

The Use of Oregano (*Origanum vulgare L*) Essential Oil as Alternative Hatching Egg Disinfectant versus Formaldehyde Fumigation in Quails (*Coturnix coturnix japonica*) Eggs

° I. YILDIRIM, °° M. ÖZSAN and ° R. YETISIR

° The University of Selcuk, Faculty of Agriculture, Departement of Animal Sci, 42031, Campus, Konya, Turkey

°° The University of Selcuk, Faculty of Agriculture, Departement of Food Sci, 42031, Campus, Konya, Turkey

Corresponding Adress :

Dr. Iskender YILDIRIM, Selcuk Universitesi, Ziraat Fakültesi, 42031 Kampus, Konya, Turkey

E-mail : iyildir@selcuk.edu.tr - Tel. : +90 332 2410041 ext. : 2820 - Fax : +90 332 2410108

SUMMARY

The present study examined the effectiveness of Oregano (*Origanum vulgare L*) (OV) essential oil versus formaldehyde fumigation (FF) to disinfect quail (*Coturnix japonica*) hatching eggshell surface and maintain hatching results. There were found significant differences between OV and FF in the hatchability of fertile eggs ($P < 0.05$). The total embryo mortality (TEM) was lower in OV group compared to FF group ($P < 0.05$). The treatment groups did not effect on eggshell conductance and eggshell conductance constant. Following OV application, total bacteria population significantly ($P < 0.01$) decreased compared with control (CON) and FF groups. These results demonstrated that the OV could be regarded as probable alternative hatching egg disinfectant versus FF without adversely affecting quail hatching eggs.

KEY-WORDS : Oregano - formaldehyde - disinfection - hatching eggs - eggshell conductance.

RÉSUMÉ

Utilisation de l'huile essentielle d'oregano (*Origanum vulgare L.*) pour la désinfection des œufs de caille (*Coturnix coturnix japonica*) avant éclosion à la place des fumigations de Formaldéhyde. Par I. YILDIRIM, M. ÖZSAN et R. YETISIR.

La présente étude examine l'efficacité de l'huile essentielle d'oregano (*Origanum vulgare L.*) (OV) par comparaison avec les fumigations de Formaldéhyde (FF) pour désinfecter la surface coquillière des œufs de caille (*Coturnix japonica*) au moment de l'éclosion tout en maintenant un bon rendement à l'éclosion. Il existe des différences significatives entre OV et FF sur l'éclosabilité des œufs fécondés ($P < 0,05$). La mortalité embryonnaire totale (TEM) est plus basse dans le groupe OV que dans le groupe FF ($P < 0,05$). Les différents traitements n'ont pas d'influence sur la conductance de la coquille des œufs.

Après application de OV, la population bactérienne totale a diminué significativement ($P < 0,01$) comparée à celle du groupe témoin (CON) et du groupe FF.

Ces résultats montrent que OV se révèle comme un bon désinfectant des œufs par rapport à FF qui affecte l'éclosabilité des œufs de caille.

MOTS-CLÉS : Oregano - Formaldéhyde - désinfection - éclosion des œufs - conductance de la coquille.

Introduction

Assuming that the egg is sterile during formation, microbial contamination of the eggshell occurs during passage through the cloaca and following oviposition. Eventually, bacteria penetrate the shell and infect the embryo, causing losses in hatchability, poor quality, chicks or poults and infection in growing birds [21].

An effective hatchery sanitation program is critical to achieve a high level of hatchability and ensure the production of high quality chicks. To date, Formaldehyde (FF) has been recommended as fumigant used in hatcheries, due to its effectiveness and ease application. The sanitizing of hatching eggs is a requirement under the poultry breeding flock and hatcheries. Fumigation with formaldehyde has been the

method used by most producers to achieve that, but the implication of the control of substances hazardous to health (COSHH) legislating is causing many procedures sanitizing techniques [20, 22]. Moreover, FF is an irritant for the eyes and the nose and has a lingering noxious odor; venting of its vapors is difficult [24]. And most importantly, recent actions by the environmental protection agency that regulate the use of FF under the toxic substances control act due to its suspected carcinogenicity [3]. Thus, effective alternative disinfectants are needed to replace FF in the event that the EPA (environmental protection agency) bans its use [25].

