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Disinfection of hatching eggs by formaldehyde fumigation – a review

Desinfektion von Bruteiern durch Begasung mit Formaldehyd – eine Übersicht

S. Cadirci

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Introduction

Microbial contamination of hatching eggs is a main concern of poultry producers as it causes poor hatchability and chick performance. It is evident that high standards of hygiene must be practised in hatcheries in order to minimise the soiling of eggs but, further disinfection of eggs is also necessary to limit bacterial numbers. Methods used include the application of disinfectants by wiping, spraying, and dipping but, arguably, the most effective way of disinfection of hatching eggs is fumigation with formaldehyde. Eggs can be fumigated during incubation or at pipping (during or just after the transfer to the hatcher), but most commonly prior to incubation.

Formaldehyde, besides being an excellent anti-microbial agent, is also a toxic chemical and, as such, can seriously damage the dormant embryo if fumigation is carried out improperly. The part of the egg most exposed to the fumigant is the outmost organic layer, the cuticle, an important barrier to microbial invasion. Any damage of the cuticle may have serious consequences during incubation. The aim of fumigation is therefore twofold: to reduce microbial count and to minimise the adverse effects of contamination whilst causing as little damage to the developing embryo as possible. In order to fumigate effectively, the balance must be found.

The importance of fumigation in hatchery practice is undoubtable, yet an overall and detailed review on the subject is still lacking. Moreover, results are sometimes conflicting or even misleading. The present review is an attempt to provide a summary of the literature on fumigation with a particular emphasis on the circumstances of this way of disinfection and the possible dangers to the developing embryo. It was also an aim to clear the contradictions and offer possible explanations for consideration.

Since fumigation is usually performed prior to incubation, the following review concentrates primarily on investigations on the conditions and effects of this practice.

Contamination of hatching eggs

At the time of laying, the number of bacteria on the shell of an egg may range from 300 to 500 (Mauldin, 1999). In an adequate environment, this number may increase rapidly so that one hour after the egg is laid there can be 20,000 to 30,000 bacteria present (North and Bell, 1990). If eggs are dirty, there can be up to 80,000 bacteria on the egg surface (Mauldin, 1999). Typical contaminants are Micrococcus, Salmonella, Pseudomonas, and Escherichia (Mayes and Takeballi, 1983), but various types of moulds have also been identified (Bruce and Johnson, 1978).

It has been demonstrated that if hatching eggs are not sanitised prior to incubation, excessive bacterial contamination and subsequent growth can lead to decreased hatchability, poor chick quality, growth and performance (Scott and Swetnam, 1993), and increased mortality (Reid et al., 1961). In some circumstances, as much as 20% loss of stock can occur due to bad hygienic standards (Willingham et al., 1996).

Fumigation of eggs with formaldehyde

Formaldehyde (H_2CO, formalin, formol) is a gas at room temperature and it is readily soluble in water. It is commonly used as a disinfectant, as it is cheap, not corrosive, and kills most bacteria and fungi (including their spores) (Braswell et al., 1970; Acklund et al., 1980; Williams, 1980). The biocidal efficacy of formaldehyde is due to its ability to act on the proteins and nucleic acid bases of microorganisms (Fraenkel-Conrat et al., 1945; Staehelin, 1958; Grossman et al., 1961; Russell, 1976). By attaching itself to the primary amide and amino groups of proteins, formaldehyde forms stable methylene bridges and, hence, intermolecular cross-linkages (Habeeb and Hiramoto, 1968). In addition, formaldehyde also alkylates the nitrogen atoms of purine and pirimidine bases in DNA and RNA.

Its first reported use as a disinfectant was in 1892 (cited by Hugo and Russell, 1992) but, apparently, Pernot (1908) was the first investigator to demonstrate the use of formaldehyde fumigation of eggs and incubators as a means of controlling poultry diseases. Formaldehyde may be applied as a liquid but it is more effective when used as a gas (Harry, 1954). The gas can be generated by several methods, but the most common way used in the poultry industry was the addition of formalin to potassium permanganate ($KMnO_4$) in 2:1 ratio (v/w). This is now being superseded by the volatilisation of the polymer paraformaldehyde under controlled temperature.

Factors influencing the effectiveness of formaldehyde disinfection

The effect of formaldehyde on microorganisms is influenced by 1) the concentration of formaldehyde and the

Department of Animal Science, Faculty of Agriculture, Harran University, Sanliurfa, Turkey

duration of exposure, 2) humidity (water content in the microorganisms), 3) temperature and 4) the amount of organic matter on the shell surface.

