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THE EFFECTS OF EGG WASHING SOLUTIONS ON HATCHABILITY AND BACTERIAL CONTENT OF INCUBATED DUCK EGGS

By

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ABSTRACT

THE EFFECTS OF EGG WASHING SOLUTIONS ON HATCHABILITY AND BACTERIAL CONTENT OF INCUBATED DUCK EGGS

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White Pekin Duck hatching eggs from three Michigan commerical breeder farms were used in this study. Eggs were randomly divided into four groups and were dipped into one of three commercially available egg washing products (Amway; Bioguard; Roccal II) or warm water, prior to incubation. Hatchability varied from 79% to 84%. The differences were not statistically significant ($p>0.05$). At ten days of age, the weight gain of ducklings hatched from eggs dipped in Roccal II solution was significantly ($p<0.01$) greater than the weight gain of ducklings hatched from the other treatment groups.

Periodic bacterial isolation from dead duck embryos during incubation indicated that Escherichia coli, Streptococcus and Proteus species were the principal contaminants. These isolates did not show a direct relationship to hatchability. There was however, both an increase in Proteus species isolation and a significant ($p<0.01$) increase in embryonic mortality near the end of

incubation. This result suggests the possibility that *Proteus* species might have influenced embryonic mortality.

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INTRODUCTION

Poor hatchability of fertile duck eggs is a problem for several large commercial hatcheries. An increase of as little as 1% in hatchability would represent a difference of many dollars to these hatcheries. It is therefore important to study factors that can affect hatchability, including genetics, egg shell condition, egg size, environmental conditions, and egg shell contamination. Changing any of the environmental conditions in a commercial duck hatchery, such as egg washing solutions or washing techniques, may help to improve the rate of hatchability.

Eggs which rot during storage have been the subject of many investigations (Brooks and Taylor, 1955; Board, 1965; Board and Board, 1968; Board, 1969; Seviour and Board, 1972), while incubated chicken eggs (Harry, 1963; Hodgetts, et al., 1976; Bruce and Johnson, 1978; Furuta and Maruyama, 1981; Hacking, 1982) and duck eggs (Seviour et al., 1972; Moudgal et al., 1976; Joyce and Chaplin, 1978) seem to attract little attention.

Microbiological studies have shown that Gram-negative bacteria are the principal contaminants of rotten eggs. Examples include Pseudomonas species from rotten table eggs (Board and Board, 1968; Board, 1969) and Escherichia coli

from duck eggs that became rotten during incubation (Seviour et al., 1972). However, Gram-positive organisms have been reported as the predominant contaminants of unhatched chicken eggs (Bruce and Johnson, 1978).

In a study on the effects of washing hatching eggs, it was reported that unwashed dirty eggs have a higher surface bacterial count (Brooks and Taylor, 1955) and lower hatchability than clean eggs (Furuta and Watanabe, 1978; Joyce and Chaplin, 1978). However, Bruce and Johnson (1978) failed to prove a direct relationship between any specific, isolated, microorganism and hatchability. Furuta and Sato (1977) reported that dipping eggs in disinfectant solutions, such as phenolic derivatives or iodophore, reduced bacterial counts on the eggs, but did not increase hatchability. It was also demonstrated that the hatchability of the disinfected eggs was better when the eggs were dipped for a shorter period of time (4 minutes) as compared to those eggs which were dipped for a longer period of time (8 minutes) (Furuta and Watanabe, 1978).

Improving farm and hatchery sanitation programs have resulted in both increased hatchability (Hodgetts et al., 1976; Joyce and Chaplin, 1978; Hacking, 1982) and higher quality chicks and ducklings (Chute and Gershman, 1961; Gentry et al., 1962; Hodgetts et al., 1976; Joyce and Chaplin, 1978; Shane, 1981).

Based on the assumption that a good sanitizer will reduce bacterial contamination on eggs and indirectly

increase hatchability, commercially available egg washing products were compared and bacterial contents of incubated duck-eggs were identified in this experiment. The objectives of this experiment were to (1) determine the effects of washing duck eggs prior to incubation in three different detergents and/or detergent sanitizers, on both hatchability and the early performance of the hatched ducklings; (2) identify bacteria which were isolated from the contents of the duck embryos that died during incubation; and (3) seek answers in regard to the relationship between bacterial content of duck eggs and hatchability.

REVIEW OF LITERATURE

I. Egg Defense

The egg can be viewed as an ecosystem with a need only for exchange of respiratory gases, a source of heat, and regular movement by turning (Board, 1969). The egg structure is a complex physiochemical system in which enzyme-mediated energy transfer and chemical transformation to the cells of the blastoderm takes place (Brooks and Taylor, 1955).

The shell provides the prebrooding and prenatal stages with protection against damage by impact or crushing, as well as allowing the exchange of respiratory gases. The shell is perforated with 7.17×10^3 pores having an average diameter of 9-35 μ (Board, 1969). Although the pore size is large enough to allow the passage of microbial organisms, both the shell membrane and cuticle help to act as a barrier against this invasion.

The structure of the yolk and white are important in the egg's defense. The albumen can be viewed as a medium that is unsuitable for microbial growth, since it contains the following:

1. Lysozyme, with its lysis action, destroys bacterial cell walls by causing flocculation of the bacterial cells and the hydrolysis of 1-4 β glycosidic bond (Board, 1969).

2. Conalbumen (ovotransferrin) chelates iron, copper, and zinc and inhibits microbial growth in the presence of low hydrogen ion concentration (Board, 1969).

The viscosity of the albumen and the gelatinous nature of the albuminous sac are important components of the egg's defense, since actual rotting does not occur until organisms reach the yolk (Board, 1969). For this reason, it seems that regular turning of the eggs during incubation maintains tension in the chalazae, thereby ensuring that the yolk is retained in a central position.

II. Egg Microbiology

A. Egg Microbial Content

Brooks and Taylor (1955) and Board (1968) have concluded that microorganisms are absent from the majority of eggs laid by healthy hens. Even when contamination does occur, the type of organisms in the egg at oviposition differ markedly from those in rotten eggs at or those which failed to hatch (Harry, 1963).

B. Infection

1. Microorganism Strength

The structure of the cell wall and the cytoplasmic membrane of the eubacterial cell is considered to have a role similar to that of the egg shell (Board, 1969). It

gives the cell a characteristic shape and protection against damage that could ensue from sudden changes in osmotic pressure. The outer layers of the cell wall of Gram-negative bacteria provide a permiability barrier to egg lysozyme. Different organisms showed different degrees of resistance to egg conalbumen, i.e. Micrococcus is more sensitive to conalbumen than Bacillus species (spp.) and the latter are more sensitive than Gram-negative bacteria (Board, 1969).

2. Characteristics of Rot-producing Organisms

Rot-producing organisms are characterized by one or more of the following (Board, 1969):

a. Production of pigment, e.g. Pseudomonas fluorescent caused "pink-rot", and Pseudomonas putida produced "fluorescent green-rot" (Board and Board, 1968);

b. Digestion of protein with or without the production of hydrogen sulfide, e.g., Aromonas and Proteus spp. have been isolated from rotten eggs and they produced "black-rot" type I and II, respectively. Enterobacter with its proteolytic activity caused "custard-rot" in-vitro (Board and Board, 1968).

c. An ability to attack lecithin.

