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Microbiological Response of Japanese Quail Eggs to Disinfection and Location in the Setter During Incubation

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The aim of this study was to evaluate the effectiveness of ethyl alcohol (75%) disinfection of Japanese quail (*Coturnix coturnix japonica*) hatching eggs and analysis of microbial contamination of eggs during incubation, depending on their location in the setter. Disinfected eggshells were found to have lower total bacteria (TBC) and fungi (TFC) count. Concerning the vertical location of eggs (top, middle, bottom), disinfected eggs were characterized by similar values of the TBC ($\bar{x}$ = 1.54 log CFU/shell surface). For eggs without disinfection, it was found that those from middle and bottom levels of the setter had similar and lower TBC (by about 1.22 log CFU/shell surface) as compared to eggs from the top level. No statistically significant differences between levels were found in the case of TFC. Hatch breakouts (dead-in-shell embryos) from non-disinfected eggs were characterized by higher TBC (on average 0.37 log CFU/g). Disinfected eggs, located at the middle and bottom levels of the incubator, had similar and lower TBC in comparison with eggs from the top level. There were no microscopic fungi inside disinfected eggs of hatch breakouts. On the other hand, the non-disinfected eggs, placed on trays from the middle level of the incubator, had greater TFC (by about 0.9 log CFU/g) than those from top and bottom levels. Regardless of whether the eggs were disinfected or not, the largest group of microscopic fungi included *Aspergillus* and *Penicillium*.

Key words: Japanese quail, eggs, bacteria, fungi, contamination, disinfection, incubation.

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One of the elements of the strategy for obtaining large numbers of healthy chicks is to carry out incubation based on optimal microclimate parameters in the setter (temperature, humidity, ventilation, etc.) and proper handling of eggs prior to incubation (e.g. storage). The effective disinfection of incubator and hatching eggs also plays an important role (TURBLIN 2011). Conditions inside the incubator, i.e. high temperatures and considerable humidity, foster growth and reproduction of many microorganisms including pathogens are dangerous for developing embryos. Studies carried out so far have focused mainly on bacteria, primarily of the genus *Salmonella* (BRUCE & DRYSDALE 1983; CASON *et al.* 1994; COX *et al.* 2000; GAST *et al.* 2005; KIZERWETTER-ŚWIDA & BINEK 2008). However, only a few studies have investigated the contamination of hatching bird eggs with microscopic fungi and their impact on embryogenesis (LÁBAQUE *et al.* 2003; GIGLI *et al.* 2009; NOWACZEWSKI *et al.* 2011). It was shown that the consequence of the presence of microscopic fungi in the egg is embryo mycosis. Besides, mycotoxin produced by fungi can contribute to embryo abnormalities and mortality, and even infertility of adult birds (TANGNI *et al.* 2009; JACOBSEN *et al.* 2010).

In intensive poultry production conditions, in large hatcheries, formaldehyde is usually applied for egg disinfection (CADIRCI 2009). Smaller hatcheries and amateur bird breeders use safer, in terms of human health and environment, disinfectants for this purpose (UV rays, ozone, hydrogen peroxide or ethyl alcohol). Thus experiments are carried out to determine the suitability of various

chemical compounds (preparations), as an alternative disinfectant of eggs, incubators and livestock houses than formaldehyde – a human carcinogen which is toxic via inhalation (SANDER & WILSON 1999; GEHAN 2009; WELLS *et al*. 2011).

Although microclimatic conditions in the incubator are usually precisely programmed, they are not identical in every part of the setter. This is often caused by setter construction and air flow through hatching eggs during incubation (FRENCH 1997). For example, significant differences have been shown in temperature of chicken eggshells depending on egg location in the incubator (MEIJERHOF 2000; LOURENS 2001). Similarly, the influence of Japanese quail egg location in the setter on the shell temperature during incubation and hatchability was documented by NOWACZEWSKI *et al.* (2012). Therefore, it can be expected that with such a diversity of environmental conditions inside the setter during egg incubation, the appearance and development of many groups of microorganisms may also be different.

There are no available studies describing the effectiveness of disinfection methods for quail eggs prior to incubation, and monitoring of microbial contamination during embryogenesis. The only available results concern table quail eggs contaminated with bacteria of the genus *Salmonella* and *Campylobacter* (VASHIN *et al.* 2008; OBAIDI *et al.* 2011). On the other hand, the optimization of hatches with multithreaded analysis is a very important element in the production of Japanese quail. Moreover, many new ways to improve the reproductive performance of these birds have been described (PETEK & DIKMEN 2004; ALKAN *et al.* 2008; MANI *et al.* 2008; NOWACZEWSKI *et al.* 2010a,b).

