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EFFECT OF ANTIBIOTIC, DISINFECTANT AND FORMALDEHYDE GAS ON HATCHABILITY OF BROILER EGGS

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ABSTRACT

The present study tested: 1) the biocidal effectiveness of antibiotic, quaternary ammonium disinfectant and formaldehyde gas in disinfecting broiler hatching eggs. 2) effectiveness of packaging eggs in polythene bags to reduce contamination and 3) effectiveness of changing nesting materials in controlling egg shell contamination. Experimental broiler eggs were laid between 8.00 and 9.00 am. From these eggs, samples were collected hourly and disinfected using antibiotic, quaternary ammonium disinfectant and formaldehyde gas. Five egg collections were made. Antibiotic and disinfectant were sprayed on the egg shell using a manual hand sprayer. Bacteriological analysis involved determination of aerobic plate counts. Eggs were transported to the hatchery in two batches. Batch one was packed in polythene bags while batch two was left open. Saleable hatchability significantly increased to 60%, 80% and 75% for antibiotic, disinfectant and formaldehyde gas respectively compared to 6% for control that were not disinfected. There was a reduction in aerobic plate counts of 87%, 99.1% and 91.1% for antibiotic, quaternary ammonium disinfectant and formaldehyde gas respectively. Packaging in polythene bags significantly reduced egg shell recontamination. Changing nesting materials daily did not significantly reduce egg shell contamination when eggs were collected within one hour after lay. Hazard analysis critical control points emerging from this study are; environment where the egg is laid, collection and sanitation time after lay, method of sanitation and mode of conveying eggs from the farm to the hatchery. In conclusion, antibiotics and disinfectants have a lot of potential in sanitising hatching eggs in developing countries.

Key words: Hatching eggs, Sprayed, Disinfected, Packed in polythene bags, Hatchability, Aerobic plate counts

INTRODUCTION

Hatching eggs are disinfected to kill micro-organisms on the surface of the shell. This allows the production of healthy chicks (Futura, 1981). Disinfection using formaldehyde gas is widespread (USDA, 1975; Furuta and Sato, 1977). The study of hatching egg contamination, sanitation and hatchability has been a key issue in poultry development. Several studies have been undertaken in this respect. Furuta and Murayama (1981) evaluated the effect of bacterial contamination on eggs during incubation and hatching, and of fluffs of newly hatched chicks. Kirk *et al.*, 1980, reported on factors affecting the hatchability of eggs from broiler breeders. Brake and Sheldon, did some work on egg sanitation using quaternary ammonium egg sanitizer. These studies have however, concentrated only on general aspects of contamination. Very little attention has been paid to egg contamination aspects between the point of lay and the hatchery with the aim of identifying critical control points relevant to developing countries.

The average hatchability of broiler eggs in Kenya is low (68%) (Kenchic, 1994; ADS/NFU, 1978) despite fumigation using formaldehyde gas. This makes broiler day old chicks more expensive in Kenya than other countries. The present study was designed to; assess the effectiveness of antibiotics, quaternary ammonium disinfectant compared

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to formaldehyde gas in disinfecting broiler hatching eggs, identify critical control points in handling hatching eggs between the point of lay and the hatchery. The hypothesis is that the biocidal effect of antibiotics and disinfectant are more or less the same as formaldehyde gas. H_0: r = 0 against H_A: not all r; = 0. VR is being the test statistic where VR follows F distribution.

MATERIALS AND METHODS

Eggs

Experimental hatching eggs were laid by 37 weeks old commercial duel purpose brown broiler parent hens held at Kenchic Athi River breeding farm. The hens were reared under deep litter. They were fed a standard breeder ration (28 % CP, 2800 Kcal of ME per g). All the eggs were laid between 8.00 and 9.00 am in a nest box with fifteen laying points. The nesting material (wood shavings) was changed before laying started. The box was screened off after the laying period using an aluminium metal sheet. All the eggs were held in the box for one hour after lay (9.00 - 10.00am). Eggs were collected hourly and taken into a washed and disinfected room within the poultry shed. A total of five collections were done.

