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EFFECT OF HATCHING EGG SANITATION ON THE MICROBIAL LOAD AND HATCHABILITY OF BROILER BREEDER EGGS

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SUMMARY

The present study was conducted to examine the effect of hatching egg sanitizers on the microbial load and hatchability of broiler breeder hatching eggs. For this purpose, five commercial egg sanitizers were used, A (H_2O_2), B (quaternary ammonium compound), C (H_2O_2& peracetic acid), D (gluatraldehyde & quaternary ammonium compound) and E (formalin). A total of 1225 hatching eggs were allotted into 7 groups each of 165 eggs. The first four groups were treated with a mixture of {H_2O_2+ quaternary ammonium compound (QA)} in a low & high concentration, both as a dip and as a spray treatment. The fifth and the sixth groups were treated with C and D respectively, while the last group was fumigated with formalin. Results showed that complete reduction of the total bacterial count from egg shell was obtained when eggs were treated with (H_2O_2 + QA) in high concentration and formalin fumigation after 24 hours of application. For the total fungal count results, none of the used disinfectants reduced it after 24 hours except formalin, but the high concentration of (H_2O_2 + QA dipping) and peracetic acid could produce 85.7% reduction . Regarding hatching results, the highest hatchability of fertile eggs (95.2%) was obtained when eggs were treated with peracetic acid or with the mixture of H_2O_2 + QA (in a low concentration as a dip). While the least percentage of hatchability of fertile eggs and the highest total embryonic deaths (91.7&7.8%) were obtained when eggs were treated with the high concentration of H_2O_2+ QA. Formalin fumigated eggs recorded 93.7 % hatchability of fertile eggs and the highest percentage of culls 4.9%. So, the proper use of the disinfectant in chicken hatcheries is essential and the evaluation of alternative disinfectant to formalin should not only based on its antimicrobial activity but also on its ability to maintain the hatching potential as well as its safety for hatchery employees.

INTRODUCTION

The importance of effective sanitation program in the hatchery is to achieve high percentage of hatchability and to ensure the production of high quality chicks (Brake and Sheldon 1990). Assuming that the egg was sterile during formation, microbial contamination of the egg shell may occur during passage through the cloaca and following oviposition from the surrounding environment either in the production farm or from chicken hatcheries (Sacco et al., 1989). Hatchery environment and its surrounding may become contaminated with microorganisms from various sources. Microorganisms on or in a few hatching eggs can be easily distributed throughout the hatchery by air movements during hatching, thus contaminating or infecting other chicks in the hatcher. Control of microbial populations on the egg shell surface requires a sanitizing agent that is effective in controlling contamination but which does not adversely affect the embryo (Bailey et al., 1996). Fumigation of hatching eggs with formaldehyde gas had been used successfully in the poultry industry to control microbes on the shell surface. Williams (1970) and Gehan Moustafa (1995) reported that formaldehyde fumigation eliminated the majority of bacteria (>94%). However, several disadvantages are associated with using such sanitizer, as the disinfectant vapours being inhaled by hatchery workers, in addition to the lingering unpleasant odor left by the disinfectant following decontamination (Proud foot et al.1985) and most importantly recent actions by the Environmental Protection Agency that regulated the use of formaldehyde and classified it as toxic substance due to its suspected carcinogenicity (Chemical and Engineering News, 1984).

Nowadays, there are several alternative methods for egg sanitation as recommended by many investigators among which Barbour et al. 1985 who suggested the use of iodine preprations, Patterson et al. 1990 who suggested the use of chlorine preprations, Sheldon and Brake 1991, Rodgers et al.2001 who recommended the use of H_2O_2 and Scott et al. 1993 who suggested the use of virkons and glutraldehyde for the same purpose. However, the choice of a hatching egg disinfectant should not depend only on their efficiency in reducing the microbial load but also on their safety for egg embryo and hatchery workers. So, the primary objective of the present study is to determine the effect of some available disinfectants on egg shell microbial load and to assess their effect on hatchability.

