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ANALIZA EFEKATA SPROVEDE DEZINFEKCIJE POVRŠINA I OPREME U RAZLIČITIM PROIZVODNIM CELINAMA NA FAZANERIJI PRIMENOM RASTVORA NA BAZI PERSIRĆETNE KISELINE I FORMALDEHIDA

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Kratak sadržaj: Fazanerije predstavljaju proizvodne objekte poluzatvorenog tipa sa nekoliko proizvodnih celina koje su ciklično povezane i u kojima se gaje jedinke različitih starosnih kategorija. Proizvodne celine čine volijera za matično jato, prostorija za skladištenje jaja, inkubatorska stanica, prostorije za odgoj mladih fazana i volijere sa ispustima gde se vrši odgoj fazana do momenta ispuštanja u lovište. Vodeći računa o prethodno navedenom, a u cilju postizanja dobrih proizvodnih rezultata, sa dobijanjem jedinki dobrog zdravstvenog statusa, za uspešnu farmsku proizvodnju fazanske divljači, neophodno je kontinuirano sprovođenje biosigurnosnih mera u svim fazama tehnološkog postupka proizvodnje. Jedna od ključnih biosigurnosnih mera, koja se mora kontinuirano sprovoditi u farmskim objektima, jeste dezinfekcija. U radu je praćen efekat sprovedene dezinfekcije u različitim fazama proizvodnje, na različitim površinama, primenom rastvora preparata na bazi persirćetne kiseline i para formaldehida. Praćenjem mikrobiološkog statusa površina u okviru proizvodnih celina ustanovljena je redukcija ukupnog broja bakterija, gljivica i plesni u manjem ili većem obimu u zavisnosti od mesta uzorkovanja i vrste proizvodne celine.
Ključne reči: fazanerija, dezinfekcija, persirćetna kiselina, formaldehid, mikrobiološki status

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UVOD

Očuvanje brojnosti fazana u prirodi, atraktivnost lova fazana, kako poligonskog tako i lova u otvorenim lovištima, uslovio je potrebu povećanja obima proizvodnje na fazanerijama. Uspešnost farmske proizvodnje fazana uslovljena je poštovanjem određenih tehnoloških normativa i kontinuiranim sprovodjenjem neophodnih biosigurnosnih mera u skladu sa biosigurnosnim protokolom, u cilju dobijanja vitalnih jedinki dobrog zdravstvenog statusa. (Anon, 2012; Đorđević, 2009)

Fazanerije predstavljaju specifične farmske objekte koji se sastoje od nekoliko proizvodnih celina, gde se unutar farmskog objekta vrši istovremeni uzgoj jedinki različitih starosnih kategorija. Sa aspekta zdravstvenog rizika po jedinke, ovakav način farmskog uzgoja fazana predstavlja veliki zdravstveni rizik, jer se unutar jednog ekonomskog dvorišta nalazi više starosnih kategorija, što uslovljava potrebu kontinuiranog sprovođenja biosigurnosnih mera koje se definišu biosigurnosnim protokolom, uz stalno praćenje zdravstvenog statusa jedinki u različitim fazama proizvodnje (Pavlović, Floristean, 2004). Ispitivanje efekata sprovedene dezinfekcije vršeno je u sledećim proizvodnim celinama: volijera za matično jato, prostorija za skladištenje jaja, inkubatorska stanica, prostorije za odgoj mladih fazana, volijere sa ispustima gde se vrši odgoj fazana (2–6 nedelja) i volijera sa ispustima za odgoj fazana od 6 nedelja starosti do momenta ispuštanja u lovište.