Since Gram-positive bacteria such as Bacillus, Micrococcus and Streptococcus failed to change the appearance of the yolk or white, when inoculated in fresh eggs (Board and Board, 1968), they are not considered rot-producing organisms.

3. Dominant Microorganisms

Gram-negative bacteria were the principal contaminants of rotten eggs from commercial duck hatcheries, incubated eggs (Seviour et al., 1972) and from hen eggs which have been designated for human consumption (Board and Board, 1968; Board, 1969). They were identified as *Pseudomonas spp.* from the rotten table eggs, and as *Escherichia coli* from the rotten incubated duck eggs. However, Gram-positive bacteria were the principal contaminants of unhatched chicken eggs, and they were identified as *Micrococcus spp.* (Bruce and Johnson, 1978).

C. Factors Affecting Contamination

1. Egg Strength

Since the egg shell membrane imposes a barrier to microbial movement following invasion of the shell, the infection remains confined to the shell membrane for up to 15-20 days (Board, 1969).

2. Organism Strength

The dominance of Gram-negative bacteria in rotten eggs might be due to their simple nutritional requirements and their ability to grow in the presence of conalbumen (Seviour et al., 1972).

3. Temperature

The dominance of certain microorganisms occurs while they are retained in the shell membrane. Temperature is an important factor for their selected growth. Thus,

Pseudomonas and related organisms were dominant in eggs held at room temperature, while Coliforms were predominantly found in eggs held at 37°C (Seviour and Board, 1972).

It has been reported that eggs laid on the floor tend to cool rapidly (The Shaver Technical Bulletin, 1982), as do eggs that are dipped in a cold bacterial suspension (Board, 1969). This causes the liquid contents of the egg to contract, thus aiding in the penetration of organisms. For this reason, floor-laid eggs should not be used for hatching (Joyce and Chaplin, 1978), and hatching eggs should be washed in a solution warmer than the temperature of the eggs (Moats, 1978).

4. Flock-age

It was noted by Bruce and Johnson (1978) that there was a significant increase in the incidence of contamination of incubated hens eggs as the flock aged. The Shaver Technical Bulletin (1982) further explained that when breeder flocks are young, the nest litter is relatively clean, and eggs have strong, thick shells, so bacteria have difficulty penetrating. However, in the older breeder flock, there is a build up of droppings in the litter, and the flock lays larger eggs with thinner egg shells. Thus, organisms can penetrate the shells more rapidly.

D. Source of Egg Contamination

The Shaver Technical Bulletin (1982) reported that eggs from healthy birds, when laid, were clean and free of

organisms. However, the egg is an ideal host for organisms because it is warm and moist. The egg shell can be infected when passing through the hen's vent by feces or by contact with other dirty surfaces (Board, 1969). Therefore, poor hygiene in the nesting areas provided ample opportunity for the shell to acquire organisms of fecal origin (Seviour et al., 1972). It is believed that Coliforms are the dominant contaminating organisms during the egg incubation. However, Burce and Johnson (1978) reported that Enterobacteriaceae and Streptococcus spp. were isolated more frequently. It is commonly agreed that the organisms on the shells originated from a common source, namely feces, and that the bacteria, ever present in the pens, were carried by the hen's feet and feathers to contaminate nest litter and eggs.

III. Egg Washing

A. Washing

Although washing eggs was widely condemned because it resulted in increasing spoilage losses during long term storage (Board, 1969), it is now a common practice and is required in plants operating under Federal Grading Services (Moats, 1978). However, the importance of washing eggs in water warmer than the eggs to reduce bacterial contamination is well established (Moats, 1978). Furuta and Maruyama (1981) showed that unwashed dirty eggs from floor pens have significantly higher bacterial counts than do clean eggs.

When the dirty eggs were washed with running tap water at 40°C, a significant decrease in the viable bacterial count was observed.

B. Fumigation

Since washing hatching eggs may introduce microorganisms into the eggs, it is not always recommended in the poultry hatching egg industry. Instead, almost all hatching eggs are fumigated with formaldehyde (Furuta and Sato, 1977; Nesheim et al., 1979). Furuta and Maruyama (1981), using the agar plate culture method, recovered no bacteria from clean eggs after fumigation with formaldehyde. However, they isolated a small number of bacteria from three out of five dirty eggs even though they were fumigated. Recent laboratory tests at the Shaver Company (1982) illustrated that many antibiotics and most disinfectants are not effective against Pseudomonas spp. but when formalin was used as an antimicrobial agent, it proved to be most effective.

C. Disinfection

Treating the eubacterial cell with an alkaline solution such as Ethylenediaminetetraacetic acid will disrupt the surface architecture of the cell wall and increase the sensitivity of the bacterium to the egg's lysozyme (Board, 1969). Many investigations have been concerned with the disinfection of egg shells. Furuta and Sato (1977) found that disinfectant solutions, such as phenolic derivatives or

iodophore, have a complete bacteriocidal effect on bacteria in broth culture within two minutes. However, organisms still survived on egg shells even after the eggs were dipped in the same solutions for eight minutes. Therefore, they concluded that the efectiveness of a disinfectant solution on bacterially contaminated egg shells could not be assessed by its effect on bacteria in culture broth. This conclusion agreed with the result reported by Moats (1978), who believed that treatment of hen eggs with a sanitizing chemical does not necessarily destroy bacteria that are embedded in the egg shells.

D. Methods of Disinfection

According to Furuta and Sato (1977), the results of using disinfectant on contaminated egg shells applied by spraying were not satisfactory. A better degree of disinfection was observed when shell contaminated eggs were dipped in the disinfectant solution. However, a complete disinfection of the egg shells was not achieved by either spraying or dipping methods.

IV. Farm and Hatchery Hygiene

Improvement of sanitary measures, both on the farms and at the hatchery, together with improvement of egg handling before and during incubation has led to an overall substantial increase of 10% in hatchability and also an improvement in chick quality (Hodgetts, et al.,1976). Joyce and Chaplin (1978) showed that clean duck-eggs, collected

from nest boxes, have an approximately 20% higher hatchability than floor eggs. First quality ducklings, produced from clean nest eggs, were found to be 6.2% heavier than those from dirty eggs. Therefore, the importance of a good sanitation program in both the poultry house and hatchery in achieving a high level of hatchability (Hodgetts et al., 1976; Joyce and Chaplin, 1978; Hacking, 1982) and ensuring the production of high quality chicks (Chute and Gershman, 1961; Gentry et al., 1962; Hodgetts et al., 1976; Shane, 1981), and ducklings (Joyce and Chapplin, 1978) has been firmly established.