MATERIALS AND METHODS

Hatching eggs:

Hatching eggs used in the present study were obtained from a breeder farm raising commercial strain of Hubbard broiler breeders of 40 weeks age. At 20 weeks of age the male: female ratio was 1:10. Birds were housed in closed dark-out houses with fully controlled environment, re-

ceived a total of 16.5 hours photoperiod per day throughout the production period. The feeding program was in accordance with the management guide of the breeding company. All houses were provided with a number of nests sufficient to allow one nest for each five birds. Eggs were collected manually 4-5 times per day. No excessively dirty, thin shelled or otherwise abnormally shaped eggs were used. After collection, eggs were randomly allotted into 7 groups each of 165 eggs for the different egg treatments.

Disinfectants:

Five commercial disinfectants were used in the present study; disinfectants composition and dilutions are shown in table (1).

Table (1): Disinfectant type and dilutions:

Disinfectant	Supplier	Active ingredient	Dilutions
A	Henkel (Germany)	H_2O_2 50% Dihydroxybenzole 100 ppm	5%
B	*Antec internat LTD U.K.*	Quaternary ammonium compound	300 ppm
C	Henkel (Germany)	H_2O_2 35% Peraceticacid 5%	2%
D	*EWABO Chemkalien Gmbh*	Gluteraldehyde Quaternary ammonium compound	0.5%
E	*Helliopolis Pharm.Chem.CoEgypt*	Formaldehyde solution 40%	40 ml formalin+20 gram potassium permanganate

Egg treatment:

The seven groups of eggs were sanitized using one of the methods described in table (2)

For spraying treatments, eggs were placed on clean plastic flats and sprayed with a hand sprayer until dripping. Egg dipping treatments were done in sterile suitable container containing the used disinfectant. Eggs were allowed to stand for two minutes in the dip solution before being trayed in the egg flats. Fumigation was done in the fumigation room during the routine work of the hatchery using 40 ml formalin and 20 grams of potassium permanganate/ m3 for 15 minutes. Following treatments eggs were left to dry then examined microbiologically.

Table (2) : Egg treatment

Egg treatment no.	Disinfectant	Method of application
1	1.5% dil. A+ 0.5% dil. B	Spraying
2	1.5% dil. A+ 0.5% dil. B	Dipping
3	2% dil.A +0.5 % dil. B	Spraying
4	2% dil.A +0.5% dil. B	Dipping
5	2% C	Spraying
6	0.5% D	Spraying
7	E	Fumigation

Microbiological examination of hatching eggs:

The survived contaminants of egg shell were enumerated using sterile swab moistened with sterile normal saline to swab an area of 2 cm^2 of each of five eggs per treatment using sterile flexible template. The used swabs were received into a sterile cotton plugged test tubes contained 5 ml sterile normal saline. In the laboratory, 0.1ml of each sample was plated on sterile plates of plate count agar for total bacterial count and Sabouroud `s dextrose agar for total fungal count. The inoculated plates of plate count agar were incubated for 24-48 hours at 37°C while those of Sabouroud `s dextrose agar were incubated for 3-5 days at 25°C. The total bacterial and the total fungal count per 2 cm^2 of egg shell surface were calculated and recorded. Eggs were sampled before treatment, after 60 minutes of treatment, at setting (one day post disinfection), at three days of incubation, at transfer (18 days of incubation) then at hatching day. At the 10 th day of incubation, eggs were candeled to exclude the infertile eggs and the early embryonic deaths. At hatch, all of the unhatched eggs were opened and examined for evidence of embryonic development to calculate the number of early (7th day) and late embryonic deaths (14 th day) and dead-in- shell. The hatchability of fertile eggs, number of healthy chicks and culls was calculated and recorded.

