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Effects Of Various Sanitizing Treatments On Hatchability Of Broiler Breeder Eggs

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DEDICATION

This work is dedicated to :

My father and mother

To my wife and my children

To all brothers and sisters

To my friends especially Ahmad Eid.

And Abed Alfatah Saleem

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الإقــرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

Effects Of Various Sanitizing Treatments On Hatchability Of Broiler Breeder Eggs

تأثير معاملات تطهير مختلفة على نسبة التفقيس في بيض أمهات دجاج اللحم

اقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باستثناء مـــا تمـــت الإشارة إليه حيثما ورد، وان هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أية درجة علمية أو بحث علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

List of Abbreviations

QA	Quaternary ammonium.
ppm	Parts per million.
HCHO	Formaldehyde.
UV	Ultra violet.
nm	nano-meter.
CFU	Colony forming units.
PHMB	Poly hexamethylene biguanide hydrochloride.

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Abstract

In the commercial broiler breeder farms and hatcheries, formaldehyde fumigation is routinely carried out to disinfect hatching eggs, Dipping of hatching eggs has not been practiced as means of disinfection locally. The proper use of disinfection is essential. This study was carried out to determine the effect of different disinfection practices on hatchability, egg weight loss, embryonic mortality and early chick performance. Nest–clean and dirty eggs were exposed to formaldehyde fumigation (control) or immersed in worm water (40C) followed by dipping for 5 minutes in one of the following disinfectant solutions: 1% formalin, agri – germ, or 3% hydrogen peroxide solution. Early chick mortality and hatchability of fertile eggs were not effected by treatments, but they were numerically greater in eggs dipped in hydrogen peroxide solution. None of the treatments affected egg weight loss throughout the first 18-days of incubation. The use of one-step dipping rather than the common fumigation in- farm and in-hatchery did not adversely affect hatchability. Body weights, chick mortality and weight gains did not differ by treatment. The use of one-step disinfection , and the use of hydrogen peroxide as an in-farm dip for hatching eggs may be a possible alternative to formaldehyde fumigation.

CHAPTER ONE

INTRODUCTION

Introduction

Penetration of the hatching egg shell by microorganisms may result in embryonic mortality, weak chicks, high chick mortality and poor chick growth. The most effective disinfection procedure involves sanitizing the eggs as soon as they are collected from the nest. It is common that hatching eggs are treated with a fumigant (i.e. formaldehyde) or other types of disinfectants to control the number of microorganisms on the shell surface.

Scott *et al*., (1993) examined 23 sanitizers for positive and negative characteristics with respect to their use in the hatchery. They rated sanitizers for user-and environmental - friendliness based on general characteristics, environmental impact and safety precautions, health concern, reactiveness and potential fire hazards. Adverse health effects associated with the use of formaldehyde as dip or fumigant has diverted the current research attention to effective and safe egg sanitizers.

Commercial broiler breeder hatching eggs sanitizers are available in the local market contain formaldehyde and other antifungal and antiviral ingredients. However, bacterial contamination, especially salmonella, is predominant contaminant of broiler breeder hatching eggs (Lesson and Summers, 2000). Poor nest management and untrained young pullets result in eggs being laid outside the nest. The use of floor, usually contaminated eggs is a controversial issue. Some farms don't send floor (dirty) eggs to the hatchery, while others dry-clean them and mix them with the regular eggs. Some farms use fine sand – paper to dry-clean floor eggs. However this procedure can lead to removal of cuticle and the physical clogging of the pores with fecal material and shell dust (Lesson and Summers, 2000).

Under field conditions, hatching eggs are taken from the nest, inspected, stored for 3-4 days in farm and finally transported to the hatchery. Eggs are usually fumigated using formalin in the storage room then are re-fumigated once arrived at the hatchery.

The current research was aimed at evaluating hydrogen peroxide solution as a substitute for formaldehyde and commercial disinfectants in sanitizing broiler breeder hatching eggs. Another aim of the current study was to investigate a one-step disinfection as a substitute for the common commercial procedure. Another aim of the current study is to disinfect sand – paper cleaned floor eggs. This, hopefully, may reduce embryo mortalities that occur due to problems of incorrect egg holding temperature, and because of excessive movement which can cause shell damage.

CHAPTER TWO

LITERATURE REVIEW

Literature Review

2.1 Production of clean hatching eggs

Broiler breeder management system is necessary to produce clean (nest-clean) hatching eggs. Several practices have been utilized to ensure production of clean eggs. Hatching eggs should be collected 3-4 times daily to aid in the prevention of contamination with diseases causing organism (North, 1984).

Birds are usually maintained on wire, plastic, wooden slatted floors or litters floors. It has been reported that number of nests, available nesting materials, eggs collecting equipment, and personnel cleanness are important factors in production of clean eggs (North, 1984). By providing one nest for every four hens, this could prevent contamination of eggs by reducing the percentage of floor eggs (Ernst, 2004). It has been found that collecting eggs on clean, sanitized plastic flats or in clean baskets aids in production of clean hatching eggs (North, 1984). Also it has been reported that excluding the hens from nests at night reduces development of broody hens and keeps nests cleaned (Ernst, 2004).

It has been reported that sanitizing hatching eggs as soon as possible after collection did not kill all of the microbes that have penetrated the shell but aid in killing the microbes on the outside of the shell (North , 1984).

Egg cooling overnight before placing them in cases reduces egg contamination but when eggs are stored before setting they should be placed in a clean room held at 17-22C and 75 % relative humidity (Ernst, 2004). Preventing eggs sweating during transferring from cold storage into

a warmer room resulted in maintaining quality of nest – clean hatching eggs (North, 1984).