Poultrymen may also aid egg shell bacterial penetration by damaging the cuticle which covers the shell pores, by rough handling, wet litter, improper washing, improper dipping or by using an improper dry-cleaning practice. The shells might be damaged during collection, shipping, cleaning or traying (The Shaver Technical Bulletin, 1982). Thus, no aspect of cleanliness or proper handling should be ignored.

V. Hatchability

Hatchability can be affected by many factors such as fertility, storage conditions (Moudgal et al., 1976), egg size, nutrition of the dam, conditions of the egg shell, genetic constitution of the embryo, incubation temperature, humidity, gaseous environment, and disease (Nesheim et al., 1979).

A. Relation of Washing and Hatchability

Furuta and Watanabe (1978) studied the hatchability of chicken eggs disinfected by formaldehyde or certain other disinfectants. Formaldehyde, phenolic derivatives, iodophore and soap were used to wash eggs by dipping for variable lengths or time (4, 6 and 8 minutes). Hatchability rates were not significantly different among those disinfectant groups. However, hatchability rate varied according to the length of dipping time. The hatchability was lower when eggs were dipped in the disinfectant solution for 6 or 8 minutes, as compared to the hatchability of eggs dipped in the disinfectant for 4 minutes.

B. Relation of Contamination and Hatchability

It has been anticipated that as the level of contamination increased, the level of hatchability decreased. Bruce and Johnson (1978) found that the relationship between egg contamination and hatchability was not clear. However, there was a reduction in hatchability where multiple contamination occured and as the flock aged. Likewise, Joyce and Chaplin (1978) showed that dirty floor eggs resulted in significantly lower hatchability than clean floor eggs, which in turn were significantly lower than the hatchability of nest eggs. Furthermore, the presence of Pseudomonas spp. showed a significant correlation with decreased hatchability (Bruce and Johnson, 1978). Pseudomonas organisms were believed to be the major

cause of the exploding egg problem that occurs in all hatcheries from time to time (Shaver Technical Bulletin, 1982).

According to Furuta and Watanabe (1978), hatchability of fumigated eggs obtained from a Specific Pathogen Free (SPF) chicken flock was significantly higher than that obtained from eggs from a commercial chicken flock. In another investigation, Furuta and Maruyama (1981) used clean fumigated SPF eggs for hatching and found that, at the end of hatching, eggs which contained dead embryos were highly contaminated. These results indicated thst even when they used fumigated SPF eggs for hatching, contaminating bacteria were still present.

MATERIALS AND METHODS

I. Hatchability of Duck Eggs

Source of Eggs

A total of 1440 White Pekin duck eggs were acquired from three commercial breeder flocks in Michigan. Since ducks habitually lay their eggs in the early hours of the morning (Joyce and Chaplin, 1978), 480 of these eggs were collected early on February 15, 1983 from each farm. They were randomly divided into four equal groups. Each group consisted of 120 eggs from each farm, for a total of 360 eggs per group.

Egg Washing Products

Each group of eggs were dipped in one of the commercially available egg washing solutions. Each product was used according to the manufacturer's directions as follows:

1. LOC organic cleaner, an Amway[1] product which contains no phosphorus compound, was used at a concentration of 57.8 millilitres (ml) in 68.3 litres (l.) of water. Eggs were dipped in this solution at 42°C for 2 minutes (min).

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[1]Amway Corporation, Ada, Michigan 49355

2. HEDS detergent sanitizer[2], a Bio-Lab product which contains Sodium carbonate 29%; Sodium metasilicate 25%; Alkyle (C_{14} 90%, C_{12} 5%, C_{16} 5%) dimethyl benzyl ammonium chloride 5.96%; Alkyle (C_{14} 90%, C_{12} 5%, C_{16} 5%) dimethyl ethyl ammonium bromide 0.96%; Ethylenediaminetetraacetic acid, tetra sodium salt 0.38%; Alkenyle (C_{18} 75%, C_{16} 25%) dimethyl ammonium acetate 0.19%;Bis (2 hydroxyethyl) alkyle (as in fatty acids of coconut oil) ammonium acetate 0.19%; Dodecyl benzyl alkyle (C_{12} 70%, C_{14} 30%) and dimethyl ammonium chloride 0.19%. It was used at a concentration of 28.4 grams (gr.) in 13.7 l. of water. Eggs were dipped in this solution at 42-45°C for 3 min.

3. Roccal II 10% sanitizer and germicidal, a Lehn and Fink[3] product which contains alkyl (C_{14} 50%, C_{12} 40%, C_{16} 10%) dimethyl benzyl ammonium chloride 10%, ethylalcohol 1.26% and water 88.73%. It was used at a concentration of 7.1 ml/4.6 l. of water. Eggs were dipped in this solution at 42°C for approximately 30 seconds.

Egg Handling Procedures

1. Before Incubation

The eggs were handled with care and clean hands. Soiled and cracked eggs were discarded. Equipment such as

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[2]Bio-Lab, Inc., P.O. Box 1489, Decatur, Georgia 30031

[3]National Laboratories, Lehn & Fink Industrial Products Division of Sterling Drug, Inc., Montvale, New Jersy 07645

the egg setting and hatching trays, incubators, and washing containers, was cleaned, disinfected and/or fumigated with formaldehyde prior to use.

Eggs from the three different farms were washed in separate containers. The egg dipping solutions were prepared by dissolving the recommended amount (which was 19 ml. LOC, 48 gr. HEDS, or 36 ml. Roccal II) in 22.8 l. of tap water. The first group of eggs was dipped into tap water, without adding any chemical at 42°C, as a negative control. The second group of eggs was dipped into LOC solution, as a positive control. The third group was dipped into HEDS solution. The fourth group was dipped into Roccal II 10% which has been used in some research laboratories. After dipping, a sample from each dip solution was collected for bacteriological examination.

2. Incubation

The eggs were air-dried and set, with their blunt ends up, on the egg trays and placed in incubators. Four incubators (Jamesway model 252) were used for the four groups of washed eggs. Eggs from each farm were set on two trays for replicas of 60 eggs each. Each tray was marked with the date, the wash product, the source of eggs (Farm A, B or C), and the replica number (1 or 2).

This experiment is a factorial experiment with two factors and balanced data. The first factor was the wash solution (Fixed) and the second factor was the source of duck eggs (Random). The experiment consisted of 12

wash treatment combinations with two replicas each. The experimental design can be summarized as follows:

Summary of the Experimental Design

Farm	Replica	No. of Eggs--Treatment Group				Total Eggs
		Water	LOC	HEDS	Roccal II	
A	1	60	60	60	60	
	2	60	60	60	60	480
B	1	60	60	60	60	
	2	60	60	60	60	480
C	1	60	60	60	60	
	2	60	60	60	60	480
Total Eggs	6 Replicas	360	360	360	360	1440

The incubators were checked for temperature, humidity and ventillation before incubation began and repeatedly throughout the incubation period. They were adjusted as scheduled by Jamesway manufacturing company for incubating duck eggs as follows:

1. from the 1st to the 3rd day, the temperature (temp) was 37.6° centigrade (C), relative humidity (RH) was 39%, and the exhaust vent (exh) was closed;

2. from the 3rd to the 16th day, RH was 24% to 17%, respectively, temp. was 37.5°C, and exh. vent was opened to 0.6 centimeter (cm.);

3. from the 16th to 23rd day (transfer to hatcher), RH was 23% to 13%, respectively, temp. was 37.2°C, and exh. vent was opened 0.6 cm.;

4. from the 23rd to 28th day hatching, RH was 38% to 55%, respectively, temp. was 36.8°C, and the exh. vent was opened 1.3 cm.