RESULTS AND DISCUSSION

Results recorded in table (3) showed that treatment of hatching eggs with the high concentration of hydrogen peroxide + quaternary ammonium compound (H_2O_2+ QA) either as spray or as a dip and formaldehyde fumigation reduced the total bacterial count (TBC) completely (100% reduction) after 24 hours of application while the low concentration of the same disinfectants resulted in 97.8 and 96.7 % reduction for dipping and spraying treatment respectively. QA is widely used as a hatchery disinfectant but many investigators had reported different levels of bacterial resistance to it as Tenant et al.1985, Gillespie et al. 1986. Willinghan et al. 1996, Sidhu et al. 2002, and Gehan-Moustafa et al. 2004. On the other hand, H_2O_2 had been recommended as a hatching egg disinfectant at various concentrations up to 5% by many investigators among which Hafez et al.1991, Sheldon and Brake 1991, Bailey et al. 1996, Sander and Wilson 1999 and Rodgers et al. 2001. Relatively few studies are available that had examined the mixing of QA with other disinfectants to avoid its bacterial resistance except Sacoo et al.1989 who tried to wash eggs with QA and formalin, and reported that the TBC of egg shell was not significantly affectd by treatment of eggs by formalin fumigation or by washing eggs with QA or by washing eggs with QA and formalin. In contrast, Arhienbuwa et al. 1980 reported a signif-

icant lower bacterial count in egg shells treated with QA than that of formalin fumigated.

Peracetic acid and glutaraldehyde+QA recorded similar results as they both reduced the TBC of eggshell (94.4 % reduction) after 24 hours of application. The obtained results are agreeable with those of Blakistone et al.1999, Bonadonna et al.1999, Thamlikitkul et al.2001 who proved that peracetic acid is an effective bactericide and Rodgers et al.2001 who reported that peracetic acid was the most potent bactericide against staphylococus species in hatcheries. On the other hand, Rossoni and Gaylande 2000 mentioned that peracetic acid cauldnot be recommended as the sanitising agent of choice for chicken processing equipment. Regading the glutraldehyde Hegna and Clausen 1988 reported that it is an effective bactericide and less irritant than formaldehyde.

Table (3): Effect of different hatching egg sanitizers on the total bacterial count of eggshell

Egg treatment No.	Sampling time					
	Before treatment	After one hour	At setting	After 72 hours	At transfer	At hatch
1	$45x10^2$	35x10	15x10	15x10	15x10	25x10
2	$45x10^2$	30x10	10x10	10x10	15x10	20x10
3	$45x10^2$	30x10	Nil	75	10x10	15x10
4	$45x10^2$	25x10	Nil	70	10x10	15x10
5	$45x10^2$	25x10	20x10	18x10	15x10	20x10
6	$45x10^2$	20x10	20x10	15x10	10x10	15x10
7	$45x10^2$	10x10	Nil	50	75	10x10

Results of the total fungal count (TFC) as shown in table (4) indicated that none of the used disinfectants could reduce the TFC completely except formalin which produce a complete reduction after 24 hours of application. Results obtained are in accordance with those of Fujishima et al. 1990 and Gehan Moustafa 1995 who found that formaldehyde fumigation killed all the microorganisms on the egg shell after one hour of application.

The high concentration of H_2O_2+ QA as a dip and peracetic acid reduced the TFC after 24 hours of application by 85.7%. These results are comparable to those of Brake and Sheldon 1990 who found that H_2O_2 had the least effect on molds and yeast than on TBC and Bundgaard- Nielsen and Nielsen 1996 who found that 2% formaldehyde, 3% H_2O_2 and 3% peracetic acid were ineffective as fungicide. While Baldry 1983 mentioned that H_2O_2 was more effective as a fungicide than a bactericide and Jordan 1990 mentioned that H_2O_2 at low concentration was active against bacteria, viruses and fungi.

Table (4): Effect of different hatching egg sanitizers on the total fungal count of egg shell

Egg treatment No.	Sampling time					
	Before treatment	After one hour	At setting	After 72 hours	At transfere	At hatching
1	35x10	30x10	25x10	20x10	25x10	30x10
2	35x10	25x10	20x10	20x10	25x10	30x10
3	35x10	20x10	10x10	10x10	15x10	20x10
4	35x10	15x10	5x10	5x10	10x10	15x10
5	35x10	20x10	5x10	10x10	10x10	15x10
6	35x10	30x10	25x10	20x10	25x10	30x10
7	35x10	10x10	Nil	5x10	10x10	15x10

From tables (3) and (4) it could be noticed that the TBC and the TFC of the treated egg shell had increased markedly during hatching (Sander and Wilson 1999) but, it was significantly lower in eggs treated with the high concentration of H_2O_2+ QA, peracetic acid and formalin when compared with the other treated groups. Thus indicating that these disinfectants succeeded in controlling these bacterial and fungal populations. According to literature, this should result in lower degree of contamination of hatching eggs and consequently enhance the hatchability.