The aim of this study was to evaluate the usefulness of ethanol (75%) in the disinfection of quail hatching eggs and the analysis of microbial contamination of eggs during incubation, depending on their location in the setter.

Material and Methods

The experimental material comprised eggs of Japanese quail (*Coturnix coturnix japonica*) of laying type in their first year of reproduction derived from a commercial farm. Up to week 6 of age, chicks were kept on rye or triticale litter in a rearing chamber of 40 m^2 area. During the growing period, Japanese quails were fed *ad libitum* complete diets which contained: 12.08 MJ/kg ME, 240 g $\cdot$ kg^{-1} crude protein, 71 g $\cdot$ kg^{-1} crude fat, 30 g $\cdot$ kg^{-1} crude fibre and 10 g $\cdot$ kg^{-1} calcium. Experimental birds began laying in their 7^{th} week of age. During the entire reproductive period, the birds were kept in cages at 35 birds/m^2 stocking rate with 6:1 ratio of females to males. Egg fertilisation in the analysed flock was at the level of 75-80%. Adult Japanese quails were fed *ad libitum* complete diets which contained: 11.63 MJ/kg ME, 205 g $\cdot$ kg^{-1} crude protein, 50 g $\cdot$ kg^{-1} crude fat, 30 g $\cdot$ kg^{-1} crude fibre and 31 g $\cdot$ kg^{-1} calcium. Mixtures were prepared by a commercial feed label according to feeding standards for Japanese quail. However, the basis for determining the nutritional value of these mixtures was the declaration of the producer.

The hatching of Japanese quail eggs was conducted in a single-stage incubator (AVN type) of the Jamesway Company. Two experimental hatchings were carried out. Eggs for hatching were collected for a period of 1-2 days on the 45^{th} week of life (36^{th} week of laying). During storage, eggs were kept under the following conditions: temperature – 15 to 16°C, relative humidity – about 55 %.

A total of 960 eggs were set (480 for each hatching, first disinfected and second non-disinfected eggs). Eggs selected for hatching (average weight ranged from 10.5 to 12.5 g) were marked and non- or disinfected by spraying (75% ethanol). The spray was prepared by diluting ethyl alcohol (96%) with distilled water. Eggs were placed on setting trays, blunt end up (standard arrangement of hatching eggs on setting trays) during the disinfection. All eggs were sprayed using hand spray ("KWAZAR" CORP. Sc), for 3 min. and from a distance of 30 cm, using 80 ml of ethanol each time. Then they were set into the incubator using 6 wooden setting trays after disinfection, two trays on each level (top, middle and bottom) of the incubator. Each tray comprised four rows in which 80 eggs were placed side by side (20 eggs in each row). The horizontal and vertical distances between trays were 8 and 10 cm, respectively.

During each hatching, eggshell surface microbiological contamination was assessed on the 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 10^{th}, 12^{th} and 14^{th} days of incubation (5 eggs in each tray). For each assessment, eggs were chosen randomly from extreme places of the setting tray, i.e. 4 from corners and one from the center. After taking the test material eggs were marked in order not to reuse them in further analyses. Up to day 14, eggs were kept in the setting compartment in which the temperature was 37.6°C and mean relative air humidity at 52%. On day 15 they were transferred to the hatching compartment with a temperature of 37.4°C and mean relative air humidity 60-70%. Eggshell surface microbiological contamination was analysed using the traditional swabbing method. This consisted of the following stages: wiping the whole eggshell area with a swab moistened with a dilution fluid, rinsing the swab, preparation of dilutions, submerging the cultures of 1 cm^3 each into two dishes, incubation in a mi-