Concentration of formaldehyde and duration of fumigation

For effective disinfection, it is necessary to use formaldehyde in concentrations adequate for the duration of exposure. The minimum and maximum concentrations for the effective and safe use of formaldehyde have been the subject of many studies. Factors such as the size of the fumigation chamber, duration of exposure and temperature influence the amount of formaldehyde necessary for sufficient fumigation.

LANCASTER and CRABB (1953a) found that, in order to kill *S. pullorum* on the shell using a 20 minute fumigation period, a minimum concentration of 600 mg formaldehyde per m^3 (i.e. 10 g paraformaldehyde or 45 ml of 40% formalin and 30 g $KMnO_4$) at 21°C is necessary. In a later study, WILLIAMS (1970) demonstrated the effect of fumigation on microorganisms by using three levels of fumigant for 20 minutes at room temperature (25°C). He showed that fumigation using 1.2 ml formalin and 0.6 g $KMnO_4$ per cu.ft (cubic feet) (i.e. 565 mg released formaldehyde per m^3) kills 99.8% of microorganisms on the shell surface. Greater concentrations (i.e. 1696 mg released formaldehyde per m^3, and 2827 mg released formaldehyde per m^3) showed no significantly different effect.

The air space of the fumigation chamber is the main factor to be taken into account when calculating how much formaldehyde is needed to gain the desired concentration. Other factors, such as the extent of absorption by water on the walls and the rate at which the gas escapes from the chamber, also influence the amount of formaldehyde necessary (HARRY, 1954). HARRY (1954) pointed out that faulty ventilation louvres or accumulation of polymerised formaldehyde reduces the concentration in the chamber, and thus it is important to regularly determine the formaldehyde concentration at the end of the fumigation in order to confirm that an adequate level has been maintained.

Studies investigating the effect of different exposure times were also carried out. FURUTA and SATO (1977) fumigated artificially contaminated eggs using 40 ml formalin and 20 g $KMnO_4$ per m^3 (i.e. 533 mg released formaldehyde per m^3) at room temperature (25°C) for 0.5, 1, 2, and 3 hours. They found that, when the eggs were heavily contaminated (10^5 or more organisms), a small number of bacteria ($10^0 - 10^1$) always survived after fumigation regardless of exposure time; complete disinfection of the shell surface was not possible. In contrast, when the egg shell was less contaminated (up to 10^4 organisms) fumigation for 30 minutes already ensured complete disinfection.

Several investigators studied the effects of concentration of fumigant and exposure time on hatchability, and they had different results. PROUDFOOT and STEWART (1970) examined the possible effects of varying both the concentration of fumigant and the duration of exposure to fumigant. In a later experiment, FURUTA and WATANABE (1978) varied only the duration of exposure to fumigant while keeping the concentration constant. Neither of these investigations reported significant decrease in the overall hatchability. However, in a more recent work (ELIBOL et al., 2003), a significant relationship between embryonic mortality, duration of fumigation and the concentration of formaldehyde was found. The authors fumigated eggs for 20 and 40 minutes with the following concentrations in each case: 42 ml formalin and 21 g $KMnO_4$ m^3 (i.e. 560 mg released formaldehyde per m^3) and 56 ml formalin and 28 g $KMnO_4$. (i.e. 747 mg released formaldehyde per m^3). A significant decrease (8%) in hatchability was reported when the formaldehyde fumigations were used at higher duration and higher concentration.

The effective concentration of formaldehyde depends upon the temperature in the fumigation chamber. It has been demonstrated by LANCASTER and CRABB (1953b) that at an incubation temperature of 37.5°C, the terminal formaldehyde concentration for effective 20-minute disinfection should be at least 6 to 7 mg per cu.ft (212 to 247 mg per m^3). At room temperature (25°C) the value should be not less than 17 mg per cu.ft (601 mg per m^3). If a 10-minute exposure period is used at an incubation temperature of 37.5°C, the final formaldehyde concentration should be 25 mg per cu.ft (883 mg per m^3). These values were established with experiments on Salmonella (LANCASTER and CRABB, 1953a, LANCASTER et al., 1954) using the iodometric assay method developed by ROMIJN (cited by WALKER, 1953).