Several investigations have been conducted on the antimicrobial effect of various spices and their derivatives [19]. The effect of spices has been mainly attributed to their essential oil content. It seems probable that the extent of the inhibition

of oregano may be due to aspects of their chemical structures. It is well known that a phenolic - OH group is very reactive and can easily form hydrogen bonds with the active sites of enzymes [11]. Antimicrobial properties of certain spices have been reported in meats and meat products e.g., poultry meat, turkey breast and beef [12], various meats [15] and turkey frankfurter slurries [13]. Herbaceous plants grown in some parts of Turkey. Their dried and ground fruits and leaves, respectively, are wet in some meat products. Antimicrobial activities of volatile oils have been reported by numerous workers [28, 9]. *Origanum* species have been used in medicine and as a spice ever since antiquity, mainly because of their content of essential oils. While antimicrobial activities of several spices in culture media have been reported over the years, few tests have been conducted in food systems [19].

To achieve maximal hatchability and chick quality, each egg should lose an optimal amount of its mass as water vapour during incubation [2]. The most important functions of eggshell porosity is believed to be maintenance of water balance and supply enough gas to developing embryo: eggs of chickens and most other bird species lose about 15% of their initial weight until hatching by evaporation of water [17]. The shell has an outer coating, the bloom or cuticle, that is a glyco-protein (mucin) in nature [16]. Insufficient gas exchange and water loss, late in the incubation period may cause embryonic mortality in turkeys (CHRISTENSEN and McCORKLE, 1982). On the other hand, removing of cuticle increases eggshell permeability [1]. When conductance constant (k) changes the plateau stage of oxygen consumption occurs earlier or later in metabolic development and more or less vital gas exchange may be available for each unit of egg mass [7, 4]. As a result, manipulating any factors will influence eggshell permeability and hatchability. According to BRAKE (1986), the cuticle may be affected by the application of sanitizers so as to alter eggshell permeability and embryonic development.

The aim of this study is to evaluate the effects of OV instead of FF on the bactericidal activity, which is naturally occurring on eggshell surfaces and also hatching results in Quail (*Coturnix japonica*) eggs.

Materials and Methods

A) ESSENTIAL OIL :

The plant and oil used was obtained from OV and the herb was identified and voucher specimens were deposited in the herbarium of Botany Department, in our University. The air-dried plants were submitted to hydro-distillation for 3h, using a Clevenger-type apparatus. The oil was dried with anhydrous sodium sulphate, and kept in sealed dark bottles under cool conditions until used.

B) EGG MATERIAL :

All eggs samples were taken from a non-commercial strain of Quail (*Coturnix coturnix japonica*) breeder hens, at 14-wk-of age, fed a standard ration (20 % CP, 3100 kcal, ME/kg). Eggs that had been freshly laid onto cages were col-

lected twice a day (early in the morning, 9 a.m.; and late in the afternoon 4.0 p.m.) from the research farm. Faecal-contaminated eggshells and eggshells with visible checks were discarded. Following collection, the eggs were stored for no longer than 3 days at around 15-18 °C and 75 % relative humidity (RH) prior to initiation of the experiment. A total of 800 eggs were used in the study. Eggs were incubated at 37.5 °C and 55-60 % RH until the 15th day of incubation when incubator conditions were changed to 37.2 °C and 80 % RH, for the actual hatching process. At hatching all chicks were counted and eggs that did not hatch were broken. Their contents were subsequently examined macroscopically to determine true fertility and estimate the time death for the nonhatched fertile eggs. Hatchability of fertile eggs was determined by discounting all truly infertile eggs and dividing the number of chicks hatched by the total number of fertile eggs.

C) EGGSHELL CONDUCTANCE

All eggs were weighed (nearest .01 g) and eggshell conductance (G) and eggshell conductance constant (k) were determined using the technique of CHRISTENSEN *et al.* (2001). The equation is :

$$k = (G_{H20} \times I)/W$$

Where G_{H20} is the conductance of the egg, defined by Fick's first law of Diffusion as the number of milligrams of water vapor that leave the egg in a 24-h period per millimeter of mercury gradient of water vapor pressure across the shell. I is the length of the incubation period of the egg in days; and W is the initial weight of the egg in grams [7].

