Sanitizing Hatching Eggs with Essential Oils

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Increased meat and egg production leads to concomitant changes in poultry practices, including the indiscriminate use of formaldehyde to sanitize hatching eggs. Although this sanitizer aids in the increase in poultry production, its toxic potential for man and for avian embryos represents an obstacle to its long-term use.

economic gains | egg disinfection | embryological safety | egg microbiology | poultry health

1. Introduction

The large number of healthy embryos that hatch supports the hypothesis that eggs have good microbiological quality. Ensuring embryo safety in the face of microbiological challenges is not easy. The embryo's immature status makes it insecure and defenseless against infection [1]. In this case, the eggshell can have a negative effect because it contains pathogenic microorganisms [2] and has communication routes with the embryo, favoring contact between them. Therefore, the quest for healthier poultry is increasing the need to incubate eggs with minimal microbial loads in poultry hatcheries during all incubation cycles. In this case, sanitizing hatching eggs with liquid or gas is the gold standard method of achieving this goal [3]. The sanitization of hatching eggs is nothing more than an antimicrobial resource intermediated by a simple or complex system (e.g., fumigation, spraying, or immersion) that applies a sanitizing solution to the eggshells to solve poultry losses caused by microorganisms. This step must occur within half an hour after laying or immediately collection [4][5][6].

In line with the current trend towards ecologically friendly products with minimal impact on animals, the poultry industry needs to gradually adopt sanitizers that respect safety criteria for the protection of avian life. In a previously published review, Oliveira et al. [3] showed that there are various sanitizers for hatching eggs that are available to the poultry industry which are divided into two large groups (synthetic and natural). Among the natural options recommended to the industry, the authors show that essential oils derived from volatile liquids from aromatic plants are antimicrobial and safe to use. The use of essential oils as sanitizers for hatching eggs was reviewed by Oliveira et al. [5]. They reported that essential oils compete with synthetic materials for reasons that are of interest to the poultry industry, including embryo and human safety, the ability to control microorganisms in the eggshell, and increased production rates. These effects can be seen at low concentrations, which may overcome the disadvantages of essential oils where they are more expensive than synthetic compounds that require higher concentrations for effective action. Thus, validating the potential and advantageous characteristics of essential oils in the management of hatching eggs can open an important path for their inclusion in the official list of sanitizers used in poultry routines around the world.

2. Eggshell Microorganisms: Risks for Poultry Embryos and Chicks

Even as an immunologically sensitive embryo, poultry already interact with pathogenic microbes originating from any stage prior to hatching [7]. This interaction may be a consequence of horizontal transmission [8] (**Figure 1**) and puts the poultry's life in danger. Fonseca et al. [9] observed that, by contaminating the eggshell, *Campylobacter jejuni* bacteria can penetrate it, cross the albumen and reach the yolk sac, probably resulting in embryonic mortality. This led the authors to state that the immunity conferred by breeding hens to the egg/embryo may be insufficient and inefficient for certain infections. In addition, although the eggshell is an oval antimicrobial wall formed by the fusion of membranes and mineral layers equivalent to a vital organ of a living organism (it promotes the flow of nutrients, water, oxygen and carbon dioxide to keep the embryo alive) [10], it is not totally resistant to microbial entry. The eggshell is challenged when there are microorganisms trying to move from its surface to the main target (embryo). Oliveira et al. [6] reviewed the types of microorganisms that contaminate eggshells. Among the bacterial and fungal genera cited are *Alcaligenes*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Proteus*, *Providencia*, *Pseudomonas*, *Salmonella*, *Clostridium*, *Enterococcus*, *Staphylococcus*, *Streptococcus*, *Aspergillus*, *Candida* and *Penicillium*.