Pored redovnog održavanja higijene objekata i opreme, dezinfekciji se pridaje veliki značaj, jer predstavlja najbolji vid redukcije mikroorganizama koji se održavaju u ovim objektima (Ilić et al.,2008; Matković, Matković, 2006). U tabeli 1. smo prikazali najčešće korišćene dezinficijense u farmskim objektima za uzgoj živine, kao i njihova dezinfekciona svojstva. Većina dezinficijensa koji su navedeni u tabeli 1. ispoljava baktericidno, fungicidno i virulicidno dejstvo, što ih čini efikasnim za primenu u specifičnim uslovima farmske proizvodnje fazana. Literaturni podaci o primeni dezinficijensa u farmskom uzgoju fazana su dosta skromni.

Ispitivanje efikasnosti navedenih dezinfekcionih sredstava najlakše i najefikasnije se proverava uzimanjem briseva sa tretiranih površina pre i nakon sprovedene dezinfekcije. Na samu efikasnost sprovedene dezinfekcije znatno utiče, pored izbora adekvatnog dezinfekcionog sredstva, i priprema kojom se odstranjuju nečistoće organskog i neorganskog porekla, a koje, ukoliko se ne uklone, za posledicu imaju umanjenje efekata primenjenih dezinfekcionih sredstava.

Prilikom sprovođenja dezinfekcije mora se voditi računa o tome da mikroorganizmi vremenom postaju otporni na primenjena dezinfekciona sredstva, naročito ako se ona primenjuju nekontrolisano i uz neadekvatno

doziranje, kao i usled neadekvatnog postupanja sa njima.

U radu iznosimo analizu efekata dva dezinfekciona preparata (na bazi persirćetne kiseline i formaldehida) na različitim površinama u različitim fazama proizvodnje fazana u farmskim uslovima.

MATERIJAL I METODE RADA

Nakon završetka i pre početka novog proizvodnog ciklusa, svi objekti – proizvodne celine i oprema u njima su, shodno definisanom biosigurnosnom protokolu, mehanički očišćeni korišćenjem standardne opreme (metle, četke i sl.) od vidljivih nečistoća i oprani toplom vodom (do 56^{o}C). Nakon završenog mehaničkog čišćenja i sanitarog pranja svih objekata, sprovedeno je krečenje svih proizvodnih celina, dok su podovi tretirani 2% vodenim rastvorom dezinficijensa na bazi persirćetne kiseline pomoću motorne prskalice, oprema (hranilice, pojilice, baterije, kasete za jaja) je dezinfikovana potapanjem u 2% vodeni rastvor dezinficijensa na bazi persirćetne kiseline. Zemljane površine u volijerama tretirane su 3% vodenim rastvorom dezinficijensa na bazi persirćetne kiseline korišćenjem motorne prskalice. Dezinfekcija inkubatora i valjaonika nakon čišćenja i pranja rađena je formalinskim parama (na 1 m^3 prostora korišćeno je 60 ml formaldehida i 40 gr hipermangana). Kartonske kutije za transport jaja (kartonke) i slama-prostirka dezinfikovane su formalinskim parama. Rađena je fumigacija slame pre unošenja u objekat za držanje matičnog jata i objekat za uzgoj kod fazančića 2–6 nedelja starosti. Formalinske pare su ostavljane da deluju 30 minuta, a zatim su prostorije, inkubatori, valjaonici, skladišta za kartonke, kao i prostorije u kojima je rađena fumigacija slame dobro provetravane.

Utvrđivanje mikrobiološkog statusa vršeno je na površinama u proizvodnim celinama i to na zidovima i podovima, na opremi (hranilice, pojilice, kasete za jaja, baterije), na kartonkama za jaja i u prostoriji za skladištenje jaja, u unutrašnjosti inkubatora i valjaonika, zatim sa zemljanih površina volijera i prostirke. Svi uzorci su uzimani metodom slučajnog izbora i sa svake površine je uzimano po 5 briseva. Za utvrđivanje mikrobiološkog statusa su uzimani brisevi sa navedenih površina korišćenjem komercijalnih sterilnih briseva koji su prethodno nakvašeni sterilnim fiziološkim rastvorom. Pomoću plastičnog šablona (10x10cm) uzimani su brisevi uvek sa iste površine, koja je odabrana metodom slučajnog izbora, pre i 30 minuta nakon urađene dezinfekcije. Brisevi zemljanih površina unutar ispusta volijera su uzimani pomoću nazuvica koje su stavljane na obuću prilikom ulaska u volijeru i skidane pre izlaska iz iste. Uzorkovanje zemljišta iz volijera je vršeno sa 5cm dubine radi detekcije prisustva anaeroba.

