Research Note

Ergosterol as an indicator of the presence of microscopic fungi in eggs for human consumption produced in different husbandry systems

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ABSTRACT Ergosterol (ERG) content, being an indicator of fungal biomass, was analyzed in samples of eggshell, egg white, and egg yolk from eggs from farms with intensive management systems of layer hens (i.e., cage and litter housing). Moreover, analogous samples were analyzed from eggs from farms in the western central part of Poland, where layer hens were kept in the organic system. In all samples, the highest ERG concentration was found in shells and the lowest in egg white, whereas ERG was not found in egg yolk. When comparing investigated housing systems, a higher concentration of the analyzed metabolite was detected in eggs from litter housing than in eggs from cage housing. Concentrations of ERG in samples of eggs from organic husbandry were highly varied, ranging from 2.44 to 42.67 mg/kg in shells and from 0.28 to 16.11 mg/kg in egg white.

Key words: ergosterol, fungi, eggshell, egg white, egg yolk

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INTRODUCTION

One of the significant factors determining quality of eggs produced by layer hens is the environment inside the laying house (Harry, 1963). The degree of shell contamination is related to the housing system of layer hens. It was shown that in the case of eggs from hens kept on deep litter, their shells and shell membranes had bacterial counts approximately 15 times higher than eggs from hens kept in cages (Harry, 1963). Conditions in the laying house promote the development of microscopic fungi (e.g., from genera *Cladosporium*, *Penicillium*, *Fusarium, Mucor*, *Alternaria*, and *Candida*; Board and Tranter, 1986). Microscopic fungi cause numerous occupational diseases in farmers, such as inhalation and skin allergies, allergic pulmonary alveolitis, and mycoses (pityriasis versicolor, ringworm of hairless skin and of the scalp with deep reaction) (Skórska et al., 1998). In humans, apart from tissue infection with mycelium, we may also observe disease processes caused by secondary metabolites produced by most fungi [i.e., mycotoxins (dermato-mycotoxicosis professionalis)]. Microscopic fungi also pose a threat to poultry, causing not only mycoses but also mycotoxicoses. To date, there are no data on infection of egg contents intended for human consumption with microscopic fungi and researchers suggest only the possibility of fungal hyphae penetration through the eggshell to its contents (Board and Tranter, 1995). The aim of the present paper was to investigate the potential application of the method determining fungal microflora content in different parts of eggs coming from intensive management systems of cage housing and litter housing as well as eggs from free-range husbandry farms, to determine possible differences in mycoflora contents resulting from the type of animal management, and to identify potential hazards. The chemical analysis was applied for contents of ergosterol (**ERG**; ergosta-5,7,22-trienol), being a component of the cell wall in microscopic fungi, based on the assumption that ERG is not found in egg contents; thus, it may be used as an indicator of both live and dead fungal microflora. This method, widely used by the authors in the determination of mycoflora contents in cereals (Perkowski et al., 2008), so far has not been used in the poultry industry to determine contents of mycoflora in eggs.

MATERIALS AND METHODS

The analyzed material comprised samples of 300 eggs (150 eggs from management systems of cage housing and 150 eggs from litter housing) from the Poultry Farm of the Institute of Animal Production at Zakrzewo, Poland. Analyses of ERG content were also performed on samples of shells, white, and yolk from eggs from 10

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farms (20 eggs from each farm) located in west-central Poland, in which layer hens were kept in the free-range system. Eggshell, white, and yolk were analyzed. 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, Labconco, Kansas City, MO) and shells were dried in a laboratory dryer at a temperature of 50°C and comminuted.

Analysis of ERG

Samples were analyzed for the presence of ERG according to Perkowski et al. (2008). Briefly, samples of 100 mg were placed into 17-mL culture tubes, suspended in 1 mL of methanol, treated with 0.1 mL of 2 *M* aqueous NaOH, and sealed tightly. Then, the culture tubes were placed in 250-mL plastic bottles, sealed tightly, and placed inside a microwave oven (model AVM 401/WH, Whirlpool, Warsaw, Poland) operating at 2,450 MHz and 900 W maximum output. Samples were irradiated (370 W) for 20 s, after approximately 5 min, for an additional 20 s and extracted with pentane (HPLC grade, Sigma-Aldrich, Steinheim, Germany; 3 × 4 mL) within the culture tubes. The combined pentane extracts were evaporated to dryness in a gentle stream of high-purity nitrogen. Before analysis, samples were dissolved in 4 mL of methanol, filtered through 1-mm syringe filters with 0.5-μm pore diameter (Fluoropore membrane filters, Whatman, POCH, Gliwice, Poland), evaporated to dryness by a nitrogen stream, and dissolved in 1 mL of methanol. Prepared samples were analyzed by HPLC. Separation was run on a 150 × 3.9 mm (length × diameter) Nova Pak C-18 4-μm particle size column (Waters, Milford, MA) and eluted with methanol-acetonitrile (90:10) at a flow rate of 0.6 mL/min. Ergosterol was detected with a Waters 486 Tunable Absorbance Detector (Waters) set at 282 nm. Estimation of ERG was performed by a comparison of peak areas with those of an external standard (>95%, Sigma-Aldrich, Milwaukee, WI) or by co-injection with a standard. Detection level was 0.01 mg/kg.