5. relative humidity was allowed to increase from 38% to 55% as hatching progressed and the exhaust was fully opened to control RH and temp.

3. <u>During Incubation</u>

During incubation, all the eggs were fumigated with Russel Incubator Fumigant[4], 12 ml. per incubator. The eggs were also sprayed with Bioshield 902 solution (a Bio-Lab product) at a concentration of 30 ml. Bioshield per 4.6 l. of water according to a commercially recomended procedure. The schedule for fumigation, spraying and transferring the duck eggs during the incubation period is summarized as follows:

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[4]L.D. Russel Co., Kansas City, Missouri 64141

Hatching Schedule as Follwed by a Commercial Hatchery

Serial Number	Date	Instruction
Set	2-16	Set eggs, fumigate (12 ml. Russell)
1	2-17	Spray eggs with Bioshield 90% (30 ml./4.6 l.)
2	2-18	Spray " " " "
3	2-19	Spray " " " "
4	2-20	Spray " " " "
5	2-21	
6	2-22	
7	2-23	Spray " " " "
8	2-24	Spray and Candle " "
9	2-25	Spray " "
10	2-26	Spray " "
11	2-27	
12	2-28	
13	3-1	
14	3-2	Fumigate (12 ml. Russell)
15	3-3	Spray eggs with Bioshield 902
16	3-4	Spray " " " "
17	3-5	Spray " " " "
18	3-6	Spray " " " "
19	3-7	
20	3-8	
21	3-9	Spray " " " "
22	3-10	Spray " " " "
23	3-11	Transfer and Candle
24	3-12	
25	3-13	
26	3-14	Fumigate (4 ml. Russell)
27	3-15	
28	3-16	
29	3-17	Hatch

The duck eggs were candled during incubation three times as follows:

1. at the 8th day of incubation to remove infertile eggs, which showed no embryonic development, and the early dead embryos.
2. at the 23rd day of incubation (transfer day) to remove any spoiled eggs and late dead embryos.
3. at the 29th day of incubation (hatching day) to remove pipped eggs and unhatched eggs.

Early and late dead embryos, and unhatched duck-eggs were kept for bacteriological examination in part II of this experiment. Infertile eggs were discarded and recorded. Pipped eggs (cracked with live or dead embryos) were not used in bacteriological examination because of external bacterial contamination (Bruce and Johnson, 1978). Pipped eggs with live or dead embryos were recorded as unhatched. Pipped eggs with dead embryos were considered as embryonic mortality.

4. <u>After Incubation</u>

Newly hatched ducklings were wingbanded and raised for ten days on a commercially available duckling diet (Purina). Ducklilngs from each group and farm were placed in separate sections of a battery brooder (Petersime). Body weights of individual ducklings were recorded on the first and tenth days.

Results of hatchability, embryonic mortality during incubation, and the weight gain of ducklings were subjected

to statistical analysis (Gill, 1978). The variances were analyzed as in two-factor models with balanced data. Fisher's variance ratio (F-distribution) was applied to test the variation among treatments or farms. Dunnett's test method was followed to compare the mean of each experimental group with the mean of the control group (LOC group).

II. Bacteriological Examination

Sampling Procedures

Wash solution samples were collected from all twelve wash treatments in 12 sterilized half-gallon bottles and were labeled accordingly. These samples were subjected to bacteriological examinations immediately after washing the eggs. All organisms isolated were identified and recorded.

Early and late dead embryos, and unhatched eggs found during the incubation were examined for bacterial contents. Cracked eggs were excluded from the samples to minimize the incidence of external contamination (Bruce and Johnson, 1978). All dead embryos from each treatment combination (e.g. LOC treatment X farm B) were pooled as one sample for a total of 12 samples each time the eggs were candled.

All eggs were swabbed with alcohol and iodine solution and allowed to dry, then cracked at the equator with a sharp sterile knife. The egg contents including embryos at all stages of embryonic development, were poured into a sterile blender jar, mixed and ground at a high speed as suggested by Hitchner et al. (1980).

Culture Media and Biochemical Tests

Different kinds of media and biochemical tests were used in isolation and identification of bacteria from the wash solutions and egg samples. The media, the biochemical test and the purpose of using it (Schoenhard, 1979; Carter, 1978) are summarized as follows:

1. Brain heart infusion agar (BHI):

This is a general purpose medium for the growth of fastidious bacteria.

2. Brain heart infusion broth (BHI broth):

A useful general purpose broth.

3. Selenite broth (SB):

This is an enrichment medium for the isolation of Salmonella and Shigella. Proteus and Pseudomonas aeruginosa are not completely inhibited.

4. Ethelyne violet azide broth (EVA):

This is a selective medium for the isolation of Streptococcus spp.. Gram-negative organisms are usually inhibited by this media.

5. Brilliant green agar (BG):

This is a highly selective medium recommended for the isolation of Salmonellae directly from feces. Salmonella colonies appear as slightly pink-white, opaque colonies surrounded by a brilliant red zone. Lactose or sucrose fermenting organisms produce colonies that are yellow-green and surrounded by an intensive yellowish green zone.

6. Eosin methylene blue agar (EMB):

This differential medium is used for direct plating and also subculturing from Selenite and Tetrathionate broths. Colonies have the following apearances: E. Coli, metalic sheen; Aerobacter and Klebsiella, brownish; Salmonella and Shigella, transparent, amber to colorless.

7. Enterococcus agar (EA):

This is a modified selective medium for the isolation of Streptococci which appear as pink or colorless pinpoint colonies.

8. Pseudosal agar (PA) or Centrimide agar:

This is a selective medium for the isolation of Pseudomonas. The medium favors the production of pyocyanin and fluorescin pigments.

9. Gram staining (Gr):

On a clean greaseless glass slide, a smear of bacteria in a drop of distilled water was prepared, air-dried and heat fixed. It was covered with crystal violet for one minute, then washed with tap water. It was then flooded with Gram's iodine for 2 minutes and again washed. It was decolorized with acetone alcohol for a second then washed, and counterstained with Safranin for one minute. It was finally dried with bibulous paper. This slide was checked under the microscope. The shape and grouping of the organism was recorded as coccus (c) or bacillus (b) single, in groups or chains. Its color was recorded as purple for Gram-positive (G+) or pink for Gram-negative (G-).

10. MacConkey agar (Mac):

Lactose fermenting enteric bacteria produce red colonies while non-lactose fermenters do not. Included among those producing pale colorless colonies are Salmonella, Proteus spp., and Alcaligenes fecalis. Some organisms cannot grow on MacConkey agar.