Humidity

If formaldehyde is used as a vapour phase disinfectant, its activity is influenced by the relative humidity (RH) because the gas particles are carried by water droplets. Thus, the killing power of formaldehyde for bacteria is higher in a warm, humid atmosphere than in cool, dry conditions (WRIGHT and TRUSCOTT, 1954). Hence, there may be benefits in keeping the relative humidity high (75% or more) during disinfection. Furthermore, microorganisms become more active and take up the fumigant at a faster rate as the humidity increases (HARRY, 1954; EKELENBURG, 1991). In an early study, WILSON (1949) observed that the bactericidal effect of formaldehyde was maximal at 68% RH. His findings were supported by LANCASTER and CRABB (1953b). Similarly, HARRY (1954) observed that RH of 67–90% during fumigation caused an over 99.5% reduction in bacterial count. However, throughout his experiment, he varied not only humidity but also the temperature and the volume of formalin, thus it is not possible to compare the effect of differing humidities independently from the other factors. This need was satisfied later by PROUDFOOT and STEWART (1970). They investigated whether higher RH would negatively affect hatchability. The authors compared the effect of 49–58% and 60–78% RH during fumigation using 1.5 ml formalin and 1 g $KMnO_4$ per 0.02832 m^3 (i.e. 707 mg released formaldehyde per m^3) at 31°C to 37°C, and found no significant reduction in hatchability. Hence, they concluded that a relative humidity of 60–78% did not have a deleterious effect on hatchability, and can be safely used for maximum germicidal benefit. In contrast to this report, RUSSELL and HUGO (1987) considered the literature on the subject to be conflicting and concluded that no increase in efficacy occurs at relative humidities above 50%.

Finally, it has to be noted that because formaldehyde gas becomes a relatively weak bactericide when dissolved in water, the accumulation of water on the surfaces should be avoided.

Temperature

High temperature is necessary for high levels of humidity because at higher temperature the gaseous phase is able to keep the vapour in a higher concentration, that is, the saturation time is delayed (EKELENBURG, 1991; RUSSELL and HUGO, 1987). However, there is a limit to the application of high temperature. In the freshly laid egg, the embryo contains 30000–60000 cells and is usually referred to as a

Stage X embryo (Eyal-Giladi and Kochav, 1976). To maintain dormancy of the embryo, the egg should be kept below physiological zero. Temperatures ranging from 20–21°C to 25–27°C have been defined as the physiological zero for fowl (Edwards, 1902; Funk and Biellier, 1944; Lundy, 1969). Ekelenburg (1991) suggested that temperature during fumigation should not exceed 25°C. Experiments by Proudfoot and Stewart (1970), however, showed no adverse effects on hatchability when fumigation temperatures ranged from 23°C to 37°C. It is a standard commercial practice to fumigate eggs at 25°C.

Organic matter

The presence of organic matter, such as blood, faeces, soil, food residues, is one of the most important factors which will influence disinfectant activity. Organic matter on the egg surface reduces the efficacy of the fumigant because the active agents are inactivated by the contaminant (Ekelenburg, 1993). Furthermore, the antibacterial activity can be reduced due to chemical reaction between the fumigant and the organic matter. Ekelenburg (1993) suggested that formaldehyde may react with the proteins of the organic matter, so becoming neutralised, and thus a smaller amount is available for attacking microorganisms. The most effective way of avoiding the influence of organic matter on the disinfectant is to control the hygiene of the environment.

Effects of formaldehyde

During fumigation, formaldehyde comes into contact not only with the surface microorganisms but also with the egg shell itself and, if absorbed, with the embryo. The concentration of absorbed formaldehyde (in the shell and in the albumen) was determined by Williams and Siegel (1969) using 565 mg released formaldehyde per m^3. The authors concluded that the level of fumigant detected in the egg was far lower than the toxic level. However, Cadirci (1997) pointed out that the toxic level of formaldehyde for the early embryo has not yet been determined. In an experiment investigating the toxic effect of pre-incubation fumigation, he used 600 mg released formaldehyde per m^3 (a concentration near to that applied by Williams and Siegel, 1969) and exposed the eggs to 15, 30, and 60 minutes of fumigation. It was found that the toxic effect of formaldehyde is the highest at 60 minutes exposure. The experiment also showed that this effect is most pronounced in eggs obtained from young (38–39 weeks of age) hens. The effects of parental age on embryo viability in fumigated eggs are discussed in a later section of this review.