crobiological thermostat WTB Binder (Tuttlingen, Germany). Standard diluents and agar media (BTL, Poland) were used in the experiment. The results were expressed in CFU/eggshell surface. Breakouts (dead-in-shell during the first 14 days of incubation) were crushed and 10 g were transferred to a flask with 90 ml buffered peptone water. Samples prepared in this way were shaken for 15 min. From the obtained 10^{-1} dilution successive decimal dilutions were produced and inoculated using the classical plate flooding method according to Koch towards the total microbial count of aerobic bacteria (TBC). Total bacterial counts were determined using agar medium. Samples were incubated at 30°C for 72 h. Calculations were conducted for dilutions from plates, on which the number of colonies ranged from 30 to 300. Results were expressed in CFU/g. Analyses of the composition of fungi species occurring in egg probes were done. Next, 1 ml of suspension was prepared from material used during swabbing and was placed on a potato dextrose agar medium in Petri dishes and spread with the aid of a sterile glass stick on the medium surface. The Petri dishes were incubated at 25°C for 7 days. Growing mycelia were isolated on the potato dextrose agar and synthetic nutrient-poor agar mediums to identify the fungi species. The identification was carried out by colony and spore morphology with the aid of keys from RAPER &THOM (1949), ARX (1970), and DOMSCH *et al.* (1980).

After termination of the hatching process, eggs from which no chicks were hatched were opened in order to determine the number of non-fertilized eggs, eggs with dead embryos before shell piercing (early – up to 10 day, late – after 10 day) and with unhatched chicks which died during shell piercing.

The obtained results were evaluated statistically using the SAS® v. 9.2 statistical package (SAS 2011). Data were first transformed: x = log 10y, where y = trait value. The significance of differences between egg location and treatment (non- or disinfected), regarding microbiological characteristics was verified by one-way ANOVA. In case of bacteria and fungi contamination, differences between treatments within egg location were analysed using the t-Student test. All statistical differences were evaluated at the level of $P \leq 0.05$.

Results and Discussion

The results of microbiological contamination of quail eggs (non- and disinfected prior to incubation), depending on the location in the setter, are shown in Table 1. It was found that the disinfected eggshells were characterized by lower total bacterial (TBC) and fungi (TFC) count. The differences were statistically significant and, on average, amounted to 3.97 and 1.09 log CFU/eggshell surface, respectively. In an experiment carried out on laying hens FURUTA and MARUYAMA (1981) analysed the microbial contamination of eggshells during incubation (1, 3, 6, 12, 19, 20 and 21 day) after their previous disinfection with formaldehyde. The average number of bacteria showed a rate of 1.19 log CFU/egg. JAFF (2005) used ethyl alcohol (70%) for disinfection of fertilized eggs of lay-

Table 1

Effect of eggs disinfection and location in the setter on bacterial and fungal contamination during incubation[1]

Traits	Non-disinfected				Disinfected			
	Top level	Middle level	Bottom level	Overall	Top level	Middle level	Bottom level	Overall
Eggshell bacterial contamination (log CFU/shell surface)	6.33 ± 0.04a*	5.18 ± 0.61b*	5.03 ± 0.55b*	5.51 ± 0.28*	1.54 ± 0.32a*	1.34 ± 0.24a*	1.74 ± 0.32a*	1.54 ± 0.18*
Eggshell fungal contamination (log CFU/shell surface)	0.99 ± 0.20a*	1.29 ± 0.38a*	1.20 ± 0.28a*	1.16 ± 0.16*	0.09 ± 0.06a*	0.14 ± 0.09a*	0.00 ± 0.00a*	0.07 ± 0.03*
Hatch breakouts bacterial contamination (log CFU/g)	2.39 ± 0.20a	2.46 ± 0.06a*	2.66 ± 0.18a*	2.50 ± 0.10*	2.43 ± 0.17a	1.85 ± 0.04b*	2.12 ± 0.10b*	2.13 ± 0.07*
Hatch breakouts fungal contamination (log CFU/g)	0.85 ± 0.22b*	1.99 ± 0.08a*	1.33 ± 0.17b*	1.39 ± 0.14*	0.00 ± 0.00a*	0.00 ± 0.00a*	0.00 ± 0.00a*	0.00 ± 0.00*

Means ± SEM with different letters, between eggs location within variant (Non- or Dis.), differ significantly at the level $P \leq 0.05$
*Means ± SEM between variants (Non- and Dis.), within eggs location, differ significantly at the level $P \leq 0.05$
[1]Data are averaged over all sampling days.

