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THE SANITIZING EFFECT OF GLUTARALDEHYDE-QUATERNARY AMMONIUM COMBINATION (ALDEKOL GDA) ON BACTERIAL INFECTION IN HATCHING EGGS (LABORATORY AND FIELD TRIALS)

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SUMMARY

The sanitizing effect of spraying with 1% Aldekol GDA (glutaraldehyde-quaternaries ammonium combination) compound on bacterial count of egg - shell and 5-day-old embryonted chicken eggs infected with Sal. typhimurium , Proteus mirabilis and Staph . aureus was studied . The results revealed that there were decreasing in total bacterial count on egg-shell and an improvement in the rate of hatchability after using this sanitizer. In a field trial, Aldekol GDA minimized the bacterial contamination and the total bacterial count in both hatching eggs and newly hatched chicks . Obtainaed results proved the safty of this compound in controlling bacterial contamination and shell penetration with these pathogenic bacteria

INTRODUCTION

Disinfection is a process of optimising the quality of hatching eggs. Gormaldehyde has been widly used as an effective sanitizer and a cheape egg disinfectant (Smith and Conant, 1960; Ehsan-Bashandy, 1972; Sanisbury and Sanisbury, 1982 and Rudy, 1989). The disadvantage of formaldehyde hes been recently discovered as carcenogenic, irritant and mutagen agent. These disadvantages diminshed or even completely stopped its use in poultry practice (Albart et al., 1982; Kerns et al., 1983; Blair et al., 1986; Patterson et al., 1990 and Deeming, 1992). Moreover; it has embryotoxic effect in chickens and reduces hatchability when used for long period or at high level (William and Gordon, 1969; Loomis, 1979; Messier, 1984; Badway et al., 1986 and Magras, 1996).

Consequently; the need for another potent disinfectant is highly commendable to replace formalin as a sanitizer in poultry practice. Many investigators tried alkaline or acidic disinfectant com-

pounds for hatching eggs (Patterson et al., 1990; Hafez et al., 1991; Joad, 1992; Scott, et al., 1993;Mela, et al., 1994; Gehan-Zakaria, 1995 and Youseif, et al., 2000).

Poultry bacterial pathogens such as Salmonella typhimurium , Proteus mirabilis and Staphylococcus aureus are the most common important bacterial infections that causes economic losess in chicken embryos. Additionlly ; they are of public health hazard (Harry , 1957 ; Sato et al . , 1961 ; Miura et al . , 1964 ; Williams , 1972; Cohen and Blake , 1977 ; Safwat et al., 1984 ; Youseif , 1985 ; Enany et al., 1989 ; Skeeles , 1991 ; Nagaraja et al . , 1991 ; El- Gharib et al . , 1993 and Lin and Chin- Ling , 1996) .

The present work was planned to evaluate the effeciency of a compound produced by EWABO Co., Germany under the trade name "Aldekol GDA® "as a sanitizer for chicken eggs .

MATERIAL AND METHODS

Speciemens :-

(a) Two hundred and fourty hubbard fertile chicken eggs obtained from a private sector farm were used .

(b) One hundred and thirty hubbard chicken embryos obtained from a commercial poultry company were used .

(c) Nineteen thousand and two-Hundred hatching eggs were used for field trial .

Bacterial strains :-

Salmonella typhimurium , Proteus mirabilis and Staphylococcus aureus were isolated in pure culture from chickens suffering from salpingitis (for the former) and omphalitis (for the other 2 later) respectively . These strains were identified morphologically and biochemically according to Edward and Ewing (1972) and Cruickshank et al . (1975). Serological identification was also adopted for Salmonella typhimurium after Buchanan and Gibbons (1974) .

Disinfectant :-

Aldekol GDA is an alkaline sanitizer , clear , yellowish solution contain an activated glutaraldehyd (243.0 g/L) in combinaton with second generation quaternaries (Didecyldimethylammoniumchloride 22.5 g/L) and inactive ingredients inerts (distilled water ad 1 liter) was used . The product is produced by EWABO Chemikalien GmbH Chem-Pharmazeutische Produkte . KolpingstraBe 4 , D-49835 Wietmarschen Germany .