Effect of formaldehyde on cuticle

Harry (1954) reported that the union of the egg shell surface and formaldehyde was not chemical as the latter could easily be removed by soaking the shell in water. The egg shell, however, is covered with the organic layer of cuticle (protein content is 90%) (Baker and Balch, 1962), and formaldehyde alkylates the amide and amido groups of proteins (Russell, 1976). Because the cuticle is one of the shell's main physical barriers preventing the passage of microorganisms, it would be important to know whether or not fumigation damages this layer. Yet, the literature on the possible deleterious effects of fumigation on the cuticle is limited.

The first experiments on cuticle degradation caused by fumigation did not provide useful answers. Using a protein-specific dye (Edicol Supra Pea Green H) Ball et al. (1975) found that fumigation damages the cuticle. They partially covered eggs with fumigant-proof material prior to fumigation. After fumigation the proofing was removed and the eggs were stained with the dye. The staining on the exposed part of the shell was less intense indicating that fumigation had partially degraded the cuticle. When previously coloured eggs were exposed to repeated fumigation, a reduction of colour intensity was observed. The authors therefore concluded that fumigation damages the cuticle, and recommended the avoidance of unnecessary use of fumigation. However, they used formic acid (a weak acid generated by the reaction of formalin and $KMnO_3$) instead of formaldehyde (generated by the reaction of formalin and $KMnO_4$). Weak acids are commonly used for the removal of the cuticle as a standard laboratory procedure (Vadhera et al., 1970); therefore the reduction of cuticle by fumigation with formic acid was predictable. Indeed, eggs should not be fumigated with formic acid.

In a later investigation Whistler and Sheldon (1989) used shell conductance as a tool to examine the effects of formaldehyde on the cuticle. They based their hypothesis on the fact that the cuticle provides a waterproof barrier on the shell and assumed that damage to the egg shell's cuticle cover leads to increased water loss by the embryo. They fumigated eggs using 1.198 ml formalin and 0.599 g $KMnO_4$ per cu.ft (i.e. 564 mg released formaldehyde per m^3). The authors found no change of conductance with the treatment. In an earlier study, Sparks (1985) had shown that the cuticle does not normally prevent gas (water vapour) exchange between the egg and its environment, thus it does not influence the conductance of hen's eggs. Nevertheless, Whistler and Sheldon (1989) concluded – incorrectly – that the cuticle was probably not affected by the exposure of eggs to the gas. The observation made by Sparks (1985) is in agreement with the conclusions of other authors that the cuticle on the shell surface has no apparent role in the gas exchange (Wangesteen and Rahn, 1970 and 1971; Tullett and Board, 1977; Paganelli et al., 1978; Rahn et al., 1979; Kayar et al., 1981; Board, 1982; Tranter et al., 1983; Sparks and Board, 1984). Thus, it seems that shell conductance is not a suitable parameter for measuring possible damage of the cuticle.

In contrast, a connection between cuticle quality and water uptake does exist. The cuticle is a major barrier to water movement between the egg and its environment (Board and Halls, 1973; Sparks and Board, 1984; Sparks and Burgess, 1993). It has been demonstrated that eggs with damaged or missing cuticle take up more water than eggs covered with cuticle of good quality (Sparks and Board, 1984). Water uptake, as measured by a gain in weight, is therefore a good indicator of the effectiveness of an egg's integument in preventing penetration by microorganisms (Board and Halls, 1973). A further investigation by Cadirci (1997) demonstrated that fumigating hatching eggs at 25°C with 600 mg formaldehyde gas released per m^3 for 15, 30 or even 60 minutes does not significantly change the water uptake of the eggs. The same study has also shown that, although it is not an indicator of cuticle damage, conductance of the egg shell also remained unaffected by formaldehyde-fumigation at these exposures.

Effect of formaldehyde on embryo viability

Embryonic death can occur naturally and it is a generally recognised, well documented phenomenon (Payne, 1919; Byerly, 1930; Bronkhorst, 1933; Romanoff, 1949). It can be influenced by several factors such as nutrition, manage-

ment and inheritance (Beer, 1969; Abbott, 1975; Jassim et al., 1996). Embryonic mortality shows a two-peak pattern (Payne, 1919). The first phase is during the first week of incubation, in synchrony with the period of lactic acid production, and occurs during a change in carbon dioxide elimination (Jassim et al., 1996). It is also the time when mesonephrons, part of the embryonic kidney, first function (Byerly, 1930). The second, larger, peak is during the last few days of incubation. It coincides with the period when demand for oxygen increases significantly (Jassim et al., 1996).

