Microscopic fungi in eggs of ring-necked pheasants kept in aviaries

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ABSTRACT Pheasant eggs of different colors differ with regard to physical properties. This study was conducted to ascertain the intensity of infestation by microscopic fungi based on egg color. Settling intensity by microscopic fungi of pheasant egg shells and the content of eggs of different color during a 7-d storage period was investigated. The content of ergosterol (ERG) was used as a quantitative indicator of microscopic fungi and yeasts in the egg. The highest ERG content was determined in blue- and light brown-shelled eggs, both nonstored (0 d) and stored (7 d). A similar tendency was observed when assessing ERG content in the egg white. The difference between blue- and light brown-shelled eggs and the remaining eggs amounted to approximately 0.95 mg/kg. Differences in the ERG content in the whites of eggs stored for 0 and 7 d were determined to be 0.80, 1.63, 1.91, and 0.85 mg/kg for blue-, olive-, light brown-, and dark brown-shelled eggs, respectively. The performed quality analysis of the mycoflora found on pheasant eggs indicated a considerable proportion of molds from the *Aspergillus* genus as well as the occurrence of single colonies from *Penicillium* and *Rhizopus* genera. The only yeasts determined on the egg surface of the examined eggs belonged to the *Rhodotorula* genus. The performed investigations demonstrated that microscopic fungi pose a potential hazard to pheasant embryos.

Key words: egg, pheasant, microscopic fungi, ergosterol

INTRODUCTION

In the majority of pheasant reproductive farms in Poland, birds are kept in aviaries on the ground. In such circumstances, the health of these birds is threatened because several stress factors, such as changing atmospheric conditions, can affect them. Moreover, birds remain in constant contact with the ground, in which several pathogenic microorganisms as well as intermediate hosts of endoparasites live (Nowaczewski and Kontecka, 2005; Krystianiai et al., 2007). Therefore, eggs laid on the ground are exposed more than in other keeping systems to various pathogens (bacteria, microscopic fungi, and yeasts). Investigations carried out so far demonstrated that more bacteria and fungi were found both in the air and on the egg shell surface when domestic hens were kept in aviaries or on litter than in cages (De Reu et al., 2008; Vučemilo et al., 2010). This can result in reduction of microbiological quality of hatching eggs.

The shell color of eggs laid by pheasants is genetically conditioned. The following 4 basic colors can be distinguished: dark brown, light brown, olive, and light blue (Richards and Deeming, 2001). A correlation was confirmed between egg shell color and some physical egg characteristics, their fertilization, and hatchability results (Mróz and Pudyszak, 2000; Krystianiai and Kontecka, 2002; Silversides and Budgell, 2004). Investigations carried out on pheasants revealed, among other things, that blue-shelled eggs are characterized by thinner shells with fewer pores and smaller number of Haugh units, which indicates a poorer quality of shell and internal traits compared with eggs of other colors (Kożuszek et al., 2009). This may be one of the causes of poorer hatching results of blue-shelled eggs (Krystianiai et al., 2005). On the other hand, a high lysozyme content found in blue-shelled eggs can indicate a higher natural protective barrier (Kożuszek et al., 2009).

A review of the literature revealed no comprehensive analysis of microbiological contamination of pheasant eggs. However, reports have indicated that microscopic fungi and their toxic metabolites (mycotoxins) exert a significant effect on egg quality, frequently leading to deterioration of hatchability. They cause, among others, embryo mycosis, whereas mycotoxins can influence appearance of embryo abnormalities, increased embryo mortality, or infertility of adult birds (Tangni et al., 2009; Jacobsen et al., 2010). This is why attempts are undertaken to find causes of unsatisfactory reproduc-
tion results that frequently occur in pheasants. Investigations should assess the level of contamination of hatching eggs by microscopic fungi. These types of studies have not yet been conducted on pheasants.

Aviaries have the potential for contamination by microorganisms that may pose threats to health or reproduction. Because pheasant eggs of different colors differ with regard to physical properties, this study was conducted to ascertain intensity of infestation by microscopic fungi based on egg color. The objective of this study was to define the expansion of microscopic fungi of pheasant egg shells and egg content during a 7-d storage period and to identify these fungi.