Experimental design :-

[1] Total bacterial count for hatching eggs :-

Two hundred and fourty hatching eggs were equally divided into 8 groups (1 - 8) consisting of 30 each . Eggs of group 1 and 2 ; 3 and 4 as well as 5 and 6 were dipped for 5 minutes in a chilled 18 hours broth culture containing 5.2 x 10^{12} CFU ; 5.4 x10^{12} CFU and 5.8 x 10^{12} CFU per ml of Sal. typhimurium ; Prot. mirabilis and Staph. aureus respectively . Eggs of groups 7 and 8 remained with out in fection as controls. Eggs of groups 1- 6 were put in incubators at 37°C for 2 hours to dry. Those of groups 1, 3, 5 and 7 were disinfected with 1% Aldekol GDA by spraying for 3 minutes . Eggs of all groups were kept at room temperature for 72 hours with recording the bacterial count at 1 , 2 , 4 , 24 , 48 and 72 hours post disinfection (Ali et al . 1993) .

[2]Disinfection of infected 5-day-old embryonated chicken eggs (ECE) :-

One hundred and thirty , 5-day-old ECE were used . Ten out of them were subjected to bacterial examination which proved to be free from bacterial contaminations . The remaining 120 chicken embryos were divided into 8 equal groups (1 - 8) consisting of 15 each . ECE of groups 1 and 2 ; 3 and 4 as well as 5 and 6 were dipped in a chilled 18 hours culture containing 3 x 10^{8} CFU / ml of Sal. typhimurium , Prot. mirabilis and Staph. aureus respectively for 3 minutes then incubated at 37.8 c . After 6 hours ; the groups 1 , 3 , 5 and 7 were treated with 1% Aldekol GDA by spraying for 3 minutes . Embryos of group 8 remained without treatment as blank control . Embryos of all groups were incubated at 37.8 c in a separate incubator with daily candling for embryonic mortality. Bacterial reisolation was carried out on dead embryos as well as sacrificed newly hatched chicks.

[3] Field trial :-

Nineteen thousand and two-hundred hatching eggs in a commercial chicken hatchery were used in this trial . Eggs were sprayed with a spraying machine before setting in incubator with 1% Aldekol GDA for 5 mintues . Total colony count technique was applied at 0 , 5 , 9 , 17 and 19 days post sanitization . Sample of 300 hatched and unhatched eggs were taken for recording rates of fertility , mortality and hatchability .

RESULTS

The obtained results are showed in tables 1-4 .

Table (1) : Results of bacterial count after spraying of egg-shells with Aldekol GDA.

time / Group No.	Total bacterial count of the outer shell after disinfection							Reduct.
	Zero h	1 h	2 hs	4 hs	24 hs	48 hs	72 hs	%
Sal tyhimurium. + Disinf.	3×10^8	6×10^3	4.1×10^3	2.5×10^3	4.6×10^2	3.8×10^2	2.3×10^2	0.008
Sal. typhimurium.	3×10^8	6.5×10^6	1.7×10^6	2.8×10^5	8.2×10^3	4.2×10^3	1.8×10^2	0.12
Prot. mirabilis.+ Disinf.	3×10^8	1.5×10^3	3.2×10^2	1.3×10^2	6×10	0.9×10	0.7×10	0.0002
Proteus mirabilis.	3×10^8	1.4×10^7	8×10^6	6.2×10^6	4.1×10^5	6.1×10^4	2.6×10^4	0.86
Staph. aureus. + Disif.	1.5×10^8	6×10^2	2×10^2	4.2×10^2	3.6×10^2	2×10^2	1.8×10^2	0.006
Staph. aureus.	1.5×10^8	8.8×10^6	7.9×10^6	5.7×10^6	8.4×10^3	6.9×10^3	4.5×10^3	0.3
Control + Disinf.*	1.5×10^8	1.6×10^4	3.1×10^3	1.2×10^3	5.6×10^2	1.2×10^8	1.9×10^3	0.13
Control blank.*	1.5×10^8	1.4×10^7	6.3×10^6	2.2×10^5	3.1×10^3	1.6×10^3	4.1×10^3	0.27

Observations:- tabal 1 is clearly showing that gradual decrease in the total bectrial count has been accured with the time elepsing after disinfection.