Provera mikrobiološkog statusa proizvodnog objekta matičnog jata urađena je pre početka pronošenja jaja. Brisevi su uzeti sa površina hranilica, pojilica, zidova i poda objekta za smeštaj matičnog jata, kao i zemljišta u volijeri pomoću nazuvica, po prethodno definisanoj proceduri. Pre i nakon sprovedene fumigacije uzeto je po 5 uzoraka slame u cilju provere njenog bakteriološkog i mikološkog statusa. Iz prostorije za skladištenje jaja uzeti su brisevi sa površina zida i poda unutrašnjosti objekta i brisevi sa kaseta i kartonki za jaja. Iz inkubatorske stanice uzeti su brisevi sa površina zida i poda prostorije u kojoj su smešteni inkubatori, kao i brisevi unutrašnjosti svakog inkubatora i kaseta za jaja. Iz prostorije u kojoj su smešteni valjaonici uzeti su brisevi sa površina zida i poda prostorije u kojoj su smešteni valjaonici, sa unutrašnjosti svakog valjaonika, kao i sa kaseta za jaja unutar svakog valjaonika.

U objektima za uzgoj fazančića do 2 nedelje starosti uzeti su brisevi sa površina zidova i poda prostorije i sa površina praznih baterija. U objektima za smeštaj fazančića 2–6 nedelja starosti, kao i u objektima od 6 nedelja starosti do ispuštanja u lovište uzeti su brisevi sa površina zidova, hranilica i pojilica, kao i zemljišta u volijeri pomoću nazuvica, po prethodno definisanoj proceduri.

Nakon uzorkovanja brisevi su stavljani u ručni frižider i transportovani do laboratorije. U laboratorijskim uslovima vršeno je utvrđivanje brojnosti bakterija, ispitivane su kulturalne osobine i vršena je detekcija enterobakterija, salmonela, anaeroba, gljivica i plesni. Obrada briseva je rađena tako što su oni homogenizovani nekoliko minuta u 10ml slanog rastvora uz dodatak 1% peptona odakle su pravljena razređenja. Ukupan broj bakterija je rađen u seriji razređenja od 1:10 do 1:10 000 000 na podlozi za ukupan broj bakterija.

Detekcija enterobakterija rađena je na hranljivom agaru sa dodatkom 5% ovčije krvi, Briliant zeleni laktoza žučnom bujonu, Endo agaru i McConkey agaru za enterobakterije.

Potencijalno prisustvo salmonela je rađeno na podlogama za preobogaćenje – puferisana peptonska voda (Bio Merieux, Francuska) i inkubirane su 18–24 h na 37^0 C. Zatim je 0,2 ml prebačeno na podlogu za selektivno obogaćenje Rappaport Vasilliadis (HiMedia) na $41,5^0$ C i Selenit cistein bujon na 37^0 C u toku 18–24h. Nakon inkubacije 0,1ml tečne kulture je zasejana na XLD, McConkey agaru i Brilijant zelenom agaru (HimMedia). Podloge su potom inkubirane na 37^0C u trajanju od 24h nakon čega su pregledane na prisustvo kolonija koje odgovaraju *Salmonella spp*. Identifikacija bakterija rađena je ispitivanjem kulturalnih, makro i mikro-morfoloških osobina i biohemijskih aktivnosti primenom standardnih i komercijalnih testova. Za potvrdu identifikacije korišćen je BBL Crystal sistem (Becton Dickinson, USA). Utvrđivanje prisustva anaerobnih bakterija rađeno je na Tarozzi bujonu i Zeissler agaru tako što su uzorci zemlje

(1gr uzorka u 9 ml fiziološkog rastvora) zasejani u razređenju od 1 : 100 – 1 : 1 000 000 na sulfitnom agaru (HiMedia). Konačna identifikacija anaerobnih bakterija rađena je sa BBL Crystal Anaerobes ID Kit (Becton Dickinson, USA).