Hatching results for the different treated groups of eggs are recorded in table (5). The obtained results indicated that the highest hatchability percentage of fertile eggs 95.2% and the least embryonic deaths 5.5% and culls 1.4% were obtained in eggs treated with peracetic acid.

Eggs treated with H_2O_2+QA dipping at the low concentration recorded also the same percentage of hatchability (95.2%) and a relatively low percentage of total embyonic deaths (6.0%) than the high concentration of the same prepration (92.9& 91.7%) and (7.8 & 7.2%) for dipping and spraying respectively. This may indicate that increasing the concentration of these disinfectants may have an adverse effect on embryonic development although it reduced the microbial contamination of eggs. Generally, Scott et al. 1993, Sahoo et al.1995, and Gehan Moustafa 1999 proved that egg disinfection with H_2O_2 enhanced the hatchability, but Scott and Swetnam 1993 reported that H_2O_2 might have a carcinogenic effect. While Brake and Sheldon 1990 reported that spraying QA significantly increased hatchability of fertile eggs from 32 weeks old breeder flock. Gluatraldehyde+ QA recorded a hatchability percentage of fertile eggs of 94.6% and a total embryonic deaths of 6 %. These results are in accordance with Scott et al. 1993 who found that egg disinfection with glutaraldehyde had no toxic effect on embryo viability.

Hatchability percentage of formalin fumigated eggs reached 93.7 % while the total embryonic deaths and culls were 7.2 and 4.9% respectively. The percentage of culls was the highest when compared with the other disinfectants. Many investigators had recommended the use of formalin for hatching egg disinfection, among which Ehsan-Bashandy 1972, Huttner 1973 and Barbour et al. 1985 who stated that formaldehyde had no adverse effect on hatchability. But Badawy et al. 1986 found that the accidental fumigation at a higher concentration or extention of the fumigation time had a marked negative effect on hatchability, while, Furuta et al. 1989 and Gerrits 1990 reported that formalin fumigation was very harmfull to the hatched chicks. On the other hand, other investigators tried to find a new alternatives for formalin following the recommendations of the

Table(5): Effect of different sanitizing agents on hatching results

Treatment	Total no. of eggs	Fertile eggs		Embryonic deaths								Hatchability percentage of	Produced chicks	
				E.E.D		L.E.D		Dead-in-shell		TED		Incubated eggs	Fertile eggs	% of Healthy	% of culls
		No.	%	No.	%	No.	%	No.	%	No.	%				
1	165	145	87.9	3	1.8	8	4.8	nil	nil	11	6.6	81.8	93.1	91.7	1.4
2	165	147	89.0	3	1.8	6	3.6	1	0.6	10	6.0	84.8	95.2	93.2	2.0
3	165	145	87.9	1	0.6	6	3.6	5	3.0	12	7.2	80.6	91.7	89.6	2.1
4	165	141	85.5	3	1.8	6	3.6	4	2.4	13	7.8	79.3	92.9	89.4	3.5
5	165	146	88.5	2	1.2	4	2.4	3	1.8	9	5.5	95.2	95.2	93.8	1.4
6	165	149	90.3	2	1.2	5	2.4	3	1.8	10	6.0	85.4	94.6	92.0	2.7
7	165	144	87.2	3	1.8	6	3.6	3	1.8	12	7.2	81.8	93.7	88.9	4.9

E.E.D: Early Embyonic deathes
L.E.D:: Late embryonic deathes
T.E.D: Total embryonic deathes