**MATERIALS AND METHODS**

Experiments were carried out on a total 120 nondischarged pheasant eggs of blue (n = 30), olive (n = 30), light brown (n = 30), and dark brown (n = 30) egg shell color that were selected from groups of 500 eggs of each color. Birds were housed in aviaries belonging to the Animal Breeding Centre (north-central Poland). One-half of the eggs (15 eggs/color) were assessed with regard to their mycofloral infestation on the day of laying (0 d of storage) and the remaining half were examined following 7 d of storage in standard conditions commonly applied on farms (10–15°C and 65% air RH). The analyses included egg shell, white, and yolk.

The concentration of ergosterol (ERG) in the egg shell and egg content was used as the quantitative indicator of microscopic fungi and yeasts. This compound is used as a chemical marker of quantities of microscopic fungi in various kinds of materials, provided it is not a constituent of the matrix (Perkowski et al., 2008). Because eggs do not contain ERG, it could be employed for this analysis (Szablewski et al., 2010).

Eggs were broken under sterile conditions and egg contents were poured onto Petri dishes, separated, and frozen at −18°C. Next, samples were lyophilized (FreeZone Plus, Labonco, Kansas City, MO) and shells were dried in a laboratory dryer at 50°C and pulverized.

**Analysis of ERG**

Shell, albumen, and yolk samples were analyzed for the presence of ERG according to Perkowski et al. (2008). Briefly, 100-mg samples were placed in 17-mL culture tubes, suspended in 1 mL of methanol, and treated with 0.1 mL of 2 M aqueous NaOH. Then, the culture tubes were placed in 250-mL plastic bottles and placed inside a microwave oven (model AVM 401/WH, Whirlpool, Benton Harbor, MI) operating at 2,450 MHz and 900 W maximum output. Samples were irradiated (370 W) for 20 s, after 5 min, for an additional 20 s, and extracted with pentane (3 × 4 mL, HPLC grade, Sigma-Aldrich, Steinheim, Germany) within the culture tubes. The combined pentane extracts were evaporated to dryness in a gentle stream of high-purity nitrogen. Prior to analysis, samples were dissolved in 1 mL of methanol. Prepared samples were analyzed by HPLC. Separation was run on a Nova Pak C-18 column (150 mm length × 3.9 mm diameter, 4 μm particle size; Waters Poland, Warsaw, Poland) and eluted with methanol:acetonitrile (90:10) at a flow rate of 0.6 mL/min. The ERG was detected with a Waters 486 Tunable Absorbance Detector (Waters Poland) set at 282 nm. Estimation of ERG was performed by comparing peak areas with those of an external standard (>95%, Aldrich, Milwaukee, WI) or by coinjection with a standard. Detection level was 0.01 mg/kg.

**Analysis of Fungi Occurrence in Eggs**

Fungal species occurring in pheasant eggs were detected by dilution method. The diluted method was used: 1 g of sample was put in 10 mL of sterile distilled water and mixed with the magnetic stirrer for 2 min. Next, 1 mL of suspension was carried on potato-dextrose agar medium (BTL, Lodz, Poland) in Petri dishes and spread on the medium surface with a sterile glass stick. The Petri dishes were incubated at 25°C for 7 d. Growing mycelia were isolated on potato-dextrose agar and synthetic nutrient-poor agar mediums to identify the fungi species. The identification was carried out on the basis of colony and spores morphology with the aid of Arx (1970) and Domsch et al. (1980) keys.

**Statistical Analysis**

The obtained results were elaborated statistically using SAS (version 9.1; SAS Institute, Cary, NC). The significance of differences between eggs with different shell color, within day of storage (0 or 7 d), and with regard to ERG was verified by 1-way ANOVA. Differences between days of storage were determined by the Student t-test.