11. Fermentation test (with the production of acid and gas):

Five kinds of carbohydrate (CHO) media, glucose (gluc), lactose (lact), maltose (malt), manitol (manit), and saccharose (sacc), were prepared in Durham fermentation tubes containing phenol red as an indicator. The tubes were inoculated with the suspected colony, and incubated at 37°C for 24 hours. CHO fermentation patterns were identified when the media turned yellow due to the presence of acid end products. Aerogenic organisms were identified when gas was trapped in the inverted Durham tube.

12. Catalase test (Cata) (for the production of peroxidase):

On a clean glass slide, a small amount of the suspected colony was mixed with two drops of hydrogen peroxide. A positive test was indicated by the production of oxygen gas.

13. Oxidase test (Oxid) (for the production of cytochrome oxidase):

Two drops of 0.5% N, N, N', N' - tetra dimethyl aniline monohydrochloride were added to part of the pure plate culture. The positive colonies produced a dark purple color.

14. Indole test (Ind) (indole is a product of tryptophane hydrolysis):

One ml. of chloroform was added to the BHI broth culture, then a few drops of Kovac's reagent were layered on top. A red line of precipitate was formed in the chloroform layer if the culture was positive for indole production.

15. Methyl red test (MR) (indicates acidic condition):

A few drops of methyl red indicator were added to the suspected MR-VP broth culture which had been incubated at 37°C for 24 hours. A positive reaction was indicated by a distinct red color while a yellow color indicated a negative reaction.

16. Vogus-Proskauer test (VP) (to test for 2, 3 butanediol):

A few drops of Barritt's reagent solution A and solution B were added to the suspected MR-VP broth culture which had been incubated for 24 hours at 37°C. The tube was vigorously agitated for 30 seconds and allowed to stand for 2-24 hours. A positive reaction was indicated by the development of a pink color.

17. Citrate test (Cit) (for utilization of citrate):

Simons citrate agar slant was inoculated with the suspected colony. The slant was streaked and the butt stabbed. Then, it was incubated at 37°C for 24 hours. A blue color with colony growth indicated an alkaline pH, due to the utilization of citrate as the sole source of carbon.

18. Nitrite Test (Nit) (to test nitrate reduction):

The inoculated nitrate broth culture, incubated at 37°C for 24 hours, was tested for nitrite by adding three drops if nitrite reagents solutions A and B, respectively. A cherry red color was developed immediately if nitrite was present. If the test was negative, nitrate was either not reduced or reduced beyond nitrite. With a toothpick a few grains of zinc dust were added to the tube indicating negative results. If there was no change, then nitrate was reduced beyond nitrite (positive); if there was a change to cherry red, then nitrate was not reduced by the organism (negative).

19. Hydrogen Sulphide test (H_2S) (for the production of H_2S):

Triple sugar iron agar slants (TSI) were inoculated with the suspected colonies. The slant was streaked and the butt stabbed, then it was incubated for 24 hours at 37°C. A black color in the slant indicated the productin of H_2S from cysteine.

20. Motility test (Mot):

Tubes of SIM medium were inoculated with a straight stab, to a depth of about two inches. Motility was indicated by diffuse growth producing turbidity throughout the medium after 24 hours of incubation at 37°C.

21. Urea hydrolysis test (for the production of urease):

A tube of filtered urea broth was inoculated with the suspected colony and incubated at 37°C for 24 hours. A

cerise red color indicated an alkaline pH due to the production of ammonia from the hydrolysis of urea.

22. Oxidation fermentation test (O-F):

This test demonstrated whether the breakdown of sugars was by oxidation or fermentation. Two tubes of Hugh and Leifson's O-F medium were heated for five minutes in boiling water prior to inoculation. One of the tubes was covered after inoculation with "Vaspar" seal (one part petrolatum and one part paraffin). Those bacteria that oxidize show acid production in the open tube only, while those that ferment produce acid in both tubes.

23. Spore stain:

A smear was fixed and flooded with malachite green (5%); steamed gently over a flame for 30 seconds; washed with water and stained with safranin for 30 seconds; washed with water, dried and examined for the presence of green endospore inside of and free of red vegatative cell.

Isolation and Identification

The microbiological work of isolation and identification was performed under aseptic conditions. From the wash-solutin samples, microbial isolation and identification procedures were carried out as follows:

1. 0.1 ml. sample was spread onto each BHI, BG, EMB, and PA agar plate. They were incubated at 37°C for 24 hours;

2. Different types of single colonies, i.e. shape, size, and color from the BHI agar culture were picked up with a sterile platinum loop and transferred onto fresh BHI agar plates;

3. The suspected single colonies, such as Salmonella (pink), E. coli (metalic sheen), Streptococcus (red or colorless), or Pseudomonas spp. (green or yellow) from the BG, EMB, EA, or PA agar culture-plates, respectively, were transferred separately onto fresh BHI agar plates for purification, and were incubated at 37°C for 24 hours;

4. The purity of the replated-isolates was tested by a second replating on BHI agar plates, which were then incubated at 37°C for 24 hours.

5. Each of the pure colonies was subjected to different biochemical tests for identification.

From the egg samples, the isolation and identification were carried out as follows:

a. A one ml. sample was inoculated into five ml. of BHI broth, SB, and EVA broth. This step was done to help the growth of certain specific bacteria if present in the sample and, at the same time, to dilute the suspended egg particles. The three tubes from each sample were incubated at 37°C for 24 hours.

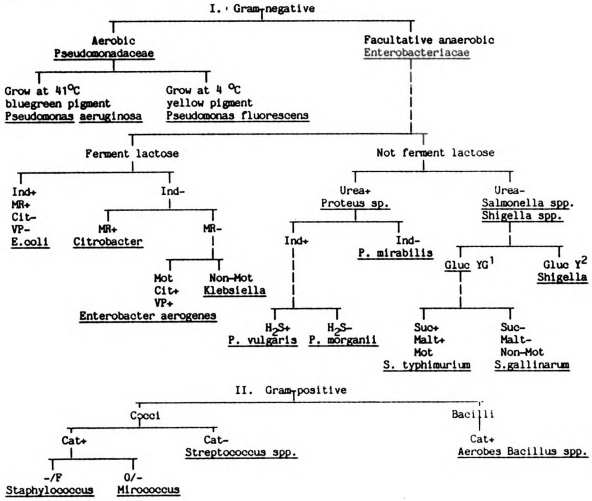
b. One ml. of the incubated BHI broth culture was spread onto the BHI, BG, EMB, EA, and PA agar plates. Also, one ml. of the SB culture was inoculated onto the BG agar plate,

and one ml. of EVA culture onto the EA agar plate. The plates were all incubated at 37°C for 24 hours.

c. The same procedures for isolation and identification were used as in steps 2 to 5 for the wash solution protocol.