It is known (Russell, 1976) that formaldehyde acts on proteins and also on nucleic acids. It is feasible that formaldehyde gas diffused into the egg at an early stage of embryonic development will alkylate the nitrogen atoms of purine and pirimidine bases in DNA and RNA thus inhibiting their function. This, in turn, it can block embryonic development at an early stage, even prior to incubation. Fumigation near the time of hatching can also result in embryo mortality. The reason is that formaldehyde a hazardous gas may damage airways and lungs, when inhaled.

Relationship between embryo viability and storage of eggs before and after fumigation

Clarenburg and Romijn (1954) noted that prolonged storage of eggs at low temperatures prior to fumigation resulted in poor quality of hatch and approximately 10% difference in hatchability between the fumigated and unfumigated group. However, the authors recorded neither the storage time nor the temperature. Post-fumigation storage has also been reported to decrease hatchability if the eggs are packed immediately after treatment, with a severe decline in hatchability if the eggs are stored for up to 15 days. This was first demonstrated by Proudfoot and Stewart (1970). The authors suggested that it may be caused by a combination of polymerised formaldehyde deposited on the egg surface and free formaldehyde diffusing through the shell during fumigation. They noted that 24 hour ventilation is necessary for optimum hatchability. Furuta and Watanabe (1978) demonstrated that hatchability was not reduced if the eggs were stored for up to 14 days after fumigation with fumigant generated with 40 ml formalin and 20 g $KMnO_4$ per m^3 (533 mg formaldehyde per m^3), for 0.5, 1, 2, and 3 hours.

It is generally accepted that hatching eggs can be stored for up to 14 days prior to placing them into setters for incubation. Storage within this period does not influence the rate of mortality provided fumigation is carried out properly and eggs are aired throughout afterwards. After this period, however, embryos become more sensitive and the likelihood of embryonic death or damage due to fumigation increases.

Relationship between embryo viability, flock age and strain

Williams and Gordon (1970) carried out an experiment to investigate the effects of formaldehyde on hatching eggs in relation to flock age and strain (White Leghorn and White Rock). They fumigated eggs for 20 minutes using 6 ml formalin and 3 g $KMnO_4$ per cu.ft (i.e. 2827 mg released formaldehyde per m^3). The loss of embryos was significantly greater from eggs of 55-week-old hens than from those of 35-week-old ones. Moreover, at candling on the 11th day, mortality was found to be higher in brown (White Rock) than in white eggs (White Leghorn). The authors suggested that, in both cases, the observed adverse effects were associated with changes in shell surface structure which interfered with gas exchange. This conclusion was partially based on an earlier study (Williams and Siegel, 1969) where no significant concentrations of formaldehyde could be detected under the shell or in the albumen of eggs exposed to 565 mg released formaldehyde per m^3. It has been suggested that the cuticle may have a role in regulating the gaseous conductance between the egg and the embryo by acting as a barrier to the loss of water vapour (Peebles and Brake, 1985; Peebles and Brake, 1986; Mair et al., 1984). However, there are major problems with the work of Williams and Gordon (1970), namely, that they have not randomised the effect of flock age and strain in their experiment. Thus, it is impossible to say which one of the factors was responsible for the differences in mortality. Moreover, they based their conclusions on the assumption that the cuticle plays a role in shell conductance, although, as it was discussed in an earlier section, this might not be the case. In addition, no further studies in support of the argument of these authors have been reported.

Moreover, Sheldon and Brake (1991) demonstrated that exposure to 2827 mg released formaldehyde per m^3 (i.e. the same concentration as used by Williams and Gordon (1970)) with standard procedure did not affect conductance. They suggested that this was because formaldehyde did not have an effect on the cuticle. The relationship between flock age and the rate of mortality in fumigated eggs was also studied in a large scale investigation (involving 7520 eggs) by Cadirci (1997). The experiment was carried out on eggs from hens of different ages (38–39 and 56–57 weeks), with three different exposure times (15, 30 and 60 minutes) and constant concentration (600 mg per m^3) of the fumigant at room temperature (25°C). In contrast to the findings of Williams and Gordon (1970), Cadirci (1997) detected a significant decrease in hatchability from both flocks. When eggs were exposed to 60 minutes fumigation, the decrease in hatchability was more pronounced at the young-parent eggs. The experiment revealed that the decrease in hatchability was mainly due to the effect of fumigation on early mortality, at the two days' period after laying in particular, which is the time of organogenesis. The fumigation of eggs did not seem to be associated with any differences noted in the number of middle and late period dead embryos, and this is in agreement with earlier observations (e.g. by Williams and Gordon, 1970; Proudfoot and Stewart, 1970; Furuta and Watanabe, 1978). These observations are also in contrast to the postulation that the integrity of the cuticle is important for shell conductance. If shell conductance values were reduced, there would be an increase in the incidence of late (and not early) embryonic mortality – the embryo's requirement for respiratory gas exchange being maximal in the final week of incubation.