Table(5): Effect of different sanitizing agents on hatching results

Treatment	Total no. of eggs	Fertile eggs		Embryonic deaths								Hatchability percentage of		Produced chicks	
				E.E.D		L.E.D		Dead-in-shell		TED		Incubated eggs	Fertile eggs	% of Healthy	% of culls
		No.	%	No.	%	No.	%	No.	%	No.	%				
1	165	145	87.9	3	1.8	8	4.8	nil	nil	11	6.6	81.8	93.1	91.7	1.4
2	165	147	89.0	3	1.8	6	3.6	1	0.6	10	6.0	84.8	95.2	93.2	2.0
3	165	145	87.9	1	0.6	6	3.6	5	3.0	12	7.2	80.6	91.7	89.6	2.1
4	165	141	85.5	3	1.8	6	3.6	4	2.4	13	7.8	79.3	92.9	89.4	3.5
5	165	146	88.5	2	1.2	4	2.4	3	1.8	9	5.5	95.2	95.2	93.8	1.4
6	165	149	90.3	2	1.2	5	2.4	3	1.8	10	6.0	85.4	94.6	92.0	2.7
7	165	144	87.2	3	1.8	6	3.6	3	1.8	12	7.2	81.8	93.7	88.9	4.9

E.E.D: Early Embyonic deathes
L.E.D.: Late embryonic deathes
T.E.D: Total embryonic deathes

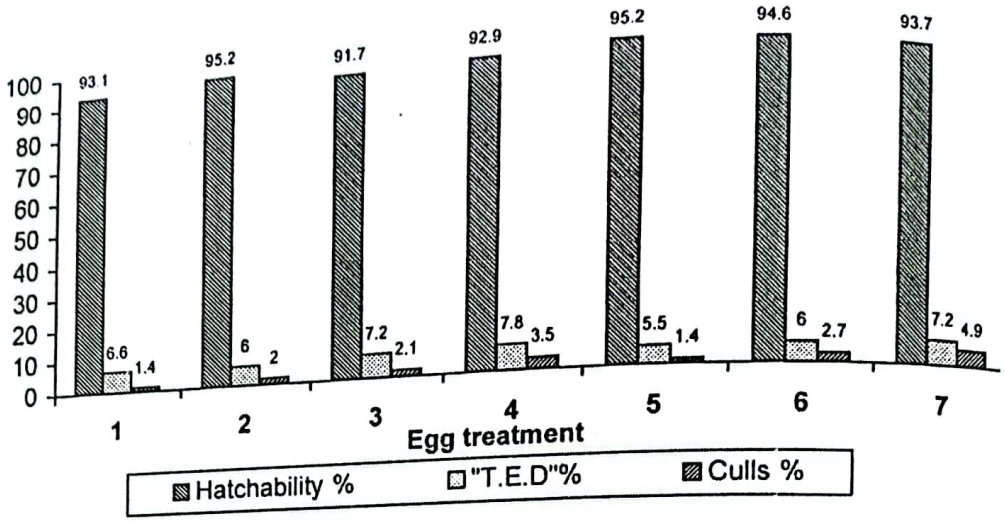

Figure (1): Hatching results for different treated groups of eggs

Environmental Protection Agency by regulating its use under the Toxic Substance Control Act (Whistler and Sheldon 1989) and, Brake and Sheldon 1990 who suggested the use of QA as a hatching egg sanitizer, Sheldon and Brake 1991who proved that there was no significant difference in hatchabililty due to H_2O_2 or formalin fumigation, and Sander and Wilson 1999 who recommended the use of H_2O_2 preprations for hatching egg disinfection.

Conculsively, the setters and hatchers by their adjusted temperature and humidity provide a favorable medium for the growth and multiplication of microorganisms.These microorganisms may penetrate the egg shell into the content causing a disease in the produced chick in addation that; such infected eggs would be considered as a source of infection to the other hatched chicks. Accordingly, the hatchabiliy percent may be reduced and the hatched chick will be of low quality and vitality. So, well-designed sanitation programme is advisable to control the different bacterial and fungal contaminants on the egg shell and for the setters and hatchers. Many available disinfectants in the Egyptian market proved their efficiency in decontaminating the hatching egg shell such as H_2O_2, peracetic acid and glutaraldehyde without excreting negative effects on hatching results.

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