All observations of the biochemical tests were recorded and used as criteria for microbial identification. The modified King's key for identification of aerobic bacteria has been listed by Carter (1978). It was useful in identifying the isolated bacteria of this experiment. In addition a scheme outlined by Schoenhard (1979) was also helpful in identification. Primary and secondary identification was determined according to the following schemes:

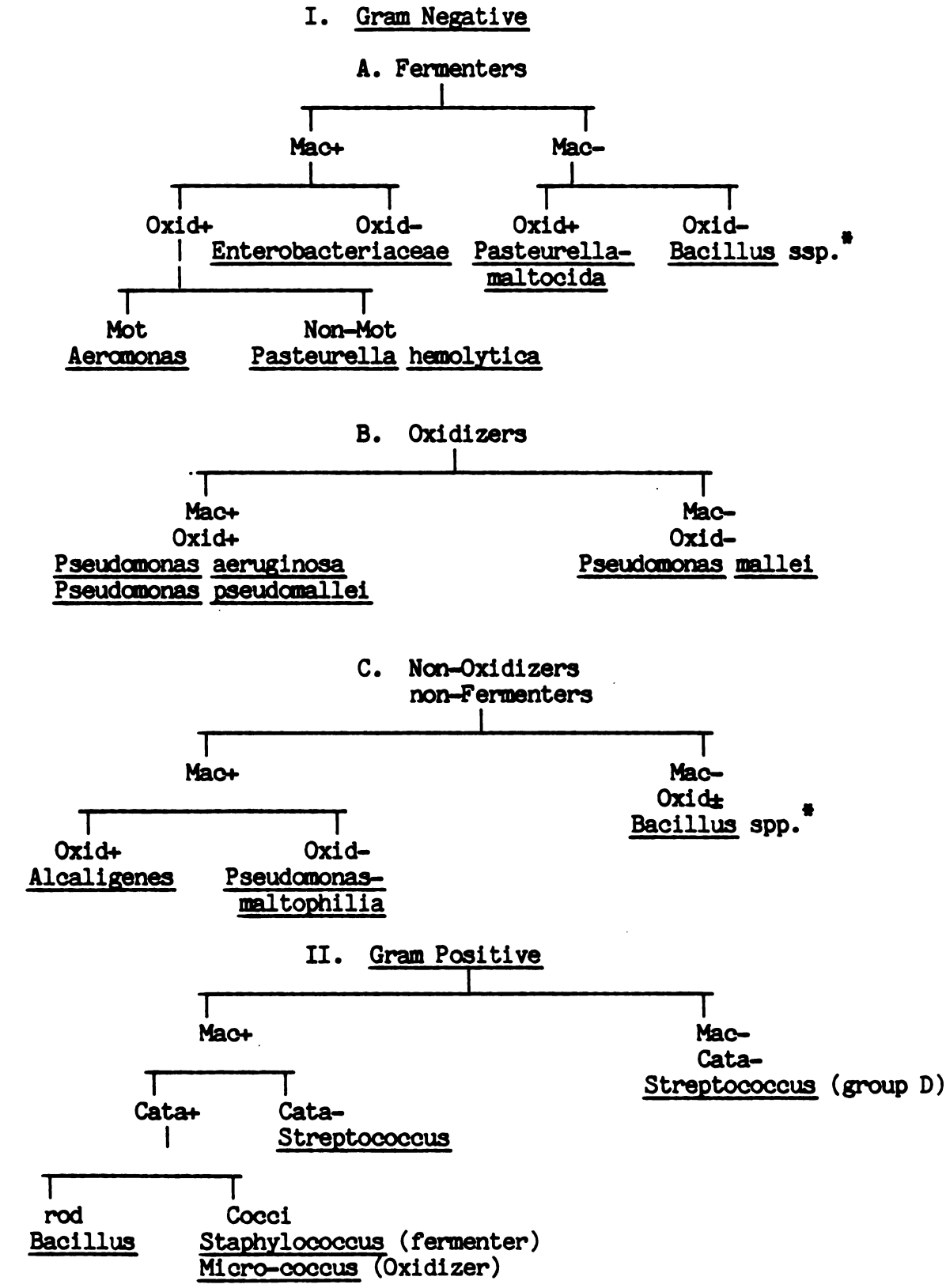
Secondary Identification Scheme



[1]YG: acid and gas

[2]Y: acid

Primary Identification Scheme



* many bacillus grow very lightly and very weakly

RESULTS AND DISCUSSION

I. HATCHABILITY OF DUCK EGGS

Hatchability Studies

Healthy, culled and dead ducklings were considered "hatched". Pipped and not pipped eggs containing dead embryos were considered as "unhatched" eggs. Results of hatchability from the four wash groups and the three farms (A, B and C) are summarized in Table 1.

Two-way analysis of variance (Gill, 1978) for the hatchability of fertile duck eggs is presented in Table 2. The hatchability did not vary significantly ($p>0.05$) among the different wash groups; water (80%), LOC (83%), HEDS (83%), and Roccal II (84%). The differences in the hatchability between the three farms, A (80%), B (86%), and C (80%), were also not significantly ($p<0.05$) different.

These results indicated that washing duck eggs prior to incubation with warm tap water, LOC, HEDS, or Roccal II produced no significant changes in hatchability. A similar result was reported by Furuta and Watanabe (1978) when they used phenolic derivatives, iodophore, and soap for washing chicken eggs.

Table 1. Hatchability Rate of Fertile Duck Eggs Dipped in Four Wash Solutions

Farm	Replica	Hatchability (%) Treatment Group: Water	LOC	HEDS	Roccal II	Av. Farm
A	1	79	83	79	77	
	2	85	86	77	77	80^{a}
B	1	81	88	83	87	
	2	83	87	88	92	86^{b}
C	1	75	70	79	86	
	2	74	81	90	85	80^{a}
Av. Treatment		80^{A}	83^{A}	83^{A}	84^{A}	

[A] Not significantly different ($P > 0.05$)

[a,b] Different letter superscripts in the farms are significantly different ($P < 0.05$)

Table 2. Two-Way Analysis of Variance for the Hatchability Rate of Duck Eggs

Source of variation	d.f.	SS	MS	F	c.v.
Wash solution	3	72.85	24.28	0.579	> 4.76
Farm	2	183.59	91.8	6.63*	> 3.89
Interaction	6	251.59	41.93		
Error	12	166.05	13.84		

d.f. degree of freedom

SS sum of squares

MS mean square

F F-distribution test

c.v. critical value

* $p < 0.05$ (significantly)

Embryonic Mortality Studies

The early and late dead embryos, the unhatched duck eggs and the dead pipped eggs were combined to give the embryonic mortality of the fertile eggs. Embryonic mortality was recorded at the 8th, 23rd, and 29th days of incubation. The results from the four wash treatments and the three farms are summarized in Table 3.