Dirty eggs with visible stains on the shell were discarded. Remaining nesting material on clean egg shells were gently removed by hand.

Experiments

Surface sanitation of egg shells. In the first experiment, lots of clean eggs were randomly arranged in clean plastic setting trays. The trays were divided into three lots of 36 eggs each. Lot 1 and 2 were sprayed with 10l/l flumisol and 10ml/l quaternary ammonium disinfectant respectively. The spraying solutions were maintained at 35 ^{0}C. The third lot was fumigated with formaldehyde gas. The fumigation was done at the farm next to the poultry shed in a 1m X 1m X 2m concrete chamber for 20 minutes. Formaldehyde was generated by mixing 40 ml formalin (containing 372 g formaldehyde/l) and 20 g potassium permanganate/m^3 room capacity in a small cylindrical metal tin. The chamber had no mechanical exhaustion systems. At the end of the fumigation time, the door was left open for five minutes before the eggs were removed. Flumisol is a synthetic anti-infective antibiotic belonging to the fluoroquinolone group, with a bactericidal activity on Gram negative (*E. coli, Salmonella, Pasteurella, Bordetella*) and some Gram positive (*Staphylococcus*). It is used only in livestock. Spraying was done using a plastic hand sprayer. The disinfectant used (TH4) is a third generation synergistic disinfectant for livestock farms. It is said to have virucidal, mycoplasmicidal, bacterial and fungicidal properties. Spraying solutions were held at 35 ^{0}C in one litre thermos flasks. Water used to mix the chemicals was previously boiled in an electric kettle and allowed to cool to 35 ^{0}C. 35 ^{0}C was chosen to stop water being drawn into the egg through the pores. Spraying at the farm was done in a 1m x 1m x 2m cabin made of 1000 gauge transparent plastic sheet. Eggs were left in the cabin for 40 minutes before samples were drawn for bacteriological analysis. Fumigated eggs were also kept in a similar cabin after fumigation. Surface treatment was done after each collection.

Transporting eggs to the hatchery. Twenty eggs from the treated lots were randomly divided into two lots of ten eggs each. Lot one eggs were individually packed in 9x15, 150 gauge polythene bags and held until the end of the experimentation. The second lot eggs were left in the setting trays. Both lots were transported to the hatchery in an open tractor drawn trailer together with other eggs. The eggs reached the hatchery at 3.00 pm.

Change of nest bedding materials. A laying nest box with fifteen laying points was used. The laying points were divided into two lots of seven each. All nesting materials were removed from the two lots and replaced with fresh clean wood shavings. Birds were allowed to lay eggs randomly in these two lots. The experiment was done for five days. Each day, nesting material was changed in the control lot by scooping off the top dirty layer

Table 1. Hatchability of control and sanitized eggs

Treatment	Hatchability (out of 10 eggs) based on collection time (Hours)					Total hatch analysis			
	1	2	3	4	5	No	%	Cull	%SH
Control	2	2	1	1	0	6	12	3	6
Antibiotic	10	10	7	4	2	33	66	3	60
Disinfectant	10	10	10	10	5	45	90	5	80
Formaldehyde gas	10	8	10	7	7	42	84	5	74

SH : Saleable Hatch

of wood shavings and replacing with clean shavings. Ten eggs were collected at random from each lot for bacteriological analysis within 20 minutes after lay.

Bacteriological analysis. For bacteriological analysis whole egg washing technique (Brake and Sheldon, 1990; Gentryand Quarles, 1972) was used to recover bacteria on the surface of the egg shell. Eggs were aseptically placed in sterile 10x20 250 gauge polythene bags and rinsed with 20 ml 0.1% peptone water. Eggs at this stage were handled with disposable surgical hand gloves. The rinse from each egg was serially diluted in 0.1% peptone water. All counts were reported per egg by multiplying the counts per ml of rinse by 20. Eggs were sampled pre and 40 minutes post surface treatment. Bacteriological analysis done were general viable count (plate count agar, 37 ^{0}C 48 hours), presumptive coliform (Violet red bile, 37 ^{0}C, 18 hours) and yeast and molds (Davis yeast salt agar acidified to a ph of 3.5, 25 ^{0}C, 72 hours).