* The isolated bacteria were gram +ve bacilli and yeast

Table (2) : Results of bacterial reisolation from egg- shells, egg membranes and yolk after disinfection .

Gr. No. / Sources of sample	Staph. aureus		Staph. aureus		Staph. aureus		Reduct.	
	Infect.	Disinf.	Infect.	Disinf.	Infect.	Disinf.	Infect.	Disinf.
1- Outer shell washing	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve*	+ ve*
2- Inner shell	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
3- Yolk	-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve

* the isolated bactria were gram + ve bacilli and yeast.

Table (3) : Results of spraying treatment with 1% Aldekol GDA for infected 5- days embryonated chicken eggs .

Group No.	Bacterial infection group	Treated group	No. of embryo	Early death			late death			Total death		Hatched chicks*	
				No.	%	Reisolation	No.	%	Reisolation	No.	%	No.	%
1	Sal. typhimurium	+	15	1	6.7	0/1	2	13.3	1/2	3	20.0	12	80.0
2	Sal. typhimurium	-	15	2	13.3	1/2	4	26.7	2/4	6	40.0	9	60.0
3	Prot.mirabilis	+	15	4	26.7	2/4	-	-	-	4	26.7	11	73.3
4	Prot. mirabilis	-	15	4	26.7	3/4	2	13.3	2/2	6	40.0	9	60.0
5	Staph.aureus	+	15	4	26.7	4/4	-	-	-	4	26.7	11	73.3
6	Staph. aureus	-	15	3	20.0	2/3	-	-	-	3	20.0	12	80.0
7	Control	+	15	1	6.7	-	-	-	-	1	6.7	14	93.3
8	Control	-	15	-	-	-	-	-	-	-	-	15	100.

* The reisolation of infected bacterial pathogenes were 100% .

Table (4) :- Results of field trial with Aldekol GDA :
(4-1) Total colony count :-

No.	Sampling time (days) of egg shell swabs	No. of swab	Average of bacterial colony count	Bacterial isolates
1	0.0 day (before treatmet)	5	Over 100-Over 300	E. coli, gram positive bacilli Yeast and colifom
2	5 days at incubator	5	1-10	Anthracoides
3	9 " "	5	1-10	Yeast
4	17 " "	5	30-100	Gram positive bacilli
5	19 at hatchery	5	1-10	Anthracoides

(4-2) Bacterial examination of representative simple (30) of eggs and chick post hatching :-

Infertile eggs		Dead embryos		Newly hatched chick					
Egg shell	Egg content	Early	Late	Liver		yolk sac		Intestine	
				culls	Ap.heal.*	culls	Ap.heal.*	culls	Ap.heal.*
Negative	Negative	Bacteria-colon (coliform)	E. coli	Negative		Negative		Bact. colon (colifom)	Negative

* Ap. heal. = Apparently healthy

(4 - 3) Hatch breakdown analysis of representative simple (300) of the incubated eggs:-

No.	Items	egg	
		No.	%
1	Infertile eggs	65	21.7
2	Fertile eggs	235	78.3
3	Early embryonic death	5	2.1
4	Late embryonic death	10	4.2
5	Hatching chicks	220	93.7

DISCUSSION

Sanitizers play an important role in the prophylactic measures to prevent the severity of the infection and contamination of hatching eggs. Good hatchery management depends on the standerds of sanitation and sound hygiene. Brake and Sheldon (1990) mentioned that the report of Environmental Protection Agency in USA listed formaldelyde under the Toxic Substances Control due to its disadvantages as carcinogenic effect for human and must be replaced by a safe disinfectant . Holte (1974) reported that formaldehyde caused irritation for eyes and respiratory membranes of employes . Moreover; formaldehyde gas is harmful to the respiratory tract of newly hatched chicks

(Furuta et al. , 1989) and is considered as embryotoxic for chicken embryos (Magras , 1996) .