Embryonic mortality data was transformed from binomial percentages to angles of equal information in degrees (arcsin) for statistical calculation (Bliss, 1937). Two way analysis of variance of the arcsin transformed embryonic mortality (Table 4) showed that it did not vary significantly ($p>0.05$) among the treatments. However, embryonic mortality significantly ($p<0.01$) varied between the farms. In order to compare embryonic mortality during the first eight days to embryonic mortality during the last six days of incubation, the data was rearranged as shown in Table 5. The differences between the two incubation periods were analyzed statistically and are presented in Table 6. The results indicate that embryonic mortality of the last six days of incubation (29th day embryonic mortality) was significantly ($p<0.01$) higher than that of the first eight days of incubation (8th day embryonic mortality). This observation may be in agreement with the findings of Board (1969), who stated that following invasion of the shell the infection remains confined to the shell membranes for up to

Table 3. The Average Embryonic Mortality Rate of Fertile Duck-Eggs At Three Different Stages of Incubation

Farms	Average Embryonic Mortality (%) Treatment Group--Days of Incubation												Average/Farm
	Water			LOC			HEDS			Roccal II			
	8	23	29	8	23	29	8	23	29	8	23	29	
A	5	0	11	4	0	11	8	1	12	4	4	10	17^{a}
B	5	4	6	4	1	7	3	1	10	3	2	6	13^{b}
C	4	5	15	4	1	15	2	5	8	3	1	5	16^{a}
Average/ Treatment		18^{A}			16^{A}			16^{A}			12^{A}		

^{A}Not significantly different ($p>0.05$).

^{a,b}Different letter superscripts in the farms are significantly different ($p<0.01$).

Table 4. Two-Way Analysis of Variance for the Arcsin Transformed Embryonic Mortality Rate of Duck-Eggs.

Source of Variation	d.f.	SS	MS	F	c.v.
Wash solution	3	64.26	21.42	0.5359	>4.76
Farms	2	55.29	27.65	17.28**	>6.93
Interaction	6	239.83	395.97		
Error	12	19.36	1.6		

d.f. Degrees of freedom
SS Sum of squares
MS Mean square
F F-distribution test
c.v. Critical value
** $p<0.01$ (significant)

Table 5. Transformed and Rearranged Embryonic Mortality Rate of Duck-Eggs in Two Periods

Sample Number	Farm	Wash Group	Transformed Embryonic Mortality (%) Days of Incubation from 1st-8th day (X)	from 23-29th day (Y)	Difference (D)
1	A	Water	13	19	6
2	B		13	14	1
3	C		11	23	12
4	A	LOC	12	20	8
5	B		12	15	3
6	C		11	23	12
7	A	HEDS	16	20	4
8	B		11	19	8
9	C		7	16	9
10	A	Roccal II	12	18	6
11	B		11	14	3
12	C		10	14	3
r=12	Mean (M)		11.59 $(M_1)^A$	17.84 $(M_2)^B$	6.25 (M_D)

r=Total number of samples

^{A,B}Different letters superscripts in the two periods are significantly different ($p<0.01$)

Table 6. Statistical Analysis of the Embryonic Mortality Rate in Two Periods

S^2_D	Variance of the difference	5.96
S_D	Standard deviation	2.44
t-Test	M_D / (S_D / r)	8.87**
c.v.	Critical value	2.71

**The difference is significant ($p<0.01$)

15-20 days, and that actual rotting does not occur until organisms made contact with the yolk because of the following reasons:

1. the egg shell membrane acts as a barrier to microbial movement;

2. the viscosity of the albumen delays the microbial movement;

3. Regular turning of the egg during incubation prevents the precipitation of the microorganisms on the yolk surface.

Those findings of Board (1969) could explain why the highest rate of embryonic mortality occurred near the end of the incubation period of this trial. Furuta and Maruyama (1981) also reported that dead embryos found at the end of the hatching period were heavily contaminated with bacteria even though the eggs were originally considered clean at the beginning of the period.

Duckling Performance Studies

The weight gains of the ten-day old ducklings were recorded according to each farm and by each wash-treatment as shown in Table 7. The ten-day average weight gains of ducklings hatched from eggs dipped in the different wash solutions were 151 gr., 153 gr., 146 gr., and 161 gr. for the water, LOC, HEDS and Roccal II groups, respectively. The ten-day weight gains of ducklings hatched from eggs from the different farms were 156 gr., 141 gr., and 161 gr. for farms A, B, and C, respectively. These results were subjected to two-way analysis of variance (Gill, 1978) and

Table 7. The Ten-Day Average Weight Gain in Grams per Duckling for Wash Treatment and Farm

Farm	Replica	Av. Weight Gain - Gr./Duckling/10 Days Treatment Group				Av./Farm
		Water	LOC	HEDS	Roccal II	
A	1	154	160	147	165	156[a]
	2	153	140	151	180	
B	1	144	155	149	147	141[b]
	2	134	137	121	140	
C	1	165	176	151	165	161[c]
	2	155	148	157	168	
Av./Treatment		151[A]	153[A]	146[B]	161[C]	

[A,B,C] Different letter superscripts in the treatments are significantly different ($P < 0.01$).

[a,b,c] Different letter superscripts in the farms are significantly different ($P < 0.01$).

are presented in Table 8. Statistical analysis showed that there is a significant difference in the average weight gain among the four wash treatments and among the three farms. Further analysis was carried out by using Dunnett's test (T_D) to compare the result of LOC (as a positive control) to results of each of the water, HEDS, and the Roccal II treatment groups. The weight gain of ducklings hatched from eggs dipped in water did not vary significantly ($p>0.05$) from that of ducklings hatched from eggs dipped in LOC solution. The weight gain of ducklings from the HEDS group was significantly lower ($p<0.01$) than that of ducklings from the LOC group. Ducklings hatched from eggs treated with Roccal II solution had the highest ($p<0.01$) ten day weight gain compared to the other treatment groups. As per manufacture's recommendations, eggs that were dipped in Roccal II had the shortest dipping period (½ min.), as compared to LOC (2 min.) and HEDS (3 min.). It could be speculated that the length of the dipping period is important to the health of the hatched ducklings. This speculation would agree with the findings of Furuta and Watanabe (1978) being that eggs dipped in the disinfectant solution for six or eight minutes (long period) had lower hatchability than those dipped for four minutes (short period). They attributed that reuslt to the persistence of disinfectant solutin on the egg shell. Therefore, they suggested that hatching eggs should be dipped in a diluted 1% of phenolic derivative solution at 40°C for four minutes,

Table 8. Two-Way Analysis of Variance for the Weight Gain of Ducklings.

Source of Variation	d.f.	SS	MS	F	c.v.
Wash solution	3	683.64	227.88	44.77**	>9.78
Farms	2	2019.09	1009.55	8.55**	>6.93
Interaction	6	30.58	5.09		
Error	12	1416.56	118.05		

d.f. Degrees of freedom
SS Sum of squares
MS Mean square
F F-distribution test
c.v. Critical value
** $p<0.01$ (significant)

then eggs should be washed with water, after dipping to remove any remaining disinfectant solution.