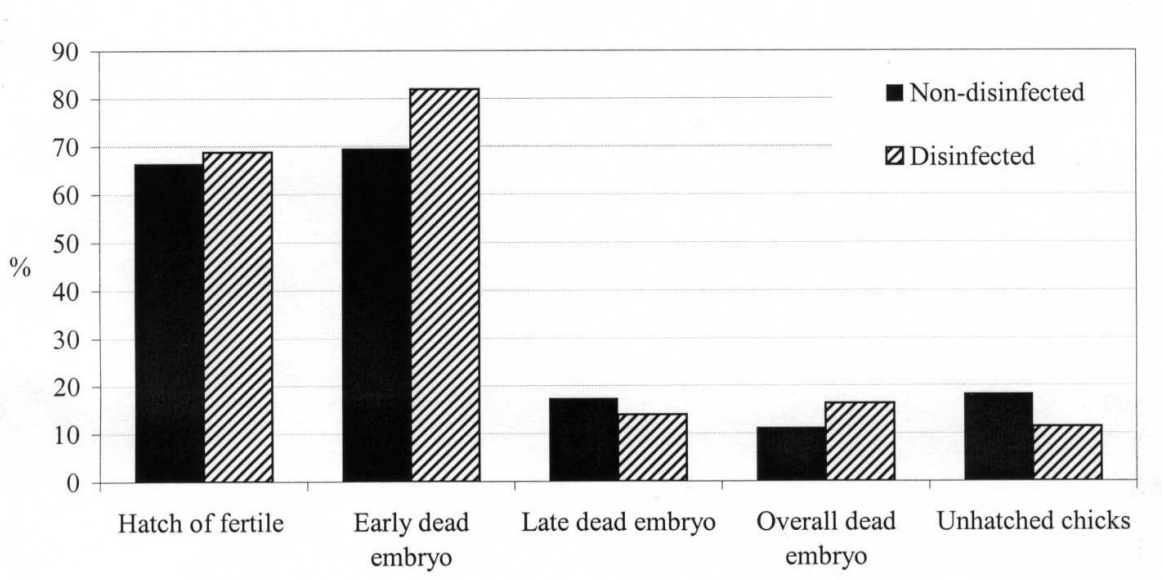

Fig. 1. Effect of eggs disinfection on hatchability results in quails[1,2].
Data was not analysed statistically
[1]Hatch of fertile + Overall dead embryo + Unhatched chicks = 100%
[2]The share of early and late dead embryos was calculated relative to the total number of dead embryos representing 100%

ing hens and observed a lower amount of aerobic and anaerobic bacteria on the shell, on average of 85.1 and 54.4%, respectively. On the other hand, WELLS *et al.* (2011) analysed the effectiveness of hydrogen peroxide and UV light (experimental group) for disinfection of eggs of broiler breeders. The authors concluded that the application of the disinfectants reduced the APC (Aerobic Plate Count) by about 2.8 $\log_{10}$CFU/egg in comparison with the control group. Notably, this meant better hatchability of fertilized eggs in the experimental group as compared to the control (74 against 68%). In our study, disinfected eggs were also characterised by slightly better hatchability from fertilised eggs (by about 2.3 percentage units, Fig. 1). Otherwise, these eggs were also characterised by a lower proportion of embryo mortality in the later period of incubation and lower number of unhatched chicks. WELLS *et al.* (2011) also observed a lower proportion of late dead embryos in eggs subjected to disinfection.

Total bacteria count in eggshells subjected to disinfection was similar, regardless of their location in the incubator. In the case of non- and disinfected eggs, no significant differences between levels of the incubator regarding the number of microscopic fungi on the eggshells surface were recorded. However, the highest fungal contamination of the shells was found on trays from the middle level (1.29 and 0.14 log CFU/shell surface for non- and disinfected eggs, respectively).

Similar to eggshell surface, hatch breakouts from non-disinfected eggs had a higher total number of bacteria (on average 0.37 log CFU/g) compared with disinfected eggs (Table 1). Assessing the results obtained for levels of setting trays, differences in the number of bacteria were only observed in disinfected eggs. Eggs located on the middle and bottom levels were characterized by similar and statistically significant lower TBC (by about 0.45 log CFU/g) compared with eggs incubated on trays from the top level. There was not fungi contamination in breakouts from disinfected eggs. On the other hand, non-disinfected eggs placed on trays from the middle level of the incubator had higher TFC (by about 0.90 log CFU/g) compared with those incubated on trays from top and bottom levels. A lack of literature on this subject does not allow for a broader interpretation of the results. However, it is worth noting that the authors have done earlier experiment in which the aim was to assess the impact of the location of Japanese quail eggs in the incubator on their temperature and hatchability (NOWACZEWSKI *et al.* 2012). It was found that eggs incubated on the middle and bottom levels of the incubator (more fungal contamination of breakouts) were characterized by significantly worse hatchability.