D) HATCHING EGG DISINFECTIONS

A total of 800 eggs were used in the experiment. The eggs were divided into four groups, each one containing 200 eggs, to investigate the effects of hatching egg sanitizers (HES) on hatching results. The second group was treated with *O. vulgare* (OV) (125 ml alcohol plus 0.2 ml OV per L solution). Eggs from the third group were treated with FF (triple strength formaldehyde gas: 3X= 119.8 ml formalin; 59.9 g potassium permanganate/ 2.83 m³). Triple strength formaldehyde gas was produced inside the setter at ambient temperature (15°C) and 90 % RH for 20 minutes. Triple strength formaldehyde is used commercially on hatching eggs (USDA, 1985).

Eggs from the last group were treated with propyl alcohol (PA) (125 ml PA per L solution) to make sure that there is any synergistic effect or not. Because, the oil has been solved in the PA. Eggs from the first one served as the control (CON) group in which no treatment was applied. The second and fourth groups were sprayed with disinfectants using hand sprayer. A room temperature of 24°C was selected, since these disinfectants does not need to penetrate the eggshell and contains no temperature-dependent ingredients. All eggs were completely wetted with the disinfectants and were allowed to air dry at room temperature (24°C) for 30 min. thereafter, all egg flats were randomly distributed into the incubator. All of the treatments were called as HES.

E) MICROBIAL INACTIVATION STUDIES

Twelve eggs were immediately placed on plastic egg flats. A whole egg washing technique was used to recover the shell associated micro-organisms for estimating the total bacteria count, Coliforms and fungi and molds counts of three eggs per treatment. Dilutions were prepared (10^{-3}), and then were inoculated into sterile Petri plate. The total bacteria, Coliforms and fungi were incubated (37 °C, 48 h) and enumerated using nutrient agar¹, violed red bile agar² and fungi and molds potato dextrose agar (oxid) respectively. Colonies were measured as cfu/mL (ÖZÇELİK, 1992)

F) STATISTICAL ANALYSES :

The data from the experiment were subjected to a one-way ANOVA using a randomized complete block design with the Statistical Analysis System-ANOVA procedure [14]. For statistical analyses, all percentage data were transformed using arc sine transformations and microbial counts were transformed to Log₁₀ prior to statistical analysis. There were 4 replicate trays for hatching results, per treatment. Means that differed significantly were separated by Duncan’s multiple range test (DUNCAN, 1955) and probabilities were based on P < 0. 05.

Results and Discussion

The effects of HES on the hatchability of fertile eggs and embryonic mortality stages are summarized in Table I. The hatch of fertile eggs in PA and CON groups have been observed slightly lower than OV, but no significant differences were found between CON, PA and OV. There were found significant differences between OV and FF in the hatchability of fertile eggs (P < 0.05). Significantly higher early embryonic mortality (EEM) were observed in PA and FF in comparison to OV and CON groups (P < 0.05). No significant differences were found in midterm embryonic mortality (MEM) and late embryonic mortality (LEM) among groups. The TEM was lower in OV group compared to FF group (P < 0.05). YILDIRIM and OZCAN (2001) observed no dif-

1. Oxoid,England.
2. Merck, Darmstadt, Germany.

ferences in the percentage of hatch of fertile eggs and TEM due to OV and FF in comparison with CON. The use of FF in the experiment appeared to be toxic for the embryo, especially during the first stage of incubation. And accordingly, the high level of EEM has led to an increase in the level of TEM. Depending on high level TEM, the percentage of hatchability of fertile eggs in FF group has been observed lesser than that of OV group. A probable explanation for the current situation is that the recommended dose of FF (3X) for chicken eggs may probably be high for quail eggs.

The effects of HES upon G and k are summarized in Table II. No significant differences were observed for G and k among the treatments groups. BRAKE and SHELDON (1990) reported that the cuticle has been described as an uneven organic layer composed primarily of protein with some polysaccharide and lipid material. The literature contains reports of cuticle providing resistance to microbial invasion and contamination. Manipulating any of the factors will influence eggshell permeability [1]. When conductance constant (conductance per unit of egg mass and incubation period) change, the plateau stage in oxygen consumption occurs earlier or later in metabolic development and more or less vital gas exchange may be available for each unit of egg mass (CHRISTEN *et al.* 1996). The cuticle may be affected by the application of sanitizers so as to alter eggshell permeability and embryonic development (BRAKE, 1986). Consequently, the treatment groups did not affect on G and k and also the high level of EEM and TEM in FF can not be attributed to conductance and conductance constant.