2.2 Egg hatching and hatcheries

Collection time is an important factor to obtain clean hatching eggs. Trained workers should watch breeding hens so that eggs are collected 10 to 15 min after laying, especially on warm days, to allow sufficient time for the cuticle to dry. Quick collection prevents damage of the embryo in hot temperatures and prevents microbial spoilage (Cooper, 2001). Button *et al*., (1994) showed that 39.5% of 114 fertile eggs tested with early to midterm embryonic death were infected, and found that greater than 19.6% infection rate of 240 infertile eggs. It has been demonstrated that microbial infection is an important cause of embryonic death, the principal microbes are environmental or fecal bacteria and fungi (Button *et al*., 1994).

The maintenance of nest hygiene is considered the simplest way of reducing microbial contamination (Deeming, 1996). The egg should be carefully gathered and wiped with a dry cloth. Holding the eggs with sterile toweling helps to prevent possible contamination from the worker's hands. The eggs are then placed in a carrying basket lined with foam rubber to prevent breakage. Before cleaning, the worker should sanitize his or her hands in a warm iodine wash or other antibacterial solution at 40 C. Common bacterial infestations arising from poor egg cleaning include *Escherichia coli, Aeromonas sp., Enterobacter sp., Acinetobacter sp., Citrobacter sp., and Streptococcus faecalis*. Fungal infestations include *Penicillium sp. and Fusarium sp.*(Foggin and Honywill., 1992). High mortality in full-term embryos that failed to hatch is caused by infection of

the yolk sac with bacteria (Deeming,1995). Welsh *et al*., (1997) described the clinical signs observed in *Salmonella*-infected breeder birds that resulted in shell-less, infertile eggs and early embryonic death.

There are two forms of the salmonella species which are specific to poultry – these are *salmonella pullorum* and *salmonella gallinarum*. The former, perhaps the oldest of all diseases diagnosed in poultry, used to be called bacillary white diarrhoea (BWD), whilst *salmonella gallinarum* is commonly called fowl typhoid. In addition we have these diseases in poultry caused by other salmonella and coming under the general title of salmonellosis or sometimes termed avian parathyroid. There are many different forms – at least 200 types of salmonella have been found in poultry but the most important is *salmonella typhimurium*, which can be a virulent killer of chicks in particular (Coufal *et al*., 2003).

If the temperature of the antiseptic solution is lower than the temperature of the egg, a reduction in the volume of the egg contents occurs, causing a negative pressure and vacuum, this results in an increase in the movement of bacteria through the shell pores and contamination of the internal contents of the egg and subsequent infection of the embryo. Such a practice has lead to high embryo mortality and yolk sac infection. Eggs are best cleaned with a dry cloth and the use of potassium permanganate- formalin mix (Huchzermeyer,1996). Disinfection of storage room is achieved by a light spray of potassium permanganate and formaldehyde (Cooper,2001). Eggs are usually gathered over period of time until adequate number of eggs can accumulate for incubation. Thus eggs must be stored properly to insure hatchability (North,1984). Eggs

should be turned to a new position once daily until placing in the incubator (North,1984). Before placing eggs in the incubator, eggs are allowed to warm slowly to room temperature since abrupt warming from 17.5 degrees to 37.7 degrees causes moisture condensation on the egg shell which leads to disease and reduces hatchability (North,1984).

It has been found that before setting hatching eggs in incubator, temperature and relative humidity should be optimized (North,1984).

The optimum incubation temperature is influenced by several factors: size of the egg shell, shell quality, genetics, age of the egg when it is set and humidity of the air during incubation (Ensminger,1990). According to North,(1984), the optimum temperature during the first 19^{th} days of incubation is 99.5 to 99.7 F (37.5 and 37.7 C), and during the twentieth and twenty-first days of incubation the optimum temperature is 97 to 99 F(36.1 to 37.2C). A relative humidity of about 75% seems optimum for most incubators at the time of hatching (North,1984).

2.3 Fertile egg quality

High quality fertile eggs should always be considered rare and fragile to successfully hatch eggs which begins with fresh , clean fertile eggs. It has been reported that fertility of hatching eggs is influenced by many factors like presence of good breeding males and healthy females. However specific gravity, egg shape, air cell and shell texture are not indicators of fertility. Fertility is an inherited factor, for example some strains of chickens have better fertility than others. Furthermore individual males and females vary in their ability to produce viable embryos. Accurate

differentiation between fertile and infertile eggs is desired. Incubated eggs should be broken out and examined, but in hatcheries differences are observed by candling. In commercial hatcheries they examine fertility by candling eggs at eight days of egg setting (North, 1984).

2.4 Disinfectants

The chemicals used for surface disinfecting are many and their values are highly variable. Sanitizers may be grouped according to their base ingredient, but there are many things that affect their potency, all of which must be understood to cause the disinfectant to produce normal effectiveness (North, 1984).

Properties of disinfectants most commonly used in broiler breeder farms and hatcheries are shown in (appendix 10). Hydrogen peroxide was tested as an egg wash in commercial egg washing machines, as a spray or fog on trays (Mauldin and Wilson, 1988). However few research have dealt with the effect of dipping hatching eggs in hydrogen peroxide.

