegetative forms. Because of this it is necessary to continuously implement hygienic measures, with continuous disinfection in order to reduce the number of microorganisms, thereby reducing the risk of emergence of potential pathogens.

The areas where it was possible to carry out quality mechanical cleaning and sanitary washing measures after the disinfection gave the best results and did not determine the presence of bacteria or it was determined sporadically.

Table 2. Results of mycological testing

Place of sampling	Prosečan broj gljivica i plesni pre dezinfekcije	Prosečan broj gljivica posle dezinfekcije
Facility for parent flock		
Wall	$2x10^6$*Mucor spp. Aspergillus spp.*	$4x10^3$*Aspergillus spp.*
Floor	$3x10^7$*Aspergillus spp.*	$3x10^3$*Aspergillus spp.*
Feeders	$4x10^5$*Mucor spp. Aspergillus spp.*	$2x10^3$*Aspergillus spp.*
Water troughs	$2x10^3$*Mucor spp. Aspergillus spp.*	>100*Aspergillus spp.*
Mat	$7x10^8$*Mucor spp. Aspergillus spp.*	$4x10^2$*Aspergillus spp.*
Earthen floor in the aviary	$5x10^4$*Mucor spp. Aspergillus spp.*	$4x10^2$*Aspergillus spp.*
Facility for egg storage		
Wall	>100*Mucor spp.*	0
Floor	>100*Mucor spp. Aspergillus spp.*	0
Egg crates	$6x10^5$ *Penicillium spp.Aspergillus spp.*	0
Egg cartons	$8x10^7$ *Penicillium spp. Mucor spp. Aspergillus spp.*	$5x10^4$*Aspergillus spp.*
Eggs	$3x10^3$*Mucor spp. Aspergillus spp.*	0
Incubation station:		
Wall	>100*Mucor spp.*	0
Floor	>100*Mucor spp. Aspergillus spp.*	0
Interior of the incubator	>100*Mucor spp.*	0
Egg crates	$5x10^4$*Penicillium spp. Aspergillus spp.*	0
Hatchers		
Wall	>100*Mucor spp.*	0

Floor	>100*Mucor spp. Aspergillus spp.*	0
Interior of hatcher	>100*Mucor spp.*	0
Egg crates	$2x10^2$*Mucor spp.*	0
Facility for 2 weeks old pheasants		
Wall	$4x10^4$*Mucor spp. Aspergillus spp.*	>100 *Mucor spp.*
Floor	$4x10^5$*Aspergillus spp.*	>100 *Aspergillus spp.*
Surfaces of empty batteries	$2x10^5$*Aspergillus spp.*	0
Facility for 2-4 weeks old pheasants		
Wall	$5x10^6$*Mucor spp. Aspergillus spp.*	$2x10^3$*Aspergillus spp.*
Floor	$5x10^7$*Mucor spp. Aspergillus spp.*	$4x10^3$*Aspergillus spp.*
Feeders	$1x10^6$*Mucor spp. Aspergillus spp.*	$7x10^2$*Aspergillus spp.*
Water troughs	$4x10^3$*Mucor spp. Aspergillus spp.*	0
Earthen floor in the aviary	$5x10^4$*Mucor spp. Aspergillus spp.*	$3x10^3$*Aspergillus spp.*
Facility for pheasants older than 6 weeks		
Wall	$3x10^6$*Mucor spp. Aspergillus spp.*	$2x10^3$ *Aspergillus spp.*
Floor	$3x10^8$*Mucor spp. Aspergillus spp.*	$6x10^4$*Aspergillus spp.*
Feeders	$4x10^5$*Mucor spp. Aspergillus spp.*	$1x10^3$*Aspergillus spp.*
Water troughs	$2x10^4$*Mucor spp. Aspergillus spp.*	0
Earthen floor in the aviary	$1x10^8$*Mucor spp. Aspergillus spp.*	$3x10^4$*Aspergillus spp.*

In Table 2 it can be seen that after the disinfection, a significant drop in the number of mold was detected. The finding of a small number of fungi and mold before disinfection in the incubator and the hatchers can be explained by the material of which they are made as with proper maintenance it is not suitable for the growth of fungi and mold.

The surfaces of walls and floors in the facility where the parent flock was located created certain problems in the realization of mechanical cleaning and sanitary washing before the disinfection procedure, due to the presence of animals. An additional problem was the feeders built of wooden materials and the fact that washing and disinfection of feeders and water troughs weren't conducted on daily basis. During the control after the disinfection, there was still a significant presence of Aspergillus mold, while the

swabs taken after disinfection revealed the predominant presence of Aspergillus flavus which can be explained by greater resistance of Aspergillus spp. spores and by previously defined problems. With the introduction of daily washing and disinfection of feeders and water troughs, satisfactory results have been achieved in the reduction of microorganisms.The problem with the presence of microorganisms on feeders after disinfection has to be solved by replacing wooden feeders with those made from materials that can be easily disinfected and washed.

CONCLUSION

By analyzing the microbiological status before and after disinfection, we can conclude that the use of peracetic acid based disinfectants shows satisfactory effects on surfaces that due to their characteristics could be adequately prepared for disinfection using mechanical cleaning and sanitary washing. On surfaces where it was not possible to carry out adequate cleaning and sanitary washing, such as wooden feeders, the results of the disinfection were not satisfactory. For this reason, it is necessary to use feeders and water troughs made of materials that can be easily washed and disinfected, thus enabling continuous disinfection as a biosafety measure. The problem of the increased number of microorganisms on egg cartons can be solved by one-time use of egg cartons with their previous disinfection according to the previously defined procedure using formalin vapor, while implementing all measures for the protection of persons who conduct disinfection.

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