For a possible explanation for higher mortality from young-parent eggs, Cadirci (1997) suggested the followings for consideration: as the quality of the shell is not constant throughout the laying period, fumigation may have different effects on embryos obtained from flocks of different ages. Moreover, older hens lay larger eggs than younger hens do, and these larger eggs have a lower specific surface area (surface to mass ratio) than the smaller eggs (noted by e.g. Peebles and Brake, 1987). Therefore, all other factors being equal, smaller eggs would absorb a relatively higher amount of fumigant than the larger eggs. Thus, embryos in smaller eggs may be exposed to a higher dose of fumigant when the gas penetrates the shell. Also, the yolk-shell distance is smaller in small eggs (young-parent) than in large ones (old-parent).

Consequently, the fumigant penetrating the shell may reach the germinal disc easier in smaller eggs (CADIRCI, 1997). In addition, according to the results of CADIRCI (1997), eggs from the younger flock have a thinner cuticle than eggs from the older flock. At fumigation, the thicker cuticle of the old-parent eggs absorbs more fumigant, thus restricting the amount of formaldehyde penetrating the shell. Moreover, proportionally more formaldehyde may react with the proteinaceous cuticle of the old-parent eggs, thus its effectiveness would be reduced.

Effect of pre-incubation fumigation

After separation from the hen at oviposition, the egg is constantly exposed to contaminants such as bacteria, viruses and moulds. Formaldehyde is a surface disinfectant, therefore it is important to destroy microorganisms while they are still on the surface of the egg shell. Once the organisms penetrate the shell, they reach the shell membrane within minutes (BEAN and MCLAURY, 1959; WILLIAMS et al., 1968) and are protected from the fumigant. Thus, fumigation should be performed soon after collection, preferably while the eggs are still warm. Indeed, commercially, hatching eggs tend to be fumigated during transport from the farm to the hatchery and at the hatchery.

Experiments investigating the effects of pre-incubation fumigation on embryo viability were first carried out by WILSON (1951). He used 1.5 ml of formalin and 1.0 g $KMnO_4$ per cu.ft (i.e. 707 mg released formaldehyde per m^3), and observed a reduction of 3.9% in hatchability. However, it was not reported whether or not this reduction was statistically significant. Later, LANCASTER et al. (1954) used 22 mg formaldehyde per cu.ft (i.e. 777 mg per m^3), a concentration greater than that recommended (i.e. 600 mg per m^3) by the Ministry of Agriculture, Fisheries and Food (MAFF) (ANONYMOUS, 1977). The authors reported no damage to the embryo and no reduction in hatchability. The same year, CLARENBURG and ROMIJN (1954) fumigated freshly laid eggs with formaldehyde at a concentration produced by 30 ml formalin added to 20 g $KMnO_4$ per m^3 (i.e. 400 mg released formaldehyde per m^3) and they found no significant difference in hatchability between the unfumigated and fumigated group. Similar findings were reported by TURK (1968), who used 10 g of paraformaldehyde per m^3 (i.e. 600 mg released formaldehyde per m^3).

A number of works have been carried out to determine the optimal duration of pre-incubation fumigation. As it was mentioned earlier, in order to kill *S. pullorum* on the shell, fumigation should last for at least 20 minutes (LANCASTER and CRABB, 1953a). SAMBERG and MEROZ (1995) examined the effects of exposure to formaldehyde on embryo mortality, and reported that fumigation for up to 60 minutes does not reduce the viability of hatching eggs. In contrast, CADIRCI (1997) showed that exposure to fumigant for this duration does significantly reduce hatchability. He demonstrated that fumigating eggs prior to incubation for 60 minutes (a period long enough for the gas to diffuse into the egg in a relatively high concentration) can cause serious damage to the differentiating cells, which increases early embryo mortality. He also showed that in the later periods of embryo development, the exposure of eggs to 60 minutes of fumigation did not result in significantly increased mortality.