Breeder flocks and hatcheries represent critical control points for salmonellae entry in commercial integrated poultry operations. Effective chemical treatment of hatching eggs will be required to intervene. In addition to surface contamination, a freshly laid egg is wet, warm, and susceptible to rapid penetration by microorganisms (Williams *et al*., 1968). Once salmonella passes through the shell and into the membranes of hatching eggs, it is difficult to prevent further invasion of the egg contents or developing embryo (Cason *et al*., 1993). After incubation, salmonella organisms entrapped in the membranes can be ingested by the hatchling as

it pips into the air cell or through the shell (Cason *et al*., 1994). Perhaps as a result of this situation, extensive reservoirs of bacteria have been established in commercial broiler and breeder hatcheries (Cox *et al*., 1990 and Cox, *et al.*, 1991). Therefore sanitizing hatching eggs is essential for achieving high levels of hatchability and producing high quality chicks (Fueng- Lin WO, 1996). Sanitation of hatching eggs is an important area of research due to the need for an effective, economic, and safe method of egg sanitation. Improved hatching egg sanitation is an important part of a pathogen reduction program within integrated poultry operations. This must be accomplished without disturbing the cuticle of the egg, which can decrease hatchability (Coufal *et al*., 2003).

About 23 sanitizers / disinfectants were examined for positive and negative characteristics in the hatchery. Each sanitizer was rated for user and environmental - friendliness based on general characteristics, environmental impact and necessary safety precautions, health concerns and potential fire hazard (Scott, 1993). Also in another research Scott, (1993) used the same 23 sanitizers for testing their effectiveness against a variety of microorganisms on the egg shell. Cox and Bailey. (1991) demonstrated that the type of organism involved and the immediacy of treatment will likely have a significant influence on the success of disinfection. The appropriate chemical should be applied as soon as economically possible after lay. Cox *et al*.(1994) have found that the effectiveness of chemical treatments to eliminate bacteria diminishes after the contamination of the egg. Surface disinfectants are most effective in the absence of organic material (North, 1984). Disinfecting is not a substitute for cleanliness, it is a means of destroying microorganisms, but is effective

only when things are relatively clean to start with (North, 1984). Control of microorganisms on the shell surface of hatching eggs requires a disinfectant effective in killing the pathogens without injury to the live chick embryo (Fueng- Lin WO, 1996). All disinfectants used in the hatchery should be highly germicidal, nontoxic to man and animal, effective in the presence of moderate amounts of organic material, noncorroding and non staining, soluble in water, capable of penetrating materials and surfaces, unassociated with pungent odors and readily available and inexpensive (North, 1984).

Cresols and Cresylic Acid are liquid yellow or brown coal tar derivatives. They have a strong odor, irritate the skin, and turn milky when water is added, but they have excellent germicidal action. Their odor may injure day – old chicks. They are effective against gram – positive and gram – negative bacteria , most fungi, and some viruses (North, 1984).

Phenols also coal tar with a base of carbonic acid. They have a characteristics odor and are effective germicides. They are effective against fungi, gram – positive and gram –negative bacteria (North, 1984).

Iodine compounds are good disinfectants in an acid situation (2-4 pH), and are effective against gram – positive and gram – negative bacteria, most fungi, and some viruses.

Chlorine is an effective constituent of certain disinfectant. It is a good disinfectant when free chlorine is available in abundance (200-300 ppm).

Chlorine is effective against bacteria and fungi, and when coming from hypo chlorites it attacks both the protein coat and the nucleic acids of viruses. Also chlorine is more active in acid solutions than in alkaline, and in warm rather than in a cold mixture, Chlorine compounds are somewhat irritating to the skin and corrosive to metal (North, 1984).

Quaternary ammonium (QA) compounds are extremely water soluble, but QA cannot be used in soapy solutions. These chemicals are effective against gram – positive organisms, and moderately effective against gram – negative bacteria, and will control some fungi and some viruses. The use of (QA) compounds on poultry farms has been met with resistance in some countries because of injurious effects on human beings when food are involved. It should be withdrawn five days before the birds are marketed (North, 1984).

Formaldehyde (HCHO). is active against most microbes. The bactericidal activity of formaldehyde is dependent upon high humidity. Formaldehyde gas has little penetrating power and is immediately dissipated on the surfaces of walls and equipments (North, 1984). In 1989, the Occupational Safety and Health Administration in United States (US) severely restricted the use of formaldehyde Chemical Engineering News, (1984).

Ultraviolet (UV) radiation is produced by the sun and is therefore a natural component of our environment. Ultraviolet radiation at 254 nm is well known and documented for its use to kill various types of microorganisms, such as bacteria, yeasts, molds, fungi, and viruses. It can be generated at high intensities by the use of low-pressure mercury-vapor

discharge lamps called germicidal lamps. Germicidal lamps are relatively easy and inexpensive to obtain and use and have been used in a variety of industries to sanitize air, water, dairy products, vegetables, meat, maple syrup, fresh cider, and packaging materials (Huang and Toledo, 1982).

Hydrogen peroxide in a colorless liquid usually produces an aqueous solution of various strengths. Hydrogen peroxide is water and an extra oxygen molecule and is an unstable powerful oxidant. It breaks down readily into water and a single oxygen molecule. Thus a single oxygen molecule is a strong oxidizing and disinfectant agent. It is stronger than chlorine, chlorine dioxide, and potassium permanganate. It can be used as water disinfectant, in the medical world, and in the food industry (Mansour, 2001). Hydrogen peroxide has been used as a sanitizers for reduction of total bacteria, and salmonella in hatching cabinet in liquid or spray forms (Mauldin and Wilson, 1988).