Ukupan broj i determinacija gljivica i plesni rađena je na Sabouraud agaru u seriji razređenja od 1:10 do 1:10 000 000.

REZULTATI I DISKUSIJA

U tabelama 1. i 2. prikazana su mesta uzorkovanja unutar proizvodnih celina i oprema, sa kojih su uzimani brisevi po prethodno definisanoj proceduri, kao i prosečan broj i vrste bakterija, gljivica i plesni pre i nakon sprovedene dezinfekcije.

Tabela 1. Rezultati bakteriološke pretrage

Mesto uzimanja uzoraka	Prosečan broj bakterija pre dezinfekcije	Prosečan broj bakterija posle dezinfekcije
Objekat za smeštaj matičnog jata:		
Zid objekata	$3x10^6$ saprofiti	$4x10^3$ saprofiti
Pod objekta	$2x10^8$ saprofiti	$3x10^4$ saprofiti
Hranilice	$5x10^7$ saprofiti	$4x10^3$ saprofiti
Pojilice	$3x10^6$ saprofiti	0
Prostirka	mali broj, saprofiti	0
Zemljište voliijere	$3x10^6$ koliforma, saprofiti	$3x10^3$ saprofiti
Prostorija za skladištenje jaja:		
Zid objekta	$7x10^5$ saprofiti	$2x10^1$ saprofiti
Pod objekta	$4x10^6$ saprofiti	$3x10^2$ saprofiti
Kaseta za jaja	$3x10^5$ koliformi, saprofiti	$1x10^2$ saprofiti
Kartoni za jaja	$7x10^5$ koliformi, saprofiti	$3x10^3$ koliformi, saprofiti
Jaja	$1x10^3$ saprofiti	0
Inkubatorska stanica:		
Zid objekta	$2x10^5$ saprofiti	$1x10^2$ saprofiti
Pod objekta	$3x10^6$ saprofiti	$2x10^2$ saprofiti
Unutrašnjost inkubatora	$4x10^3$ saprofiti	0
Kasete za jaja	$3x10^5$ koliformi, saprofiti	0

Valjaonici:		
Zid objekta	$3x10^5$ saprofiti	$4x10^1$ saprofiti
Pod objekta	$2x10^6$ saprofiti	$4x10^2$ saprofiti
Unutrašnjost valjaonika	$5x10^2$ saprofiti	0
Kasete za jaja	$2x10^4$ saprofiti	0
Objekat za smeštaj fazančića do 2 nedelje starosti:		
Zid objekta	$6x10^6$ saprofiti	$3x10^3$ saprofiti
Pod objekta	$4x10^7$ saprofiti	$4x10^3$ saprofiti
Površine praznih baterija	$2x10^4$ saprofiti	$1x10^2$ saprofiti
Objekat za smeštaj fazančića 2–6 nedelja starosti		
Zid objekta	$2x10^6$ saprofiti	$4x10^3$ saprofiti
Pod objekta	$3x10^7$ saprofiti	$2x10^4$ saprofiti
Hranilice	$5x10^7$ koliformi, saprofiti	$2x10^4$ saprofiti
Pojilice	$3x10^6$ saprofiti	0
Zemljani pod volijere	$3x10^5$ koliformi , saprofiti	$4x10^3$ saprofiti
Objekat za smeštaj fazančića starijih od 6 nedelja:		
Zid objekta	$7x10^5$ saprofiti	$3x10^2$ saprofiti
Pod objekta	$2x10^6$ saprofiti	$3x10^4$ saprofiti
Hranilice	$3x10^7$ saprofiti	$6x10^4$ saprofiti
Pojilice	$3x10^4$ saprofiti	$2x10^2$ saprofiti
Zemljani pod volijere	$4x10^6$ koliformi, saprofiti	$3x10^3$ saprofiti