However, a most recent work using transmission electron microscopy shows that pre-incubation fumigation of eggs for even 20 minutes negatively affects the tracheal epithelial cells of 18-day- old embryos and 1-day-old chick (HAYRETDAG and KOLANKAYA, 2008).

Effect of fumigation during incubation

It is not a common practice to fumigate eggs after setting and literature on the subject is limited. It includes early studies (GWATKIN, 1926 and 1928; BUSHNELL et al., 1929) investigating the effects of formaldehyde on viability, and recommending that eggs should not be fumigated between 24 and 84 hours of incubation. MARCELLUS et al. (1930) reported that developing embryos were particularly sensitive to formaldehyde gas between 24 and 96 hours of incubation, and embryonic mortality increased if exposed during this period. This finding is supported by the work of a number of researchers including INSKO et al. (1941); WILSON (1951) and HARRY (1954). LANCASTER et al. (1954) claimed that the critical period extends up to 120 hours after the setting of the eggs. On the other hand, HARRY and BINSTEAD (1961) suggested that hatchability can be negatively affected by fumigation between 3 and 9 days of incubation. Recommendations that fumigation should not been carried out during this sensitive period of incubation were also published (e.g. STOVER, 1960; HODGETTS, 1987).

Effect of fumigation at pipping

Towards the end of the incubation process (about three days before hatching) in commercially raised avian species, eggs are transferred from setters to a type of incubator called hatcher. In here, newly hatched chicks can easily get infected by microbes even if the eggs were clean (FURUTA and MARUYAMA, 1981). However, the mortality rate of 2-week-old chicks from dirty eggs can be as much as four times that of those from nest-clean eggs (MAULDIN, 1999). Fumigation of eggs immediately after transfer to the hatcher can minimise the number of pathogenic microorganisms and thus increase the number of healthy chicks. Nevertheless, fumigation at this stage of the incubation requires great care because at pipping, the embryo becomes a direct-air-breathing animal (as opposed to the embryo breathing by air exchange through the egg shell). Controlling the temperature and humidity is especially critical at this point of hatching. Levels of moisture too high or too low will interfere with the hatching process and result in increased mortality and/or poor post-hatch performance of chicks. In addition, the time necessary for all chicks to emerge from the eggs may be up to thirty hours. The use of a conventional method of disinfection (e.g. spraying, conventional automatic foggers) would produce humidity levels too high when used on a continuous basis resulting in a smaller percentage of chicks surviving the hatching process, or poor post-hatch performance. However, a gaseous disinfectant such as formaldehyde will not significantly increase the moisture level in the environment. It can also be administered continuously from the time of pipping, when the microorganism bloom occurs up until essentially all the chicks have exited from the eggs.

On the other hand, fumigation with formaldehyde near hatching is associated with the degeneration and disappearance of the epithelial linings of the respiratory tract in chicks (FURUTA et al., 1989). Morphological changes of the epithelium have also been found (SANDER et al., 1995). FAUZIAH et al. (1996) reported that exposure of hatching chicks to 130 ppm formaldehyde vapour during the last 3 days of incubation had adverse effects on the health of chicks. Moreover, ZULKIFLI et al. (1999) reported that exposure of hatching chicks to 23.5 ppm formaldehyde vapour resulted in poor production performance. The physical damage caused by formaldehyde to the avian respiratory system may predispose the animals to increased susceptibility to respiratory disease encountered in the early days of life.

Conclusion

The growth of the poultry industry has led to a rapid increase in the size of individual hatcheries, so that, at any one time, many contain half a million or more eggs. This created new problems such as the spread of disease-causing microorganisms, e.g. the Salmonella species (Cason et al., 1994). Even a low mortality rate of 1–2% would involve a very large number of chicks or poults, with final losses running into millions of Euros over a long period of time.

Reducing contamination of hatching eggs is a priority, and research investigating the fumigation of hatching eggs with formaldehyde gas has clearly demonstrated that this is an effective way of disinfection. However, care must be taken because under-fumigation does not kill the microorganisms, but over-fumigation can kill the chick embryo. Following recommendations for the amount of chemicals, time and duration of fumigation, temperature and level of humidity is of crucial importance. After fumigation, eggs should be allowed to air out for several hours before placing them in the incubator.