2.5 Methods of sanitation hatching eggs

Because wet eggs are more likely to be penetrated by bacteria, the traditional wisdom among hatchery experts was to avoid wetting the egg at all costs, especially with liquid that was cooler than the egg (Haines and Moran, 1940). By the 1940s poultry scientists were showing that wet sanitization of hatching eggs could be performed with no adverse effect on hatchability (Pritsker, 1941 , Olsen and McNally, 1947). Many research have been done to indicate the probable method for sanitizing hatching eggs and many studies have been conducted to examine different chemicals as hatching egg sanitizers to obtain the maximum hatchability, Early work implemented sodium hydroxide in concentrations of 1 and 2% and was

found to be helpful in the control of salmonella and other microorganisms without reducing hatchability (Olsen and McNally, 1947). Gordon *et al*., (1956) concluded that when eggs were dipped in disinfectant solution within 1 hr of lay, no more cleaning was needed. The need to disinfect broiler hatching eggs was recognized at least as early as 1908, when the use of formaldehyde gas to control microbial populations when first introduced. Since the Occupational Safety and Health Administration in the United States published its list of concerns in 1991 on the effects of repeated or prolonged exposure to formaldehyde, there have been numerous studies evaluating various chemical sprays or dips as potential replacements for formaldehyde (Occupational Safety and Health Administration, 1991). Bierer *et al*., (1961) agreed with earlier research in that formalin in 0.5% solution was an effective sanitizer, and showed its ability to kill *S. typhimunum.* Pritsker, (1941) however, because of the noxious characteristics of this chemical did not recommend its use. Williams, (1970) found that application of formaldehyde by fumigation was very effective in lowering bacterial populations on the surface of hatching egg. The author recommended the use of formaldehyde on the farm, noting its efficacy, lack of penetration, and lack of detrimental effect on hatchability.

Patterson *et al*., (1990) tested the use of chlorine dioxide applied to eggs as a foam in comparison to formaldehyde fumigation. Eggs were covered with a thick foam for 15 min before placement in the incubator and results compared to the old industry standard of formaldehyde fumigation of eggs in the incubator. Foam did not change hatchability compared to the untreated control group and lowered counts of inoculated *Escherichia coli*

cells as effectively as formaldehyde fumigation. Brake and Sheldon, (1990) and Scott and Swetnam, (1993a,b) provided a through review on the hazards associated with the use of formaldehyde as a disinfectant in the work place. However, Furuta and Maruyma, (1981) and Scott and Swetnam, (1993a,b) and Lesson and summers, (2000) were concerned about finding a sanitizer as effective against microorganisms as formaldehyde, and yet user and environmentally safe.

Scott and Swetnam, (1993c) found that quaternary ammonium, did not significantly affect hatchability relative to eggs treated with formaldehyde. Formaldehyde fumigation has long been the recommended treatment by hatcheries due to its effectiveness and ease of application (Funk and Irwin, 1955 and Graham and Michael, 1932). However, there are undesirable consequences of working with this toxic gas. Formaldehyde gas is intensely irritating to mucous membranes and is a suspected carcinogenic (Budavari *et al.*, 1989). Sub acute exposure of rats to formaldehyde concentrations higher than 2 ppm inhibits mucocilliary clearance of the nasal epithelium and leads to progressive histological and ultra structural lesions (Bolt, 1987). Casteel *et al*., (1987) indicated that humans exposed to formalin vapor suffer symptoms of eye and upper respiratory tract irritation. Hence, repeated exposure of the hatchery worker to these vapors could result in chronic skin irritation and respiratory problems. Given these health concerns about formaldehyde fumigation, the need for safer alternatives has become apparent (Casteel *et al*., 1987).

Scott and Swetnam, (1993a) tested a short list of chemicals that can be used as disinfectants, one of which was hydrogen peroxide in a 0.7%,

1.4%, or 2.9% solution. Earlier work (Sheldon and Brake, 1991) indicated that 5% hydrogen peroxide was suitable as an egg disinfectant, eliminating culturable microorganisms, a 5.3 log10 CFU reduction, without adversely affecting hatchability.Other researchers (Cox *et al*., 1991a,b) reported similar results with 1% hydrogen peroxide. Likewise, Pardon, (1995) found that dipping eggs twice in 6% hydrogen peroxide was beneficial as a sanitizer, lowering bacterial counts on the membranes beneath the shell by 95%, and lessening salmonellae-positive eggs by 55%, without lowering hatchability. Shane and Fuast, (1996) found that hydrogen peroxide achieved complete disinfection of the shell surface, in contrast to distilled water and phosphate buffer which only removed 83% and 48% of the *E. coli* contamination, respectively. It has been found that hydrogen peroxide resulted in removal of salmonella from 70% of the inoculated eggs (Cox *et al.*, 1990 and Cox and Bailey 1991). Scott *et al*., (1993a,b,c) found that 1.4% hydrogen peroxide lowered microbial populations without adversely affecting the embryo or hatchery personnel. Padron. (1995) found that sanitizer effectiveness could be improved by applying positive pressure to push hydrogen peroxide through eggshell pores during immersion. Cox *et al*., (1999) found that simple immersion in 1.4% hydrogen peroxide was notably more effective on *S. Heidelberg* than on *S. typhimurium.* Only 20% of the eggs treated with hydrogen peroxide as a dip were positive for *S. heidelberg.* Although the vacuum step led to a measurable increase in hydrogen peroxide effectiveness against *S. heidelberg* (10% remaining positive), dipping inoculated eggs in 1.4%. Hydrogen peroxide led to recovery of Salmonella from 43% of the eggs. Addition of a vacuum step significantly improved the efficacy of hydrogen peroxide with only 10%

(3/30) being positive after treatment. Scott and Swetnam, (1993a) compared a long list of sanitizers for "user friendliness" or hatchery personnel safety. Scott and Swetnam, (1993b) tested the chemicals' ability to lower microbial counts on the eggshell. With the exception of a product consisting primarily of quaternary ammonium compounds (Basic G + H). Scott and Swetnam, (1993b) found that quaternary ammonium did lower the total count to below detectable limits. It is important to note that the method for recovery in this study cannot detect organisms below the shell surface. Quaternary ammonium compounds are usually sprayed on eggs either stacked on collecting flats or trayed in setter racks Brake and Sheldon, (1990). In one study, the application of a quaternary ammonium sanitizer reduced levels of aerobic bacteria on shells by 99% and raised hatchability by 6% compared to levels in untreated eggs (Brake and Sheldon, 1990), Cox *et al*., (2007) determined that quaternary ammonium were effective resulting in 95% reduction of salmonella incubated to hatchery eggs. Cox and Berrang, (1994a,b) evaluated 16 different commercial hatching egg sanitizers using an egg spray sanitizing machine.