Tokom eksperimenta od koliformnih bakterija izolovane su *Escherichia coli, Klebsiella spp.* i *Enterococcus spp.*, a od saprofitskih najveći broj su činile različite vrste *Bacillus spp., Micrococcus spp.* i -hemolitičnih *Streptococcus spp.*

Na osnovu dobijenih rezultata koji su prikazani u tabeli 1. uočava se da je došlo do smanjenja broja bakterija na svim uzetim brisevima nakon sprovedene dezinfekcije preparatima na bazi persirćetne kiseline i formaldehida. Nakon sprovedene dezinfekcije, na brisevima nije utvrđeno prisustvo koliformnih bakterija, osim kod briseva skinutih sa kartonki za jaja, gde je i ukupan broj bakterija i nakon dezinfekcije ostao i dalje visok. Navedena pojava se objašnjava lošom praksom u prethodnom periodu, gde je primenjivana višekratna upotreba kartonki za jaja. U skladu sa dobijenim rezultatima, u biosigurnosnom protokolu je zabranjena višekratna primena kartonki za jaja.

Saprofitne bakterije koje su izolovane iz briseva nakon sprovedene dezinfekcije bile su uglavnom pripadnici roda *Bacillus*. Navedeni rezultati se objašnjavaju pojavom da pripadnici roda *Bacillus* u nepovoljnim uslovima sredine formiraju spore koje su znatno otpornije na delovanje primenjenog dezinfekcionog sredstva od vegetativnih oblika. U skladu sa navedenim, neophodno je kontinuirano sprovoditi higijenske mere, sa kontinuiranim sprovođenjem dezinfekcije u cilju smanjenja broja mikroorganizama, čime se smanjuje rizik od pojave potencijalnih patogena.

Površine na kojima je bilo moguće kvalitetno sprovesti mere mehaničkog čišćenja i sanitarnog pranja su nakon sprovedene dezinfekcije dale najbolje rezultate i na njima nije utvrđeno prisustvo bakterija ili je ono utvrđeno sporadično.

Tabela 2. Rezultati mikološke pretrage

Mesto uzimanja uzoraka	Prosečan broj gljivica i plesni pre dezinfekcije	Prosečan broj gljivica posle dezinfekcije
Objekat za smeštaj matičnog jata:		
Zid objekta	$2x10^6$*Mucor spp. Aspergillus spp.*	$4x10^3$*Aspergillus spp.*
Pod objekta	$3x10^7$*Aspergillus spp.*	$3x10^3$*Aspergillus spp.*
Hranilice	$4x10^5$*Mucor spp. Aspergillus spp.*	$2x10^3$*Aspergillus spp.*
Pojilice	$2x10^3$*Mucor spp. Aspergillus spp.*	>100*Aspergillus spp.*
Prostirka	$7x10^8$*Mucor spp. Aspergillus spp.*	$4x10^2$*Aspergillus spp.*
Zemljani pod voliijere	$5x10^4$*Mucor spp. Aspergillus spp.*	$4x10^2$*Aspergillus spp.*
Prostorija za skladištenje jaja:		
Zid objekta	>100*Mucor spp.*	0
Pod objekta	>100*Mucor spp. Aspergillus spp.*	0
Kaseta za jaja	$6x10^5$ *Penicillium spp.Aspergillus spp.*	0
Kartoni za jaja	$8x10^7$ *Penicillium spp. Mucor spp. Aspergillus spp.*	$5x10^4$*Aspergillus spp.*
Jaja	$3x10^3$*Mucor spp. Aspergillus spp.*	0
Inkubatorska stanica:		
Zid objekta	>100*Mucor spp.*	0
Pod objekta	>100*Mucor spp. Aspergillus spp.*	0