Among all analyzed eggs, there were 28 and 61% of positive samples (incidence of fungi) in disinfected and non-disinfected eggs, respectively. On the surface of non-disinfected eggshells

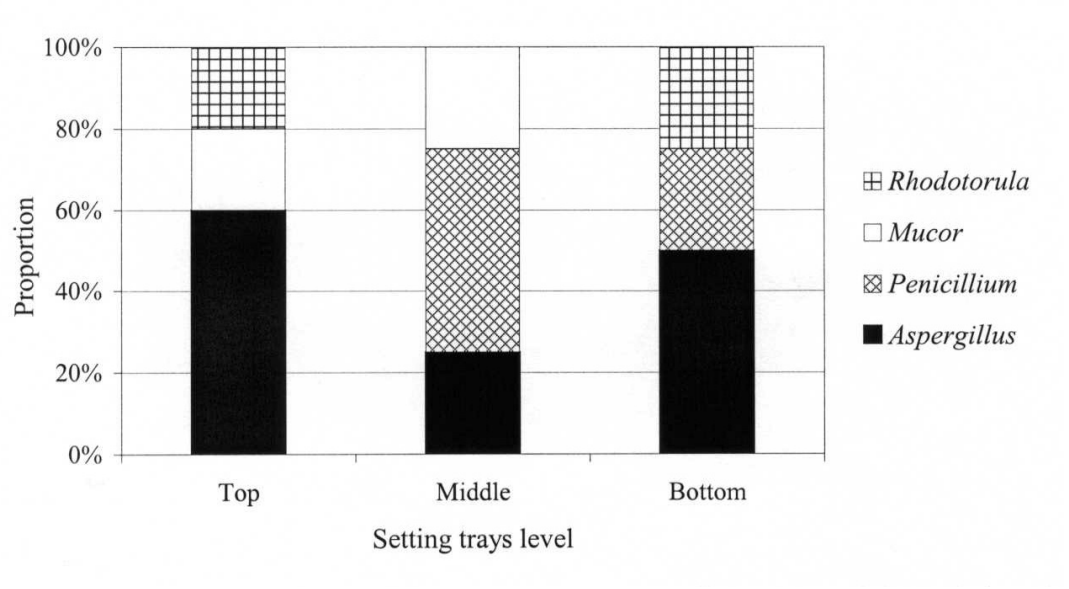

Fig. 2. Proportion of total identified fungi genus in non-disinfected eggshells.

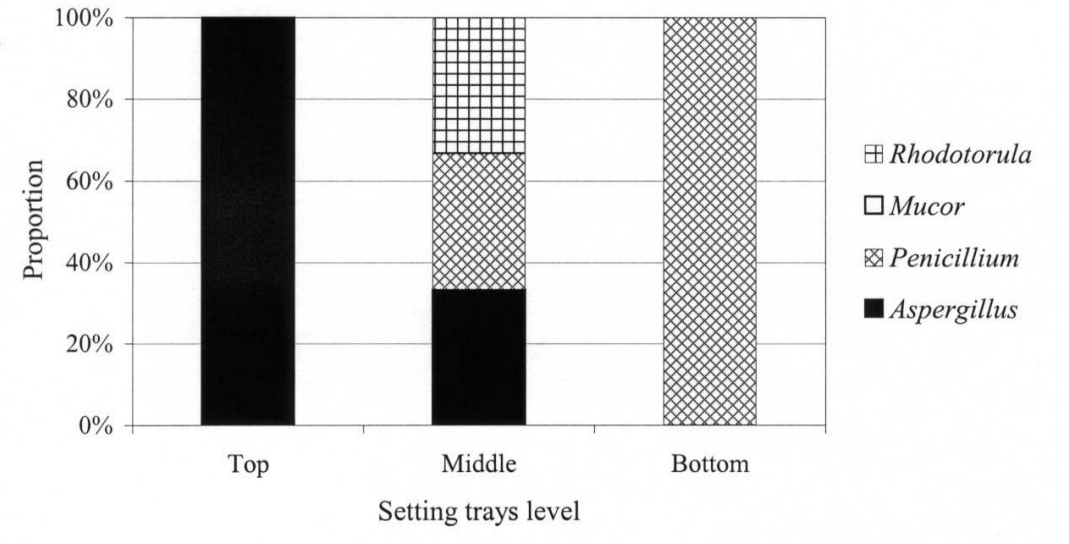

Fig. 3. Proportion of total identified fungi genus in disinfected eggshells.