Incubation at the hatchery. Eggs were set in a setter model Chick Master running at 37.5 ^{0}C with 50-55% RH. The eggs remained in the setter for 19 days. At the end of the 19th day, eggs were transferred to the hatcher model Chick Master running at 36.5 ^{0}C with 65 - 75% RH. Hatching was done on the 21st day.

Unhatched egg analysis. Analysis of unhatched eggs was done using the Chick Embryo Development Chart (Tad Pharmazeutisches werk GMBH D-2190 Cuxhaven P.O Box 720. West Germany).

Statistical analysis

Analysis of variance (ANOVA) statistical method was used to compare the sample means for test of significance on a randomised complete block design (Snedecor, G.W., and Cochran, W. G. (1967); Raghuramulu *et al.* (1983).

RESULTS AND DISCUSSION

Hatchability

Application of 10ml/l flumisol, 10ml/l quaternay ammonium disinfectant and formaldehyde gas significantly increased the saleable hatchability of eggs in the 37 week old flocks to 60%, 80% and 74% respectively compared to 6% for control (Table 1). These results contrast those of Brake and Sheldon, 1990 in which control egg samples hatched between 80% and 90%.

A drop in hatchability was noted in eggs disinfected 3, 5 and 2 hours for antibiotic, disinfectant and formaldehyde gas respectively (Table 1). The drop in hatchability was more gradual in fumigated eggs than in surface sprayed eggs. Hatching eggs should be collected and disinfected within two hours of lay for maximum hatchability.

Egg contamination

Table 2 shows Average number of bacteria (Aerobic plate count) on clean egg shell surfaces. Contamination was in the tune of 10^3 to 10^4 bacterial colonies. Furuta and Murayama, 1980 isolated 10^3 and $10^{4.5}$ bacterial colonies from clean and dirty eggs respectively. Contamination levels increased

Table 2. Aerobic plate count on control samples

Microbiological test	Aerobic plate counts based on sampling time in hours				
	1	2	3	4	5
Viable counts	1202	2100	4000	5760	6080
Presumptive coliform	6160	7200	9600	10408	14080
Yeast and molds	0	0	60	20	0

with time after lay. According to Board (1966) a contamination level between 10^4 and 10^5 is not unusual. Contamination levels however, depend on the material on which the eggs are produced. Carter *et al* (1971, 1973) observed different levels of contamination between eggs produced on litter and those produced on wire floors. In view of the increase in contamination after lay, hatching eggs be disinfected within two hours after lay.

Biocidal effect

Antibiotic, quaternary ammonium disinfectant (disinfectant) and formaldehyde treatments reduced aerobic contamination levels by 87.7%, 99.8% and 91.1% respectively as indicated by viable counts results (Tables 3 and 4). The results agree with the findings of Brake and Sheldon, 1990, in a study using quaternary ammonium sanitizer for hatching eggs. Both researchers achieved a significant reduction of aerobic plate count within 30 minutes of application.

The effects of the treatments on presumptive coliform are summarised in Table 4. Coliform was in general 60-85% of the total aerobic plate count. The counts however, decreased to the levels of 2.6%, 0% and 1.2% after the treatments with antibiotics, quaternary ammonium compound and formaldehyde gas respectively.

The pattern of yeast and molds contamination of egg shell surfaces could not be established from these studies (Tables 2). Yeast and old are not major causes of egg spoilage (Frazier and Westhoff, 1988).