Unutrašnjost inkubatora	>100*Mucor spp.*	0
Kasete za jaja	$5x10^4$*Penicillium spp. Aspergillus spp.*	0
Valjaonici:		
Zid objekta	>100*Mucor spp.*	0
Pod objekta	>100*Mucor spp. Aspergillus spp.*	0
Unutrašnjost valjaonika	>100*Mucor spp.*	0
Kasete za jaja	$2x10^2$*Mucor spp.*	0
Objekat za smeštaj fazančića do 2 nedelje starosti:		
Zid objekta	$4x10^4$*Mucor spp. Aspergillus spp.*	>100 *Mucor spp.*
Pod objekta	$4x10^5$*Aspergillus spp.*	>100 *Aspergillus spp.*
Površine praznih baterija	$2x10^5$*Aspergillus spp.*	0
Objekat za smeštaj fazančića 2–6 nedelja starosti:		
Zid objekta	$5x10^6$*Mucor spp. Aspergillus spp.*	$2x10^3$*Aspergillus spp.*
Pod objekta	$5x10^7$*Mucor spp. Aspergillus spp.*	$4x10^3$*Aspergillus spp.*
Hranilice	$1x10^6$*Mucor spp. Aspergillus spp.*	$7x10^2$*Aspergillus spp.*
Pojilice	$4x10^3$*Mucor spp. Aspergillus spp.*	0
Zemljani pod volijere	$5x10^4$*Mucor spp. Aspergillus spp.*	$3x10^3$*Aspergillus spp.*
Objekat za smeštaj fazančića starijih od 6 nedelja:		
Zid objekta	$3x10^6$*Mucor spp. Aspergillus spp.*	$2x10^3$ *Aspergillus spp.*
Pod objekta	$3x10^8$*Mucor spp. Aspergillus spp.*	$6x10^4$*Aspergillus spp.*
Hranilice	$4x10^5$*Mucor spp. Aspergillus spp.*	$1x10^3$*Aspergillus spp.*
Pojilice	$2x10^4$*Mucor spp. Aspergillus spp.*	0
Zemljani pod volijere	$1x10^8$*Mucor spp. Aspergillus spp.*	$3x10^4$*Aspergillus spp.*

Iz tabele 2. može se videti da je nakon sprovedene dezinfekcije uočen značajan pad broja plesni. Nalaz malog broja gljivica i plesni pre dezinfekcije u inkubatoru i valjaoniku može se objasniti samim materijalom od kojeg su naprevljeni, a koji po

svojim karakteristikama, uz adekvatno održavanje nisu pogodni za rast gljivica i plesni.

Površine zidova i podova u objektima u kojima je smešteno matično jato su shodno tehnološkom postupku proizvodnje stvarale određene probleme u realizaciji mehaničkog čišćenja i sanitarnog pranja pre sprovođenja samog postupka dezinfekcije, usled prisustva životinja. Dodatni problem uočen je u korišćenju hranilica sagrađenih od drvenih materijala i nesprovođenju svakodnevnog pranja i dezinfekcije hranilica i pojlica. U toku kontrole, nakon sprovedene dezinfekcije, ustanovljeno je i dalje prisustvo značajnog broja plesni iz roda *Aspergillus*, dok je iz briseva uzetih nakon dezinfekcije utvrđeno pretežno prisustvo vrste *Aspergillus flavus*, uz značajno smanjenje prisustva ostalih pripadnika ovog roda, što se između ostalog može objasniti većom otpornošću spora *Aspergillus spp*. i prethodno definisanim problemima. Uvođenjem svakodnevnog pranja i dezinfekcje pojilica postignuti su zadovoljavajući rezultati u redukciji mikroorganizama, a problem prisustva istih i nakon sprovedene dezinfekcije na drvenim hranilicama mora biti rešen isključivanjem drvenih hranilica iz upotrebe i primenom hranilica od materijala koji se lako mogu prati i dezinfikovati.