II. Bacteriological Examination

Examination of the Wash Solutions:

Several different colonies grew on the BHI agar plates that were streaked from the samples of LOC wash solutions and the wash water. These colonies were identified as Staphylococcus, Micrococcus, Streptococcus, Aeromonas species and Enterobacteriaceae from both the wash water and the LOC solution. In addition, Pseudomonas and Bacillus species were isolated from the wash water and Alcaligenes was isolated from the LOC solution. These results indicate that LOC cleanser did not kill or reduce the diversity of bacteria in the wash solutions. The eggs that were dipped in LOC could therefore have a similar degree of bacterial contamination as the eggs dipped into warm tap water.

On the other hand, samples of HEDS and Roccal II wash solutions showed no bacterial growth on the BHI agar or any other culture plates. This indicates that HEDS and Roccal II sanitizers killed all of the bacteria in the wash solutions. However, washing or treating eggs with sanitizing chemical does not necessarily destroy bacteria embedded in shells (Moats, 1978; Furuta and Sata, 1977).

Examination of the Dead Embryos

During the incubation period, a variety of microorganisms were isolated at three different stages of

incubation from dead embryos. By following the normal microbiological identification procedures, they were identified according to their morphology, gram stain appearance and biochemical results, such as fermentation patter, EMB, BG, EA, PA, Mac, O-F, Oxid, Cata, Ind, MR, VP, Cit, Nit, H_2, Mot, Urea hydrolysis, and spore stains.

According to the results of this study, a total of 123 different types of bacteria were isolated, of which 64% of the organisms were Gram-negative and 36% were Gram-positive. These results were in agreement with the classification of bacteria isolated from rotten chicken eggs by Board (1965) and Board and Board (1968) and from duck eggs by Seviour et al. (1972). However, Bruce and Johnson (1978) reported that Gram-positive bacteria were the principal contaminants of unhatched chicken eggs.

The percentage of microorganisms isolated from dead duck embryos at three different stages of incubation are summarized in Table 9. The major isolates were E. coli, Streptococcus, and Proteus spp. being 30%, 27%, and 17%, respectively, of the total isolates. The isolation of Proteus spp. increased from 12% to 14% then to 25% at the 8th, 23rd, and 29th days of incubation while the percentage of Streptococcus spp. increased from 18% to 35% then decreased to 27%. The percentage of E. coli isolated decreased slightly at the 29th day of incubation. These results seemed to suggest that the presence of Proteus spp. might have influenced the embryonic mortality near the end

Table 9. Percentages of Various Bacteria Isolated from Dead Duck Embryos During Incubation

Organism	Percentage of Isolates Days of Incubation 8th Day	23rd Day	29th Day	% of the Total Isolates
Gram-negative	66%	59%	66%	64%
E.coli	30	35	25	30
Proteus	12	14	25	17
Citrobacter	12	7	5	8
Shigella and Klebsiella	2	0	4	2
Aeromonas and Alcaligenes	10	0	7	6
Pseudomonas	0	3	0	1
Gram-positive	34%	41%	34%	36%
Streptococcus	18	35	27	27
Staphylococcus	6	0	0	2
Micrococcus	0	3	7	3
Bacillus	10	3	0	4

of the incubation period. Perhaps due to a synergistic relationship may have been demonstrated between Streptococcus and Proteus, causing the increase of embryonic mortality near the end of incubation. Harry (1957) reported that Streptococcus fecalis and Bacillus cereus have a synergistic effect to enhance their individual spoilage potential of egg yolk when studied in vitro. Board and Board (1968) have isolated Proteus species from rotten eggs. It was proven to be the cause of "black rot" in eggs. It is believed that Proteus species gained entrance to the egg before incubation, but its fatal action was delayed until the end of the incubation period, when the organism was able to contact the yolk and cause the actual rot (Board, 1969).

It is difficult to pin point the relationship between bacteria isolated from egg content and hatchability of duck eggs since it was proven that there is no statistical difference in hatchability. Even when hatchability varied, Bruce and Johnson (1978) failed to show the relationship between them.

The frequent isolation of E. coli, Streptococcus, and Proteus species during the incubation period supported the explanation that feces is the main source of egg contamination. Salmonella spp. was not isolated in this study. Although Pseudomonas spp. was numerically a minor

contaminant in this study compared to E. coli and Proteus, it has been frequently isolated from rotten hen-eggs (Board, 1965).

Bruce and Johnson (1978) believed that Pseudomonas isolated from unhatched hen eggs were correlated with decreased hatchability. Shaver Technical Bulletin (1982) reported that Pseudomonas was a frequent cause of exploding egg problems in many hatcheries. The importance of Pseudomonas was also demonstrated in our laboratory from a related research experiment to show its effect on hatchability. When hatching eggs were immersed in a culture of Pseudomonas solution before being placed in the incubator, the rate of hatchability was severly reduced.

SUMMARY AND CONCLUSIONS

This experiment was conducted to determine the effect of several preincubation washing treatments on the hatchability of fertile duck eggs, on the early ten-day performance of the hatched ducklings, and on the bacterial content of dead embryos and to find whether a relationship betrween the organisms isolated and embryonic mortality could be established. Hatching eggs were dipped in one of three commercially available detergents or detergent sanitizers [LOC (Amway); HEDS (Bio-Lab); Roccal II 10% (Lehn & Fink)] or warm tap water. The wash products were used according to the manufacturers' directions.

The results of this experiment can be summarized as follows:

I. Hatchability of Duck Eggs

1. HEDS and Roccal II have better sanitizing action on the egg contaminating bacteria than that of warm water and LOC.
2. Embryonic mortality in all treatment groups significantly increased during the last six days of incubation as compared to embryonic mortality during the first eight days of incubation.

3. Percent of hatchability achieved in this experiment appeared to be normal compared to other investigations and to commerical duck hatcheries.

4. Dipping the duck eggs in warm wawter, LOC, HEDS, and Roccal II produced no significant differences in hatchability.

5. Ducklings hatched from eggs treated with Roccal II did however, have a statistically higher initial ten-day weight gain over ducklings hatched from other treatment groups.

II. Bacteriological Examination

1. The isolatin of Proteus and Streptococcus spp. increased with a significant increase in embryonic mortality, near the end of incubation. This reslt indicates that either Proteus spp. alone is important in causing embryonic mortality, or that a synergistic action between Proteus and Streptococcus causes embryonic mortality. In conclusion, further research should be directed toward the role of Streptococcus and/or Proteus in influencing embryonic mortality.

2. E. coli, Streptococcus, and Proteus spp. were frequently isolated during incubation. This result indicates that feces are the major source of egg contamination. It is suggested that the sanitary programs on the farm and at the hatchery should be improved and concentrated on reducing Proteus, Streptococcus, and E.coli.

3. The major contaminant isolated during incubation was E. coli. However, it showed no relationship to the rate of hatchability or embryonic mortality.

4. A direct relationship between the organisms isolated, during incubation, and hatchability or embronic mortality could not be established. The fact that an organism is present in an egg which has failed to hatch does not necessarily implicate the organism in the arrest of embryonic development. Factors other than the presence of contaminating organisms can influence hatchability. Some organisms are known to reduce hatchability and cause embryo mortality, while the importance of others is this respect in uncertain.

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