microscopic fungi of the genus *Mucor* (Figs 2 & 3) were also found. It should be emphasized that regardless of whether the eggs were disinfected or not, the largest groups included *Aspergillus* and *Penicillium*. Moreover, it was found that eggs incubated on the top level of the incubator were characterized by the highest fungal contamination of the genus *Aspergillus*. Literature data indicate that the most dangerous fungi in terms of hatchability results, also identified in our study, is *Aspergillus*. This fungus was also included in the experiment carried out by NÄÄS *et al.* (2008). Moreover, BARNES and GROSS (1997) suggest that infection with *Aspergillus* is probably the most common cause of high mortality of chicks up to 3 days after hatching. A negative correlation between microscopic fungi contamination of chicks and later performance was also observed by BRAEM (1988) and CERVANTES (1995). DEEMING (1996) also provides interesting information. This author noticed that fungal infections usually stated in ostrich eggs in which embryos survived to the final phase of incubation (36-42 days).

Based on the survey, it can be said that ethyl alcohol (75%) proved to be an effective disinfectant of Japanese quail eggshells before incubation. However, it seems that it strongly influenced microscopic fungi which were not observed in hatch breakouts. The differences in eggshell surface and hatch breakout microbial contamination depend-

ing on eggs location in the setter confirms previously observed differences between microclimatic conditions in the incubator (temperature, humidity, airflow). In many ways they predispose the microorganisms to different development. In this study, the poorest results were detected for the middle level of the incubator.

References

ALKAN S., KARABAG K., GALIC A., BALCIOGLU M. S. 2008. Effects of genotype and egg weight on hatchability traits and hatching weight in Japanese quail. S. African J. Anim. Sci. **38**: 231-237.

ARX J. A. 1970. The genera of fungi sporulating in pure culture. Lubrecht & Cramer, Port Jervis, NY.

BARNES H. J., GROSS W. B. 1997. Colibacilosis. (In: CALNEK B. W. Disease of poultry. 10th ed. Ames: Iowa State University Press): 131-141.

BRAEM G. 1988. Limiting *Aspergillus* in the hatchery. International Hatchery Practice. **2**: 11-13.

BRUCE J., DRYSDALE E. M. 1983. The bacterial flora of candling-reject and dead-in-shell turkey eggs. Br. Poult. Sci. **24**: 391-395.

CADIRCI S. 2009. Disinfection of hatching eggs by formaldehyde fumigation – a review Arch. Geflügelk. **73**: 116-123.

CASON J. A., COX N. A., BAILEY J. S. 1994. Transmission of *Salmonella typhimurium* during hatching of broiler chicks. Avian Dis. **38**: 583-588.

CERVANTES H. 1995. Evaluación y manejo de los problemas respiratóriosem pollos de engorde. Avicult. Prof. **13**: 74-84.

COX N. A., BERRANG M. E., CASON J. A. 2000. Salmonella penetration of egg shells and proliferation in broiler hatching eggs – a review. Poult. Sci. **79**: 1571-1574.

DEEMING D. C. 1996. Microbial spoilage of ostrich (*Struthio camelus*) eggs. Br. Poult. Sci. **37**: 689-693.

DOMSCH K. H., GAMS W., ANDERSON T. H. 1980. Compendium of Soil Fungi. Academy Press, New York, NY.

FRENCH N. A. 1997. Modeling incubation temperature: the effects of incubator design, embryonic development, and egg size. Poult. Sci. **76**: 124-133.

FURUTA K., MARUYAMA S. 1981. Bacterial contamination on eggs during incubation and hatching, and of fluffs of newly-hatched chicks. Br. Poult. Sci. **22**: 247-254.

GAST R. K., HOLT P. S., MURASE T. 2005. Penetration of *Salmonella enteritidis* and *Salmonella heidelberg* into egg yolks in an *in vitro* contamination model. Poult. Sci. **84**: 621-625.

GEHAN Z. M. 2009. A new approach to evaluate the hygienic condition of commercial hatcheries. Int. J. Poult. Sci. **8**: 1047-1051.