ZAKLJUČAK

Analizom mikrobiološkog statusa pre i nakon sprovedene dezinfekcije, možemo zaključiti da primena dezinficijenasa na bazi persirćetne kiseline pokazuje zadovoljavajuće efekte na površinama koje su zbog svojih karakteristika mogle biti adekvatno pripremljene za dezinfekciju primenom mehaničkog čišćenja i sanitarnog pranja. Na površinama na kojima nije bilo moguće sprovesti adekvatno čišćenje i sanitarno pranje, kao što su drvene hranilice, rezultati sprovedene dezinfekcije nisu bili zadovoljavajući. Iz ovog razloga je neophodno koristiti hranilice i pojilice koje su napravljene od materijala koji se lako mogu prati i dezinfikovati, čime se omogućava kontinuirano sprovođenje dezinfekcije kao biosigurnosne mere. Uočeni problem povećanog broja m.o. na kartonkama za jaja može se rešiti jednokratnim korišćenjem kartonki za jaja, uz njihovu predhodnu dezinfekciju, po prethodno definisanoj proceduri, korišćenjem formalinskih para, uz sprovođenje svih mera zaštite lica koja sprovode dezinfekciju.

Primenjena dezinfekciona sredstva na bazi persirćetne kiseline i formaldehida ispoljila su zadovoljavajuće efekte, a uvažavajući činjenicu da se radi o biocidnim proizvodima sa prihvatljivom cenom i jednostavnim načinom aplikacije, oni se mogu preporučiti za praktičnu primenu u dezinfekciji fazanerija na površinama koje su prethodno adekvatno pripremljene.

LITERATURA

1. Annon (2012: Biosecurity procedures in poultry production, OIE - Terrestrial Animal HealthCode,Apendix6.4.1.,http://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/2010/en_chapitch_1.6.4.htm
2. Đorđević M. (2009): Biosigurnosne mere u fazanerijama, Zbornik radova XIX savetovanje, Dezinfekcija, dezinsekcija i deratizacija u zaštiti životne sredine.
3. Ilić Z., Jakic Dimic D., Pavlovic I., Zugic G., Gavrilovic M. (2008): Efficacy of same antimicrobial substance at Salmonella microorganisms isolated in poultry breeding objects 1st Mediterranean Summit of WPSA, Tthessaloniki, Greece, 07–10.
4. Matković K., Matković S. (2006): Važnost čišćenja i dezinfekcije u peradarstvu, Znanstveno stručni dio, Vol. VII, 95–99, 2006.
5. Pavlović I., Floristean I. (2004): Fazani – odgoj i zdravstvena zaštita (3), Biologija i fiziološke karakteristike (I). Živinarstvo, XXXIX(11), 12–13, 2004.

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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.

REFERENCES

1. Annon (2012: Biosecurity procedures in poultry production, OIE - Terrestrial Animal HealthCode,Apendix6.4.1.,http://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/2010/en_chapitch_1.6.4.htm
2. Đorđević M. (2009): Biosafety measures in pheasant farms, Collection of papers XIX consultation on disinfection, disinsection and deratization in environmental protection
3. Ilić Z., Jakic Dimic D., Pavlovic I., Zugic G., Gavrilovic M. (2008): Efficacy of same antimicrobial substance at Salmonella microorganisms isolated in poultry breeding objects 1st Mediterranean Summit of WPSA, Tthessaloniki, Greece, 07-10
4. Matković K., Matković S. (2006): The importance of cleaning and disinfection in poultry, Scientific Expertise, Vol. VII, 95-99. 2006
5. Pavlović I., Floristean I. (2004): Pheasants - education and health care (3), Biology and physiological characteristics (I). Poultry farming, XXXIX(11), 12-13, 2004.

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