Analysis of Fungi Occurrence in Eggs

Analyses of the composition of fungi species occurring in eggs probes were done. The diluted method was used: 1 g of dust 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 the potato dextrose agar medium (BTL, Lodz, Poland) in Petri dishes and spread with aid of a sterile glass stick on the medium surface. The Petri dishes were incubated at 25°C for 7 d. 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 and Thom (1949), Arx (1970), and Domsch et al. (1980).

RESULTS AND DISCUSSION

In analyzed samples of 3 egg parts (i.e., shells, white, and yolk), the content of ERG was found to vary. In all samples, the highest concentration of this metabolite was recorded in shells (17.01 mg/kg), whereas it was lower in egg white (6.49 mg/kg), and it was not detected in egg yolk. Contents of ERG in samples of eggs coming from the litter housing system were higher than in samples of eggs from cage housing (Table 1). Mean content of this metabolite in eggshells from litter housing was 22.49 mg/kg, whereas in the egg white, it was 4.29 mg/kg. It needs to be stressed that among analyzed shell samples, 76% fell within the range of ERG concentration from 18.22 to 26.39 mg/kg, whereas only 2% comprised samples with the highest concentration of this metabolite (i.e., from 26.39 to 41.37 mg/kg), and the rest consisted of samples with the lowest ERG content (5.24 to 18.22 mg/kg). Similarly, the distribution of ERG percentage levels was similar for these eggs in egg white samples. When analyzing samples of eggs from cage housing, the highest ERG content was also found in shells, amounting to 3.17 mg/kg, whereas it was lower in egg whites, 0.97 mg/kg. The percentage distribution of the content of the analyzed marker was opposite than in litter housing [i.e., 79% of samples of shells fell within the range of 0.97 to 3.00 mg/kg, 10% of samples were those with the highest range of ERG concentrations (8.46 to 10.14 mg/kg), whereas the rest were samples with average contents of this metabolite (3.00 to 7.61 mg/kg)]. The content of ERG in samples of shells and egg whites coming from free-range management was highly diverse and ranged from approximately 2.44 to 42.67 and 0.28 to 16.11 mg/kg, respec-

Table 1. Ergosterol content (mg/kg) in 300 samples of eggshell, egg white, and egg yolk from eggs produced in cage housing and litter housing systems

	Litter housing system (n = 150)		Cage system (n = 150)	
Type of samples	Range	Mean	Range	Mean
Shell	5.24 to 41.37	22.49[a]	0.97 to 10.14	3.17[b]
White	0.04 to 6.41	4.29[a]	0.01 to 1.55	0.97[b]
Yolk	<LOD[1]	<LOD	<LOD	<LOD

[a,b]Means within columns with different superscripts are statistically significant ($P = 0.05$).

[1]LOD = limit of detection.

Table 2. Mean ergosterol content (mg/kg) in samples of eggshell, egg white, and egg yolk from 10 farms of layer hens kept in the free-range system

Type of sample (n = 10)	Range	Mean
Shell	2.44 to 42.67	25.37[a]
White	0.28 to 16.11	14.23[b]
Yolk	<LOD[1]	<LOD

[a,b]Means within columns with different superscripts are statistically significant ($P = 0.05$).

[1]LOD = limit of detection.

tively. It was observed that mean ERG concentration in shells was similar to that found in shell samples coming from litter housing. In free-range husbandry, the mean ERG concentration in egg whites was 3 times higher than in egg whites from litter housing (Table 2). Due to the lack of available data concerning ERG concentration in eggs, the only justified comparison may be to its concentration as determined by Maupetit et al. (1993), amounting to 3 mg/kg, or by Schnürer and Jonsson (1992), with 9 mg/kg as the threshold ERG content in grain intended for human consumption. The recorded definite data indicate the presence of considerable amounts of mycoflora in the egg contents. Thus, after chemical analyses, fungi isolated from egg samples from all analyzed environments were identified. In samples of eggs from cage housing, fungi from genera *Mucor*, *Penicillium*, and *Rhodotorula* were detected; in samples of eggs from litter housing, it was *Penicillium*, *Stemphylium*, *Rhodotorula*, *Alternaria*, and *Mucor*; whereas in samples of eggs from free-range animal husbandry, these were *Mucor*, *Rhizopus*, *Penicillium*, *Fusarium*, *Alternaria*, and *Rhodotorula.*

Results obtained by us to date suggest an essential problem indicating the possibility of egg content contamination with microscopic fungi. As a consequence, this may lead to the formation of fungal metabolites, as was indicated in literature (Brake et al., 2000; Tangni et al., 2008). In the course of this study, it was found that the highest amounts of mycoflora were found in shells, whereas mycoflora was lower in egg white and there was none in egg yolk. Similar dependencies were observed in cereal kernels (Jackowiak et al., 2005; Packa et al., 2008), in which the highest ERG concentration was observed in the outer layers of kernels; kernel infestation with mold started from the outer layer, the mycelial hyphae penetrated kernels, and in its individual internal layers, ERG content was observed to decrease (Perkowski et al., 2008). These studies will continue in an extended form because this problem seems to be crucial for the understanding of the mechanism of egg infection with mycoflora.

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