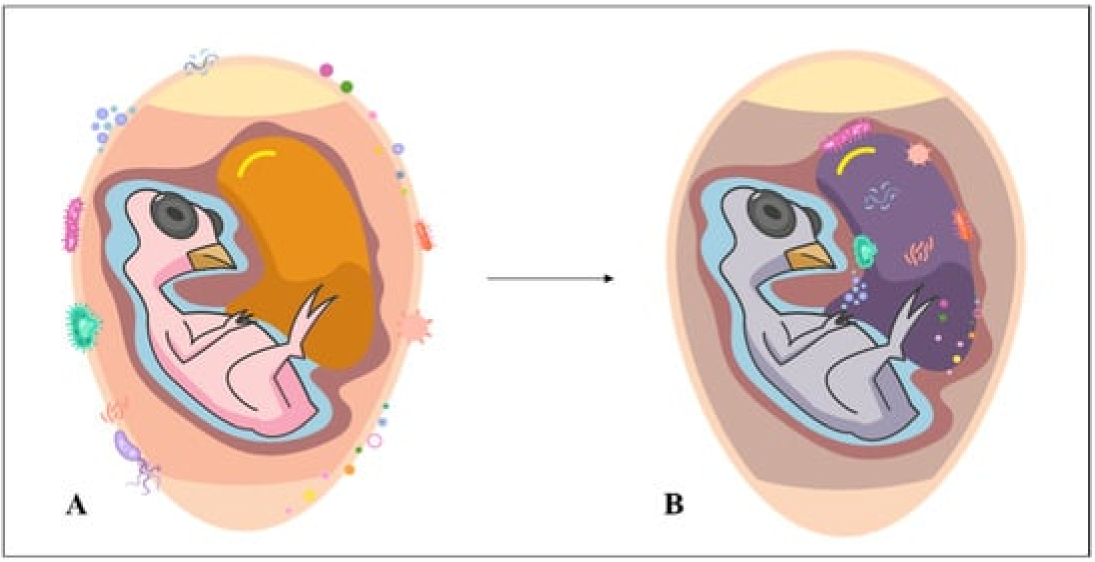

Figure 1. Horizontal contamination in hatching eggs. Microorganisms on the shell (**A**) penetrated and reached the yolk sac (**B**).

Previously published studies have reported the adverse relationship of microorganisms with embryos and/or chicks. Weil and Volentine [11] reported that contamination of the yolk sac of the chicken embryo by *Shigella dysenteriae* can cause lethal infection. Embryos from chickens killed by contamination with avian pathogenic *Escherichia coli* and *Salmonella enterica subsp. enterica serovar* Enteritidis showed signs of congestion and diffuse redness throughout the skin, head and neck, as well as microscopic lesions in the yolk sac, including congestion, inflammation, damaged blood vessels and abnormal endodermal epithelial cells [1]. Fungi of the genus *Aspergillus*, which may be responsible for causing mycoses or mycotoxicosis, have been isolated from dead chicken embryos [12]. Saleemi et al. [13] reported that aflatoxigenic fungal extracts isolated from *Aspergillus* fungi caused high embryonic mortality, weight reduction and severe alterations in the liver (fatty alteration and cell necrosis) and kidneys (congestion and tubular necrosis) of chicks. Karunarathna et al. [14] demonstrated that multidrug-resistant *Escherichia coli* and *Enterococcus* were recovered from the yolk of non-viable chicken embryos at hatching. Contamination by *Enterococcus* spp. can trigger pulmonary hypertension syndrome in chicken embryos and chicks [15]. Mortality of chicken embryos associated with *Enterococcus* contamination was reported by Karunarathna et al. [16]. Multidrug-resistant bacteria that cause yolk sac infection, including *Escherichia coli*, *Salmonella*, and *Staphylococcus*, have been recovered from dead embryos and chicks [17][18]. Far et al. [19] observed that dead ostrich embryos were contaminated with *Pseudomonas* spp., *Klebsiella* spp., *Bacillus* spp., *Citrobacter* spp., *Staphylococcus* spp., *Proteus* spp., *Aeromonas* spp., *Enterobacter* spp., as well as *Escherichia coli* with antimicrobial resistance profile.

The findings mentioned above raise concerns, especially in relation to the health of poultry and humans, since multiresistant microorganisms can spread and cause massive irreversible damage. In addition, the undue, exacerbated use of sanitizers without proven scientific tests and without the prescription of trained professionals can contribute to even worse health and economic instability. Therefore, collective efforts within the poultry industry should focus on antimicrobial interventions that involve the controlled use of broad-spectrum sanitizers focused on hatching egg sanitization.

3. Essential Oils and Their In Vitro Antimicrobial Activity

Essential oils are any aromatic, viscous and volatile oils belonging to plants. *Syzygium aromaticum*, *Allium sativum*, *Ocimum basilicum*, *Thymus vulgaris*, *Lavandula angustifolia*, *Eucalyptus globulus*, *Citrus sinensis*, *Citrus aurantifolia*, *Cinnamomum cassia*, *Rosmarinus officinalis*, *Origanum vulgare*, *Allium cepa*, *Cymbopogon winterianus*, *Cymbopogon flexuosus*, *Piper nigrum*, *Zingiber officinale*, *Protium pallidum*, *Litsea citrata*, *Satureja hortensis*, *Salvia officinalis*, *Mentha piperita*, *Cedrus deodara*, and *Cuminum cymincum* are examples of plant species that provide commercially available essential oils that may have promising futures in poultry nutrition and production such as egg coating additives and sanitizers for hatching eggs. This is because essential oils have a chemical configuration that triggers their biological properties. For example, hydrocarbons, esters, lactones, alcohols, oxides, phenols, ketones, and aldehydes are present in the chemical composition of essential oils with similar or distinct bioactive functions. Depending on the compound, these functions include antimicrobial, antiviral, antitumoral, antibacterial, stimulant, anesthetic, anti-inflammatory, anti-fungal, antipyretic, and spasmolytic [20]. The content, quality, and effectiveness of essential oil compounds depend on factors such as extraction, which can be by hydro distillation, steam distillation, supercritical CO_2 extraction, ultrasonic extraction, and cold pressing [21][22][23][24].