Considering the factors influencing the effectiveness of fumigation, the MAFF (Ministry of Agriculture, Fisheries and Food) released a bulletin (Anonymous, 1977) with recommendations for disinfection of hatching eggs. According to this publication, the advisable humidity for effective fumigation is 61-79%, and the following terminal formaldehyde gas concentrations should be achieved:

either (a) 600 mg per m^3 after 20 min at 21°C
or (b) 900 mg per m^3 after 10 min at 21°C
or (c) 230 mg per m^3 after 20 min at 37.8°C

These concentrations can be achieved by the reaction of 45 ml 40% formalin solution with 30 g potassium permanganate crystals per m^3 or by the heating of 10 g of paraformaldehyde prills per m^3 (Anonymous, 1977).

In addition to the effects of formaldehyde on microorganisms, egg shell, and the developing embryo, formaldehyde gas can potentially become hazardous to human health. Although it was not the objective of this paper to discuss the possible dangers of formaldehyde fumigation to human health, it needs to be mentioned that it is a toxic gas, a strong irritant, a potent sensitizer and a suspected carcinogen. People working with this gas must bear its hazards in mind. Casteel et al. (1987) indicated that exposure to formaldehyde gas can damage mucous membranes, resulting in symptoms of eye and skin irritation and respiratory problems such as pulmonary oedema. Therefore, direct contact with formaldehyde should be avoided by the use of gloves and suitable protective clothing, including mask. Moreover, it is recommended that hatchery workers should be screened regularly.

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Summary

Losses due to microbial contamination of hatching eggs in the poultry industry can run into millions of Euros. Fumigation with formaldehyde gas is an effective way of disinfection before and during incubation as well as near hatching. The present paper is a review of the literature on the conditions and effects of formaldehyde fumigation.

In summary: for disinfection against Salmonella species, nest clean eggs should be fumigated prior to incubation at room temperature (25°C) and ambient humidity for at least 20 minutes with a minimum concentration of 600 mg formaldehyde gas per m^3 (i.e. 10 g paraformaldehyde or 45 ml of 40% formalin and 30 g $KMnO_4$). Fumigation under these conditions kills 99.8% of microorganisms on the shell surface and is not associated with increased embryonic mortality. Fumigation should not be performed during the first 9 days of incubation. Fumigation at pipping may damage the respiratory system of chicks and have adverse effects on the health and production performance. In addition, formaldehyde is also hazardous to human health.

Key words

Disinfection, hatching eggs, formaldehyde fumigation

Zusammenfassung

Desinfektion von Bruteiern durch Begasung mit Formaldehyde – eine Übersicht

Die mikrobielle Kontamination von Bruteiern kann den Bruterfolg deutlich reduzieren und hierdurch Kosten im Bereich von Millionen Euro verursachen. Die Begasung mit Formaldehyde sowohl vor als auch während der Brut und kurz vor dem Schlupf stellt eine effektive Desinfektionsmaßnahme dar. Die vorliegende Übersicht stellt den Kenntnisstand zu den Bedingungen und Auswirkungen der Bruteibegasung mit Formladehyde zusammen.

Saubere Bruteier sollten zur Desinfektion gegen Salmonella-Serotypen vor der Bruteinlage bei Raumtemperatur (25°C) und einer normalen relativen Luftfeuchte über 20 Minuten mit einer minimalen Konzentration von 600 mg Formaldehyde-Gas pro m^3 (10 g Paraformaldehyde oder 45 ml 40%iges Formalin und 30 g $KMnO_4$) begast werden. Unter diesen Begasungsbedingungen werden 99,8% der Mikroorganismen auf der Schalenoberfläche abgetötet. Die Embryonalsterblichkeit wird hierdurch nicht erhöht. Dagegen sollte während den ersten 9 Bruttagen keine Begasung durchgeführt werden. Eine Begasung während dem Anpicken der Eier kann das Atmungssystem der Küken schädigen und negative Effekte auf die Gesundheit und die Leistungsentfaltung der Küken haben. Es sollte ferner nicht vergessen werden, dass Formaldehyde auch für die menschliche Gesundheit gefährlich ist.

Stichworte

Desinfektion, Bruteier, Formaldehyde, Begasung

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Correspondence: Sahin Cadirci, University of Harran, Faculty of Agriculture, Department of Animal Science, 63200 Sanliurfa, Turkey; e-mail: scadirciuk@yahoo.co.uk