Polyhexamethylenebiguanide hydrochloride (PHMB) at 0.035%, hydrogen peroxide at 1.4%, and Tektrol (commercial sanitizer), at 0.39% were found to be the most effective for killing salmonella on and beneath the eggshell surface (Bailey *et al*., 2001) These authors found that egg contamination did not affect PHMB or hydrogen peroxide, but feces did reduce the effectiveness of these chemicals. The buildup of fecal material washed from eggs being sanitized is almost inevitable in a machine that recycles sprayed chemicals and would likely begin to reduce the effectiveness of the PHMB or unstablized hydrogen peroxide, (Cox *et al*.,

1990). In their study, they found that immersion of eggs in PHMB with vacuum step resulted in eliminating *S. typhimurum* from 29 of 30 and *S. heidelberg* from 24 of 30 eggs. Against Salmonella cells inoculated onto the eggshell surface, 0.035% polyhexamethylenebiganide hydrochloride (PHMB) was a very effective eggshell sanitizing agent that can be applied by immersion or spray (Cox *et al*., 1994.). However, Cox *et al*., (1982) found that when salmonella was able to penetrate the shell, PHMB was no longer effective against it. PHMB was more effective than hydrogen peroxide, in eliminating *S. typhimurium*. After simple immersion in PHMB, 13% of eggs were positive; only 3% remained positive following application of PHMB and vacuum treatment. Cox *et al*., (1992) examined the immersion of hatching eggs into chemicals to eliminate inoculated salmonella. Preliminary studies suggested that hydrogen peroxide, polyhexamethylenebiguanide hydrochloride (PHMB), and phenol were the most effective of the many chemicals tested. Polyhexamethylenebiguanide hydrochloride (PHMB) proved to be the most efficient chemical, resulting in an 85% reduction in the number of positive eggs compared to the water-washed-control. Hydrogen peroxide also performed adequately, causing a 60% reduction. Also phenol was tested by Cox *et al*, (1998). They detected that within 1 min inoculum drying time, 0.78% phenol reduced the number of salmonella-positive eggs by 80%, compared to the control group which was dipped in water. North. (1984) demonstrated that in high concentration, phenols act as a protoplasmic poison, penetrating and disrupting the cell wall and precipitating the cell proteins. But in low concentrations phenol disrupts essential enzymes of the cell (Williams, 1969).

Patterson *et al*., (1990) observed that dipping, compared to foaming, hatching eggs in a chlorine dioxide solution resulted in decreased hatchability of heavily soiled duck eggs. It has been observed that chlorine dioxide (Sanimist) was quickly neutralized by components of the egg shell and was ineffective in reducing microorganisms on the egg shell (Scott *et al*., 1993b).

Ozone gas was used to reduce microorganism loads of hatching eggs (Whistler and Sheldon, 1989). However, at the levels tested (3% ozone by weight for 2 hours), embryo toxicity occurred. Ozone could cross the shell wall and destroy microorganisms which had penetrated the shell barrier. Unfortunately, this transfer across the egg shell wall also puts ozone into direct contact with the embryo. Ozone may be a serious health hazards for those working with it (Scott *et al*., 1993a). The level of ozone used in the study by Whistler and Sheldon, (1989) was extremely high, far exceeding amounts which have been recommended for food products. This high level of exposure may explain the high embryo mortality which was reported in the above study. Alternative methods to reduce microbial load on hatching eggs are currently being sought in face of restriction on the use of formaldehyde.

The potential of UV – lights as a safe sanitizers for hatching eggs and / or as a method to "scrub" the circulating air during incubation is examined (Scott, 1993). It has been reported that UV-light has potential as a safe sanitizers to the users and environment. UV-light apparently reduces bacterial loads of circulating air during incubation and hatching (Scott, 1993). Ultra violet (UV) light has been studied as a potential means

to sanitize hatching eggs. Scott, (1993a) tested the use of UV lights in hatching cabinets. Compared to formalin dipping, the author found that UV light is ineffective as a pre-incubation treatment to lower total bacterial counts as detected by rinsing egg surfaces. When applied in the hatching cabinet. Scott *et al*., (1993a) found that UV light helped to prevent cross contamination of pre-treated eggs from eggs that were not pre-treated. Although did not point out to UV as a promising technique for egg sanitization. Scott *et al*., (1993b) did not report the intensity of UV light that was used, nor did they attempt to increase intensity to test effectiveness. It has been found that when eggs were inoculated with a drop of salmonella suspension on the surface. UV light at an intensity of 600 µW\cm² significantly lowered (but did not eliminate) the number of positive eggs. However, when eggs were inoculated with Salmonella in a smear of feces, UV light at intensities as high as 1600µW/cm² was ineffective (Berrang *et al*., 1995). Earlier research, Kuo *et al*. (1997) showed that UV light at 620µW/cm² did lower the *S. typhimurium* total cell count. While UV light may hold some promise as an egg sanitizing agent, its usefulness is limited to clean eggs (without fecal staining) (Kuo *et al*., (1997). Because it is potentially harmful UV light is most promising for application inside closed hatching cabinets as suggested by (Scott *et al*., 1993).