Packaging in polythene bags

Packaging in polythene bags reduced recontamination of egg shells after sanitation. Shells of unpacked eggs were - thus heavily re-contaminated after sanitation (Table 5). The levels of re-contamination were higher with both antibiotic and disinfectant compared to formaldehyde gas (Table 5). This is due to wetness of the surface due to spraying making dust stick on the egg shell. Bacterial analysis results on packed samples taken on arrival at the hatchery show lower levels of bacterial contamination compared to level 40 minutes after disinfection (Table 5). This indicates that residual antibacterial effect of the treatments continued in packed samples. This is another advantage of packaging hatching eggs in polythene bags as it not only stops re-contamination, but also creates a micro-environment in which

Table 3. Biocidal effect of sanitation on viable counts

Treatment	Reduction in viable counts per sampling time				
	1	2	3	4	5
Control	1202	2080	4000	5760	6080
Antibiotic	70 93.8%	75 96.4%	200 95%	802 86.1%	1200 80.4%
Disinfectant	0 100%	0 100%	0 100%	0 100%	60 99.6%
Formaldehyde gas	66 94.6%	80 96.2%	150 96.3%	591 97.4%	800 86.8%

Table 4. Biocidal effect of sanitation on coliform counts

Treatment	Reduction in coliform counts per sampling time				
	1	2	3	4	5
Control	6160	7200	9600	10400	14080
Antibiotic	0	0	0	0	60
	100%	100%	100%	100%	99.6%
Disinfectant	0	0	0	0	0
	100%	100%	100%	100%	100%
Formaldehyde gas	0	0	0	10	10
	100%	100%	100%	99.9%	99.9%

bactericidal activity continues. Hatching eggs should therefore be packed in polythene bags while on transit form the farm to the hatchery.

Change of nesting material (Wood shaving)

Table 6 shows that the levels of bacterial contamination increase when the nesting material is not changed. The level of contamination is however, lower than eggs left longer before collection and disinfection. This further emphasizes the need to collect and disinfect hatching eggs immediately after lay. This observation disagrees with the findings of Kirk *et al.*, 1980 who indicated that collecting eggs hourly rather than after five hours after lay reduces hatchability.

CONCLUSION

The following conclusions can be drawn from this study:

1. Hatching eggs should be collected and disinfected hourly after lay.
2. Leaving hatching eggs uncollected in the nest boxes leads to increase in bacterial contamination resulting in low hatchability.
3. Hatching eggs be packed in polythene bags before transporting to the hatchery from the farms.
4. Where it is not possible to change nest materials ones per week, hatching eggs be collected and disinfected hourly.
5. Quaternary ammonium disinfectants can be used to disinfect eggs instead of formaldehyde gas.
6. Use of antibiotics in disinfecting hatching eggs requires further investigations.
7. Four key hazard analysis critical control points have emerged from this study.

Table 5. Changes in aerobic viable counts due to packaging

Treatment	Viable counts per sampling time				
	1	2	3	4	5
Control	1202	2100	4000	5760	6080
Antibiotic	70	75	200	802	1200
Open	*3600	*1600	*1100	*100	*60
Packed	10	30	35	60	80
Disinfectant	0	0	0	20	20
Open	*1200	*800	*800	*100	*110
Packed	10	0	0	20	80
Formaldehyde gas	60	80	150	600	800
Open	*800	*400	*260	*388	*400
Packed	10	40	80	70	30

Table 6. Effect of changing nest materials anaerobic plate count

Treatment	Aerobic plate count per sampling time in days				
	1	2	3	4	5
Viable counts	*1080	*870	*960	*1100	*1030
	1080	1120	1144	1357	1560
Presumptive coliform	*5800	*4870	*4620	*6000	*5860
	5800	6680	9800	11200	12600
Yeast and old	*0	*10	*5	*0	*0
	0	5	0	20	36

Key: * = Control

These are:

a. Hatching egg collection time.
b. Hatching egg disinfection time.
Packaging hatching eggs in polythene bags during transportation.
d. Training and capacity building of personnel handling hatching eggs.

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