The effects of HES on antimicrobial activities on the eggshell surface are summarized in table III. No significant differences were observed for molds and coliforms among the groups. However, following OV application total bacteria population significantly (P < 0.01) decreased compared with

Groups	G H ₂ O (mg H ₂ O/d/mmHg)	k(G/g)
CON	2.75 ± 0.7	1.12 ± 0.3
OV	2.54 ± 0.5	0.98 ± 0.2
FF	2.79 ± 0.7	1.16 ± 0.3
PA	2.29 ± 0.4	0.92 ± 0.1

TABLE II. — Effect of applying HES on the G and k values of the eggs for quails

----- $\bar{X} \pm SE$ -----

Groups	Hatch of fertile egg	EEM	MEM	LEM	TEM
CON	65.17 ^{ab} ± 2.5	6.25 ^a ± 3.1	10.09 ± 1.8	18.49 ± 2.6	34.83 ^{ab} ± 2.5
OV	73.00 ^a ± 3.7	4.43 ^a ± 1.2	6.82 ± 2.6	10.71 ± 3.3	26.99 ^b ± 3.7
FF	50.53 ^b ± 5.7	16.70 ^b ± 2.8	17.92 ± 1.2	14.85 ± 3.3	49.47 ^a ± 5.7
PA	63.76 ^{ab} ± 7.4	17.20 ^b ± 5.6	9.62 ± 4.6	9.41 ± 2.5	36.24 ^{ab} ± 7.4

a,b; Different superscripts in the same column indicate significant differences (P<0.05) among mean values

TABLE I. — Effect of applying HES on the hatchability of fertile eggs and embryonic mortality stages (%).

Groups	Fungi	Coliforms	Total Bacteria ⁰
CON	11.67 ± 1.7	4.67 ± 1.2	44.70 ^a ± 10.1
OV	5.00 ± 1.7	0	10.00 ^b ± 2.5
FF	5.50 ± 2.5	0	24.00 ^a ± 2.3
PA	11.67 ± 3.2	0	18.00 ^{ab} ± 2.1

a,b; Different superscripts in the same column indicate significant differences ($P < 0.01$) among mean values.

TABLE III. — Antimicrobial activity (cfu per egg)* 10^{-3} HES on the microflora of hatching eggs (geometric means $\log_{10} \pm SE$, $n = 3$).

CON and FF groups. The high level of total bacteria population can be responsible for low hatchability of the fertile eggs in FF group. But the same approach cannot be thought for CON group in regarding to hatching results for this study. A possible explanation of this situation in FF group may be associated with the other environmental conditions during or before incubation or the other factors during the application of FF, such as, high level of heat energy emerged from FF application and also the distance of eggs from the FF source. Because the data from this study shows us that only high level of total bacteria cannot be responsible for the low percentage of hatchability of fertile eggs. Otherwise, the percentage of hatchability of fertile eggs in CON group must have decreased, but it did not. Some researchers (YILDIRIM and OZCAN, 2001; BRAKE, 1986; BRAKE and SHELDON 1990; WHISTLER and SHELDON, 1989; YILDIRIM and YETISIR, 1999) reported that FF has been used successfully in the poultry industry to control microbes on the eggshell surface and SACCO *et al.*, (1989) observed that FF eliminated the majority of bacteria of the eggshell microorganism population. But, the data from the current study does not support that FF can eliminate the total bacteria populations naturally occurring on the eggshell surface.

These results demonstrated that the use of OV on the quail hatching eggs could be used successfully to make a reduction of total bacteria populations. The other thing is that the projects planning for FF should measure the level of heat energy emerged from FF and the proper distance of the hatching eggs from the FF source during the application. Although the quails have commonly been accepted as lab. animals and the results obtained from them can be applied to the other poultry species, the data in the study was quite different at those from the chicken.

Results of this study suggest that hatchery managers may improve hatchability by using OV instead of FF in the disinfections of hatching eggs. But this approach requires further clarification by other comprehensive studies and with eggs from other poultry species.

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