Latala and Wakula- Radzik, (1990) exposed large numbers of hatching eggs to UV-lamps (1 m away from eggs) for 1,3**,** or 5 minutes prior to setting. Gram negative bacteria numbers on the eggshell were reduced by 90% after 3 minutes of exposure. At higher exposure times (8 minutes), early embryonic mortality increased from 2.4 to 3.3%. It was

concluded that one minute of exposure to UV-light resulted in a reduction in bacterial load equal to that realized with formaldehyde fumigation. Ultraviolet (UV) light is a rapid, easy method that has no detrimental effect on the embryo and, therefore, may provide a means to evaluate cuticle quality of large numbers of eggs prior to setting (Stanley *et al*., 2003).

2.6 Floor eggs sanitization

Broiler hatching eggs that are classified by the producer as dirty due to adhering fecal or litter material are most likely not sent to the hatchery. The producer and the integrated poultry company lose the revenue associated with potential chicks from these eggs. Although wetting or washing hatching eggs has been thought to lower hatchability (Berrang, 1997). Research over the years has shown that proper wet egg sanitization does not adversely affect hatchability **(**Olsen and McNally, 1947 and Lancaster *et al*.,1952 and Brake and Sheldon, 1990). Washing dirty eggs with a spray sanitizing machine and sending the cleaned eggs to the hatchery can provide an appreciable increase in economic gain from a breeder flock, providing the eggs do not harbor undetected microbial contamination (Buhr *et al*., 1996.). The economic benefit of sanitizing dirty eggs is especially noticeable if the flock is laying a high number of eggs on the floor (Berrang, 1997). There are several reports which support the idea that a dirty environment and subsequently a dirty egg will decrease hatchability, (Scott *et al*., 1993a.) and Tullet. (1990) compared floor ("dirty") eggs to nest run eggs and observed a 10% to 15% decrease in hatchability. Factors other than bacterial contamination of these floor eggs

were also indicated (Tullet, 1990). Mowry *et al*., (1980) observed that performance of 50 day-old broiler chick was improved by sanitizing eggs prior to incubation. Buhr and Mauldi, (1994a) found that hatchability of eggs set and hatchability of fertile eggs were similar for clean and dirty hatching eggs. Also nest clean eggs consistently have greater hatchability than dirty eggs. The difference in hatchability between nest clean eggs and dirty eggs was due to embryonic mortality following transfer into the hatcher for dirty eggs. Chick weight on day of hatch and at one week post hatch did not differ between nest clean and dirty eggs.Also first week chick mortality was low (< 1%) and did not differ significantly between nest clean and dirty hatching eggs (Buhr *et al*., 1994).

In summary, It has been known that salmonellae can penetrate the shells and membranes of hatching eggs. We now know that this can critically affect the salmonellae contamination on the final product (the processed broiler carcass). As the freshly laid egg cools, the contents contract, producing a negative pressure that in turn can draw bacteria such as salmonellae into and through the shell and adhering membranes.

The salmonellae may then be ingested by the embryo as it emerges from the egg (Cason *et al*., 1994) or the salmonellae may proliferate as the embryo develops and be spread in the hatching cabinet (Cason *et al*., 1993). Very few salmonella cells are required to colonize the gut of the young animal. Cox *et al*., (1990) producing a seeder bird that will then spread contamination to the intestinal tract, skin, and feathers (Bailey and Cox, 1991) of other birds in the flock. Several studies around the world have shown that the same Salmonella serotypes originating from hatcheries and

breeder flocks can be found on fully processed broiler carcass (Blankenship *et al.*, 1993, Goren *et al.*, 1988 and Lahellec and Collin, 1985). Therefore, early contamination of the freshly laid fertile egg is a very important critical control point for preventing the entry of salmonellae and other human foodborne pathogens into poultry production and subsequent processing. Research has shown that when salmonella are able to penetrate the shell and membranes, they can avoid the killing effect of a chemical by avoiding direct contact with the chemical. Hydrogen peroxide has been shown to be an effective sanitizer for eggs by several researchers (Cox *et al.*, 1994 and Padron, 1995 and Shane and Fuast, 1996). However, hydrogen peroxide, like other chemicals, cannot be effective against salmonella cells that have penetrated the shell, thereby avoiding direct contact with the chemical (Cox *et al*., 1998). In an attempt to reach these microorganisms with a chemical treatment, researchers have explored the possibility of pushing or pulling chemicals deeper into the egg. (Padron, 1995) found that hydrogen peroxide was a more effective bactericide when positive pressure was applied to push the chemical deeper in the egg during immersion treatment. Others have used a negative pressure approach. by placing an egg in a solution and evacuating the air within the egg with vacuum, a negative pressure is formed within the egg. When the vacuum is released, the liquid surrounding the egg is drawn deeper into the egg. This has been shown to be effective with chemical hydrogen peroxide disinfection of a salmonella-contaminated fertile egg (Cox *et al*., 1998). Also, surfactants have been shown to assist chemicals to penetrate deeper into fertile eggs (Cox and Bailey, 1992).

CHAPTER THREE

MATERIALS AND METHODS

Materials and Methods

3.1 Broiler breeder eggs

All eggs were obtained from fourty weeks old broiler breeders in Auja-Jericho farms of Sinokrot Broiler Breeder Company. A1830 nest-clean and floor eggs used in this study had been collected over and processed at the end of each day. Eggs were then transported to a commercial hatchery in Jenin and stored there, until incubated, at 18.3C and 75% relative humidity.