GIGLI A. C. S., BARACHO M .S., NÄÄS I. A., SALGADO D. D., ALVARENGA D. P. 2009. Environmental conditions in broiler multi-stage setter – a case study. Sci. Agric. (Piracicaba, Braz.) **66**: 145-149.

JACOBSEN I. D., GROSSE K., SLESIONA S., HUBE B., BERNDT A., BROCK M. 2010. Embryonated eggs as an alternative infection model to investigate *Aspergillus fumigatus* virulence. Infect. Immun. **78**: 2995-3006.

JAFF B. M. A. 2005. The risk of bacterial contamination in hen eggs of Sulaimani poultries. J. Zankoy Sul. **8A**: 63-71.

KIZERWETTER-ŚWIDA M., BINEK M. 2008. Bacterial microflora of the chicken embryos and newly hatched chicken. J. Anim. Feed Sci. **17**: 224-232.

LÁBAQUE M. C., NAVARRO J. L., MARTELLA M. B. 2003. Microbial contamination of artificially incubated Greater Rhea (*Rhea americana*) eggs. Bri. Poult. Sci. **44**: 355-358.

LOURENS A. 2001. The importance of air velocity in incubation. World Poult. **17**: 29-30.

MANI A. U., GARNDAWA I. I., USMAN B. A. 2008. Effects of pre-incubation storage on the hatchability of quail (*Coturnix coturnix japonica*) eggs in the Sahel region of Nigeria. Int. J. Poult. Sci. **7**: 350-354.

MEIJERHOF R. 2000. Embryo temperature as a tool in the incubation process. Incubation and Fertility Research Group, WPSA Working Group 6 (Reproduction), St. Edmand's Hall, Oxford, UK.

NÄÄS I. A., BARACHO M. S., ALMEIDA PAZ I. C .L., SALGADO D. D. 2008. Estimating the impact of environmental conditions on hatching results using multivariable analysis. Braz. J. Poult. Sci. **10**: 215-222.

NOWACZEWSKI S., KONTECKA H., ROSIŃSKI A. 2012. Effect of Japanese quail eggs location in the setter on their weight loss and eggshell temperature during incubation as well as hatchability results. Arch. Geflügelk. **76**: 168-175.

NOWACZEWSKI S., STUPER K., SZABLEWSKI T., KONTECKA H. 2011. Microscopic fungi in eggs of ring-necked pheasants kept in aviaries. Poult. Sci. **90**: 2467-2470.

NOWACZEWSKI S., WITKIEWICZ K., KONTECKA H., KRYSTIANIAK S., ROSIŃSKI A. 2010b. Eggs weight of Japanese quail vs. eggs quality after storage time and hatchability results. Arch. Tierz. **53**: 720-731.

OBAIDI F. A., SHADEEDI S. M. J. DALAWI R. H. 2011. Quality, chemical and microbial characteristics of table eggs at retail stores in Baghdad. Int. J. Poult. Sci. **10**: 381-385.

PETEK M., DIKMEN S. 2004. The effects of prestorage incubation of quail breeder eggs on hatchability and subsequent growth performance of progeny Anim. Res. **53**: 527-534.

RAPER K. B., THOM C. 1949. A manual of the penicillia. Williams & Wilkins, Baltimore, MD.

SANDER J. E., WILSON J. L. 1999. Effect of hydrogen peroxide disinfection during incubation of chicken eggs on microbial levels and productivity. Avian Dis. **43**: 227-233.

SAS 2011. User's Guide. Statistical Analysis System Institute, Inc. Cary North California

TANGNI E. K., WAEGENEERS N., VAN OVERMEIRE I., GOEYENS L., PUSSEMIER L. 2009. Mycotoxin analyses in some home produced eggs in Belgium reveal small contribution to the total daily intake. Sci. Total Environ. **407**: 4411-4418.

TURBLIN V. 2011. Good quality chicks from disinfected eggs. World Poult. **27**: 1-3.

VASHIN I., STOYANCHEV T., ROUSSEV V. 2008. Prevalence of microorganisms of the campylobacter genus in quail (*Coturnix coturnix*) eggs. Bulg. J. Vet. Med. **11**: 213-216.

WELLS J. B., COUFAL C. D., PARKER H. M., KIESS A. S., YOUNG K. M. MCDANIEL C. D. 2011. Hatchability of broiler breeder eggs sanitized with a combination of ultraviolet light and hydrogen peroxide. Int. J. Poult. Sci. **10**: 320-324.