**RESULTS AND DISCUSSION**

The performed analyses of ERG revealed its presence on the shell as well as in the white of the examined eggs, although no detectable quantities of this compound were identified in the yolk. The highest quantities of this fungal metabolite were detected in eggs with blue and light brown shells, both nonstored (0 d) and stored (7 d; Table 1). The difference in ERG quantities between shells of these eggs and olive and dark brown eggs (P < 0.05), on average, was 1.59 mg/kg. Similar tendencies were observed when assessing the amount of ERG in egg whites. The difference between eggs with blue and light brown shells and the remaining eggs was approximately 0.95 mg/kg. The difference between ERG concentrations in the whites of eggs stored for 0 and 7 d was 0.80, 1.63, 1.91, and 0.85 mg/kg for blue, olive, light brown, and dark brown eggs, respectively (Table 1). The content of ERG in pheasant egg shells established in our experiments was in the range demonstrated for egg shells of laying hens (2.4–42.7 mg/kg).
maintained in a free-range system (Szablewski et al., 2010), although the mean value of this index in hens was clearly higher, reaching 25.4 mg/kg. A similar situation was observed in the case of hens kept indoors on litter (π = 22.5 mg of ERG/kg). This can indicate a better effectiveness of the first protection barrier in pheasant eggs (i.e., cuticle), but this could also have been caused by a less-infected substrate in the aviary. On the other hand, the ERG content in the white of pheasant eggs was similar to that found in the eggs of hens kept in cages (π = 0.97 mg/kg), but concentrations of this sterol were several times higher in the eggs of free-range hens (Szablewski et al., 2010). The determined higher levels of ERG in blue and light brown eggs appear to corroborate with the greater number of egg shell pores, through which molds and fungi can penetrate eggs. As shown by Kożuszek et al. (2009), their numbers, in comparison with olive and dark brown eggs, were greater by 7.4/0.25 cm² of egg shell. Similar to chicken eggs (Szablewski et al., 2010), no ERG was detected in yolks of pheasant eggs.

The mycofloral analysis of pheasant egg shells revealed a significant proportion of molds from Penicillium (35% of total identified genus fungi; TIGF), Aspergillus (26% TIGF), and Rhodotorula (13% TIGF) as well as single colonies representing the Rhizopus genera (9% TIGF) and deuteromycetes (4% TIGF). Similar results were reported by Salem et al. (2009) who also identified Penicillium spp. and Aspergillus spp. in shells of chicken eggs, although they failed to identify yeasts on those egg shells that were identified in pheasants. On the other hand, the fairly large numbers of molds from the Aspergillus genus determined on pheasant eggs is worrisome. Experiments demonstrated that these molds, in particular Aspergillus fumigatus, may multiply in the inner shell membrane near the egg air cell because of the presence of oxygen, given that molds are oxygenic microorganisms (Williams et al., 2000). It was found that it referred, in particular, to eggs in which embryos died early (1–4 d of incubation). Although adult birds are relatively resistant to this pathogen, when infected they can respond with anorexia, increased mortality, deteriorated feed conversion ratio, and, consequently, worsened BW gains (Müller et al., 1970).

The performed investigations demonstrated that microscopic fungi pose a potential hazard to pheasant embryos. In addition, conditions found in incubators favor development of these microorganisms and, consequently, production of mycotoxins. This can lead to numerous diseases during the embryonic stage as well as during postnatal life. The present experiments signaled a previously uninvestigated problem in this species. In the future, the authors intend to study the content of mycotoxins and analyze their cytotoxicity in relation to embryo tissues.

**REFERENCES**


**Table 1. Ergosterol content (mg/kg) in eggshell, white, and yolk of eggs laid by pheasants kept in aviaries**

<table>
<thead>
<tr>
<th>Item</th>
<th>Blue (n = 30)</th>
<th>Light brown (n = 30)</th>
<th>Olive (n = 30)</th>
<th>Dark brown (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shell</td>
<td>6.85±0.35a,b</td>
<td>4.29±0.35b,a</td>
<td>5.25±0.35b,a</td>
<td>5.72±0.35c,a</td>
</tr>
<tr>
<td>Range</td>
<td>6.75–6.95</td>
<td>4.22–4.35</td>
<td>5.16–5.32</td>
<td>6.45–6.81</td>
</tr>
<tr>
<td>White</td>
<td>1.27±0.35a</td>
<td>2.07±0.35b</td>
<td>0.27±0.35C,a</td>
<td>0.28±0.35C,a</td>
</tr>
<tr>
<td>Range</td>
<td>1.09–1.43</td>
<td>2.03–2.14</td>
<td>0.26–0.28</td>
<td>0.27–0.29</td>
</tr>
<tr>
<td>Yolk</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
</tbody>
</table>

a,bSecond small letter within a row means eggshell color between days of storage differ significantly (P < 0.0001).

A–DMeans within a rows (0 d) with different letters differ significantly (P < 0.0001).

1LOD = limit of detection.