3.2 Sanitizers and Sanitizing procedures

3.2.1 Stock solution preparation

Four stock solutions were prepared prior or immediately at the time of hatching eggs treatment. The first solution was prepared by mixing 18 gram potassium permanganate, 21ml water and 21 ml formalin to achieve the standard recommendations in commercial practices (control). the second stock solution was prepared by adding 50 ml agri-germ 2000 to 10 liters of tap water to achieve the standard recommendations for the control of bacteria. A third stock solutions of 1% (v\v) formalin was prepared by adding 10 ml concentration (38%) to 990 ml tap water. The final stock solution was prepared by adding 30 ml hydrogen peroxide (3%) to 970 ml tap water to achieve 3% solution (v\v).

3.2.2 Sanitizing procedure

Experiment 1 , 720 nest-clean eggs were divided randomly to four equal treatment groups. Each group was divided into three 60-eggs

subgroups (replicates), All eggs in each replicate were numbered and initial weight was record for 10-eggs from each replicate. Eggs in treatment 1 (control) were fumigated by using formalin and potassium permanganate solution. Eggs in the second treatment were immersed in warm water (40C) for 5 minutes, then transferred to agri-germ solution and dipped for 2 minutes. Eggs in treatment three were immersed in warm water (40C) for 5 minutes, then were dipped in the 1% formalin solution (at 4C) for 2 minutes. Eggs in the fourth treatment were immersed in warm water (40C) for 5 minutes then transferred to 3% hydrogen peroxide solution and dipped for 2 minutes. Eggs in treatments 2,3 and 4 were fumigated as in treatments before transported to the hatchery.

Experiment 2, in experiment 2 similar treatments to experiment 1, of eggs in were used except that eggs in treatments 2,3 and4 were not fumigated following immersion into the sanitizers solutions.

Experiment 3, 270 nest-clean eggs were equally divided into three treatments according to time (1,3 or 6 hours) of dipping after collection.The eggs were then immersed in warm water for 5 minutes then transferred to a 3% hydrogen peroxide solution (4C) and dipped for 2 minutes Following. dipping, eggs stored until transported to the hatchery.

Experiment 4, in experiment 4, 120 floor (dirty) hatching eggs were divided into two treatment groups and eggs in each group were replicated twice. All eggs in each replicate were numbered and initial weights were recorded for the first ten eggs in each replicate, Eggs in the first (control) treatment were cleaned using sand paper, then were fumigated in the storage room using formalin and potassium permanganate. Eggs in the

second treatment were cleaned using sand paper, then eggs were immersed in warm water and dipped in 3% hydrogen peroxide solution for 5 minutes.

3.3 Incubation and hatching

All eggs were transported to a commercial hatchery located in the West Bank city of Jenin. Subsets of eggs per treatment in experiment 1 and experiment 4 were weighed prior to setting, then at day 8 ,15 and 18 of incubation. Eggs were incubated at 37.7c and 60% relative humidity. Due to logistic considerations related to commercial practices, egg were not candled until the 14th day of incubation when fertility was initially reported. On the 18^{th} day of incubation , the eggs were transferred to the hatcher and incubated at 37.2 C and 70% relative humidity for 3 days. At hatching **,** the alive chicks in each experimental treatment were counted, and all unhatched eggs were transported to the Faculty of Agriculture Laboratories. Unhatched eggs were then opened and the stage of embryo death was determined by visual inspection (early dead, 1-7 days; middle dead , 8-14 days; late dead , 15-21 days). Eggs that did not show early embryonic death were counted as infertile.

3.4 Post hatching performance of chicks

At hatching four sub-sets of 10 chicks each from each treatment in experiment 1 and experiment 4 were placed in 24 separate floor pens. Chicks were reared to 8-days period, during this period they were exposed to standard management conditions (Ensminger, 1990 , and North, 1984).

3.5 Statistical Analysis

Hatchability parameters, embryonic death, egg weight and chick performance of all experiments were subjected to (ANOVA) by treatment using the general models procedure of SAS (SAS institute, 2000). Duncans multiply range test compared means of different treatments within experiments, significance was accepted at ($P \leq 0.05$).

CHAPTER FOUR

RESULTS

RESULTS

4.1 Nest – clean hatching eggs sanitization

4.1.1 Experiment 1

Hatchability of fertile eggs was not influenced by the sanitation method. Eggs sanitized by means of fumigation, and agri-germ had numerically the highest hatchability. Percentages of embryo that died early after incubation were not influenced by the sanitization method, but dipping in formalin solution resulted in the highest number of early – dead embryos (table 1).

Table (1): The effect of chemical sanitizers on hatchability of nest-clean eggs and embryo mortality (experiment 1)

	Treatment			
Variable	**Control**	**Agri-germ dipping**	**Formalin dipping**	**H2O2 dipping**
Fertility (%)	**93.9a**	**94.4a**	**82.2b**	**92.8a**
Hatchability of all eggs (%)	**82.2a**	**82.2a**	**65.0b**	**80.0a**
Hatchability of fertile eggs (%)	**88.3a**	**87.7a**	**78.9a**	**68.2a**
Early dead (%)	**4.72a**	**2.97a**	**10.0a**	**5.97a**
Med dead (%)	**3.55a**	**2.38a**	**4.0a**	**6.0a**
Late dead (%)	**2.94a**	**4.63a**	**6.5a**	**3.58a**

(a,b) means within the same row with different superscripts are significantly different ($P \leq 0.05$)

4.1.2 Experiment 2

Eggs dipped in formalin solution (1%) and the controls had significantly the lowest hatchability compared to those dipped in agri-germ and hydrogen peroxide (table 2). Similarly numbers of early dead embryos

were significantly lower for eggs dipped in hydrogen peroxide and agri-germ solutions.