In the present work the results illustrated in table (1) showed that egg shells infected groups (1 - 6) with Sal. typhimurium , Prot. mirabilis and Staph. aureus that treated with aldekol GDA (groups 1,3 and 5) lowered the shell becterial count within 72 hours post infection from 3×10^8 CFU / ml. (for sal.and prot.) and 1.5×10^8 (for staph.) before treatment to 2.3×10^2 CFU (0.008%), 0.7x10 CFU (0.0002%) and 1.8×10^2 CFU / ml.(0.006%). post treatment respectively as compared with those untreated groups (2,4 and 6) (table 1). While in the control treated group (7) and control untreated group (8) the microbial count at zero hour was 1.5×10^8 CFU / ml. (gram positive bacillus species and yeasts) and it declined within 72 hours into 1.9×10^3 CFU (0.127%) and 4.1×10^3 CFU / ml. (0.27%) for groups 7 and 8 respectively . These results are in agreement with the previous studies by Mela et. al., (1994) who suggested that the quaternary ammonium was affective on Staph. aureus and that obtained by Rubbo et al., (1967) who reported in vitro the rapidly inactivation of Pseudomonas. aerginosa to low concentation with 0.05% glutaraldehyde. Willinghan et. al., (1996) found that enterococcus species had low resistance to glutaraldehyde.

On the other hand , contrary to our results Willinghan et al., (1996) stated that the bacillus species were highly resistance to glutaraldehyde and phenol . Also Bierer et al. (1961) ; Townsend et al ., (1984) and Tennent et al., (1989) who mentioned that Sal.typhimurium and Staph . aureus were resisted to quaternary disinfectant .

Regarding our resulte illustrated in table (3); groups 1 and 3 that were infected with Sal. typhimurium and Prot. mirabilis and sprayed by 1% Aldekol GDA , the rate of hatchability improved and reached 80% and 73.3% respectively as compared with 60% in untreated groups 2 and 4 . These results are in complete accordance with that abtained by Scott et al ., (1993) who found that there was no gross toxic effect on embryo viability treated with glutaraldehyde .

On the other hand, a mild decrease in the rate of hatchability was observed in group (5) that infected with Staph . aureus and treated with aldekol GDA (73.3%) as compared with infected untreated group 6 (80 %) . These could be explained in the view recorded by Townsend , et al., (1985); Lyon and Skurray (1987); Yamamoto et al., (1988) ; Tennent et al ., (1989) and Rouch et al.,(1990) who mentioned that the resistance of Staph. aureus is attributed to plasmid mediated and in some instance linked to resistance to various antibiotics and DNA - binding compounds .

The rate of hatchability in control uninfected groups (7 and 8) were 93.3 % and 100% for groups treated with 1% Aldekal GDA and untreated groups respectively . The slight bad effect on embryonic hatchability might be due to presence of quaternary which causes low early embryonic loss Scott et al.(1993) .

Bacterial reisolation from treated dead - in-shell embryos reached 33.3% , 50% and 100% from Sal. typhimurium , Prot. mirabilis and Staph. aureus groups (1 , 3 and 5) respectively . While in the control infected groups (2 , 4 and 6) reached 50% , 83.3% and 66.6% respectivelly . These results are indicating the decrease in rates of reisolation after using Aldekol GDA disinfectant (group 1-4) . The bacterial reisolation from uninfected control groups (7 and 8) proved to be negative . The bacterial reisolation from sacrificed hatched chicks reached 100% positive in all examined groups (1 - 6) .

Results of the field trial are illustrated in table (4). The rate of hatchability reached 93.7% after using Aldekol GDA for hatching eggs with minimising bacterial conatmination and bacterial counts in both hatching eggs and newly hatched chicks. This results are confirming the above mentioned results . Regarding results presented in table 4, the sanitizing effect of Aldekol GDA was clear on gram negative bacteria while no effect could be recorded on gram positive bacilli and yeast which might be attributed to the ability of gram positive bacilli to sporulate which can resist anti-bacterial chemical agents Jawetz et al . (1978) and Baron (1982) . However , it seems that the yeast can resist Adekekol GDA disinfection .

In conclusion ; taking in consideration the disadvantages of formalin it seems that Aldekol GDA can be used as an alternative , safe and sanitizing compound which can be used for disinfection of the outside of eggs for controlling possibly present bacterial infection or contamination . Moreover ; it improves the rate of hatchability .

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