In vitro antimicrobial screenings initially detect the potential viability of essential oils before they are used as in vivo antimicrobial agents. These screenings demonstrated that essential oils are effective against standard Gram-negative and positive bacterial strains and avian isolates, as well as standard and avian-isolated fungi. Among the bacteria are *Salmonella enterica subsp. enterica serovar* Enteritidis, *Salmonella enterica subsp. enterica serovar*

Typhimurium, *Salmonella enterica subsp. enterica serovar* Infantis and avian pathogenic *Escherichia coli* (APEC), which are important pathogenic bacteria for poultry and public health. The antimicrobial effectiveness of essential oils ranges from mild to very strong. In fact, some of them have been shown to be more effective than conventional antibiotics [25][26]. Thymol, eugenol, carvacrol, linalool, citral, limonene, trans-cinnamaldehyde, geraniol and citronellal are some compounds that are part of the composition of some essential oils that can act as protagonists in antimicrobial action. The main mechanisms responsible for making the bacterial [27] and fungal [28] cells unfeasible are listed below:

- Bacteria:
- Cell membrane alteration and increased permeability.
- Stops energy production.
- Blocks active transport.
- Fungi:
- Cell membrane disruption, alteration, and inhibition of cell wall formation.
- Dysfunction of fungal mitochondria.
- Inhibition of efflux pumps.

Essential oils can promote beneficial actions for human health by reducing pain and inflammation, protecting and healing wounds, neutralizing or stopping the development of carcinogens, neutralizing oxidative stress and possessing antiviral, antibacterial, antifungal, cardioprotective, antidiabetic, and insect-repellent properties; among other benefits, they can also potentially treat central-nervous-system-based disorders [29][30][31][32]. The safety of a stock of essential oils including *Ocimum basilicum*, *Zingiber officinale*, *Lavandula officinalis*, *Cymbopogon citratus*, *Mentha piperita*, *Rosmarinus officinalis*, *Thymus vulgaris*, *Eugenia caryophyllata*, and *Allium sativum* has been documented and they received the generally recognized as safe (GRAS) seal [33]. However, the intake of essential oils needs to be monitored, as they can, like any other edible food, cause an inappropriate effect.

4. Comparing Essential Oils and Formaldehyde for Sanitizing Hatching Eggs

Formaldehyde is still preferably used in the practice of sanitizing hatching eggs [3][34][35]. Antimicrobial effectiveness and cost are two of the main reasons why formaldehyde remains in use in the poultry industry. Even its strong toxicity to poultry embryos [34][36][37] and humans [38][39][40] has not managed to have it removed from the practice of sanitizing hatching eggs. However, researchers are strongly committed to continuing to alert the poultry industry that, from a health point of view, formaldehyde is not compatible with a sustainable and safe poultry chain.

The sanitization of hatching eggs with natural sanitizers is based on a sanitary practice of microbial control of eggshells without synthetic chemical treatments, which aims to contribute to the production of healthy chicks free of pathogenic microorganisms using exclusively substances derived from plants and friends of living organisms [5][41][42][43]. Comparing natural sanitizers made from essential oils with synthetic sanitizers made from formaldehyde, there should be conscious support for the transition from sanitization systems that involve aggressive products to those that use green and responsible products. In addition to the antibiotic profile capable of significantly reducing the microbial count of hatching eggshells, one of the main advantages of using essential oils as sanitizers for hatching eggs is the productive results promoted in terms of hatchability, which, on average, are not inferior to those of sanitization with formaldehyde (**Table 1**). Thus, the application of essential oils to hatching eggs does not require additional or different practices to promote the production of the same number of poultry than is routine in the conventional poultry sector. The prioritized use of synthetic chemicals in hatching egg management can be minimized by replacing them with essential oils.

Table 1. Comparison between the efficiency of essential oils and formaldehyde after application in hatching eggs.

Compounds	Bacterial Count (log) [a]	Hatchability (%) [a]	Significance [b]	Most Efficient	Study
Origanum onites	<0.47	>1.98	* TBC [ns] Hatchability	Essential oil	[44]
Formaldehyde	<0.06	>1.89			
Thymus vulgaris	<1.68	>6.95	*	Formaldehyde	[45]
Formaldehyde	<1.81	>9.70			
Syzygium aromaticum	<1.19	>10.66	ns	Similar	[41]
Paraformaldehyde	<1.26	>7.84			
Origanum vulgare	<6.33	>12.05	*	Essential oils	[36]
Cuminum cyminum	<6.13	>11.70			
Formaldehyde	<3.03	<2.01			

[a] Comparison of essential oils and formaldehyde with non-sanitized eggs; [b] Comparison between essential oil and formaldehyde; * Significant; [ns] non-significant, TBC, Total bacteria count.

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