Table (2): The effect of chemical sanitizers on hatchability of nest-clean eggs and embryo mortality (experiment 2).

Treatment				
variable	**Control**	**Agri-germ dipping**	**Formalin dipping**	**H2O2 dipping**
Fertility (%)	**94.44b**	**93.33b**	**93.33b**	**100.0a**
Hatchability of all eggs (%)	**80.55b**	**83.9b**	**83.3b**	**99.44a**
Hatchability of fertile eggs (%)	**85.34b**	**90.0a**	**89.2b**	**99.44a**
Early dead (%)	**8.23a**	**7.1a**	**2.38a**	**0.00b**
Med dead (%)	**5.27a**	**2.9a**	**4.8a**	**0.55a**
Late dead (%)	**1.15a**	**1.71a**	**2.4a**	**0.00a**

(a,b) means within the same row with different superscripts are significantly different ($P \leq 0.05$)

4.1.3 Experiment 3

Results show that eggs dipped in hydrogen peroxide after one hour of collection had significantly the lowest hatchability compared to those dipped 3 and 6 hrs after collection. Eggs dipped in hydrogen peroxide after three hours of collection had significantly higher hatchability as percentage of all fertile eggs (table 3). Percentage of embryos that died early after incubation was influenced by the time of sanitizing eggs with hydrogen peroxide after collecting. Eggs dipped after three hours had significantly the highest hatchability compared to those dipped after one and six hrs of collection.

Table (3): The effect of time of dipping chemical sanitizers on hatchability of nest- clean eggs and embryo mortality (experiment 3).

Treatment			
variable	**H2o2 dipping (1 hour)**	**H2o2 dipping (3 hours)**	**H2o2 dipping (6 hours)**
Fertility (%)	**91.1a**	**95.55a**	**95.55a**
Hatchability of all eggs (%)	**74.4b**	**88.89a**	**83.3a**
Hatchability of fertile eggs (%)	**81.6b**	**92.94a**	**87.2a**
Early dead (%)	**10.85a**	**0.00b**	**6.94a**
Med dead (%)	**2.56a**	**3.53a**	**1.15a**
Late dead (%)	**4.95a**	**4.86a**	**3.5a**

(a,b) means within the same with different superscripts are significantly different ($P \leq 0.05$).

4.2 Sanitation of floor hatching eggs

Experiment 4

Hatchability of Fertile dirty eggs dipped in hydrogen peroxide (3%) was numerically higher than that of eggs sanitized by fumigation (formalin and potassium permanganate) (table 4). Hatchability of all eggs was not influenced by sanitization method. Also embryos died early in incubation were not significantly different.

Table (4): The effect of chemical sanitizers on hatchability of floor eggs and embryo mortality (Experiment 4).

Treatment		
variable	**Control**	**H2o2 dipping**
Fertility (%)	**86.6b**	**95.0a**
Hatchability of all eggs (%)	**65.0a**	**78.3a**
Hatchability of fertile eggs (%)	**75.0a**	**82.57a**
Early dead (%)	**13.46a**	**13.9a**
Med dead (%)	**3.84a**	**1.78a**
Late dead (%)	**7.7a**	**5.3a**

(a,b) means within the same with different superscripts are significantly different ($P \leq 0.05$).

4.3 Egg weight loss

Moisture loss data for samples of incubated eggs in experiments 1 and 4 are shown in tables 5 and 6 . Loss of egg weight (moisture loss) was not significantly different in the eggs dipped in agri -germ , formalin or hydrogen peroxide when compared with the control eggs.

Table (5): Egg weight and percentage weight loss of nest clean eggs (Experiment 1).

Treatment				
Variable	**Control**	**Agri-germ dipping**	**Formalin dipping**	**H2O2 dipping**
Egg weight loss (gm) Day 0 - day 8	**3.64**	**3.2**	**3.34**	**2.67**
Egg weight loss (gm) Day 8 - day 15	**6.9**	**7.1**	**6.9**	**6.0**
Egg weight loss (gm) Day 0 - day 18	**9.45**	**8.34**	**9.8**	**8.7**
Egg weight loss (%) Day 0- day 8	**5.5**	**4.76**	**5.0**	**4.0**
Egg weight loss (%) Day 8 - day 15	**10.5**	**10.5**	**10.4**	**9.2**
Egg weight loss (%) Day 0- day 18	**14.3**	**12.2**	**14.8**	**13.2**

A significantly greater loss off egg weight was found in floor eggs in control treatment compared with those dipped in hydrogen peroxide solution during the first eight days of incubation. This differences was not significant by the 18^{th} day of incubation. However, the control eggs lost numerically more weight by the 18^{th} day of incubation.

Table (6): Egg weight, and percentage weight loss of floor eggs (Experiment 4)

Treatment		
Variable	**Control**	**H2o2 dipping**
Egg weight loss (gm) Day 0 - day 8	**3.2a**	**1.75b**
Egg weight loss (gm) Day 8 – day15	**8.6a**	**6.7a**
Egg weight loss (gm) Day 0 - day 18	**12.25a**	**8.98a**
Egg weight loss (%) Day 0 - day 8	**4.75a**	**2.6b**
Egg weight loss (%) Day 8 - day 1	**12.95a**	**10.0a**
Egg weight loss (%) Day 0 - day 18	**18.43a**	**13.4a**

(a,b) **values with the same row followed by different superscript as significantly different (p≥0,05**).

4.4 Early chick performance

The data obtained from tables (7and 8) show that there were no significant differences in mortality percentage for chicks that hatched from clean eggs and sanitized with different methods; control group, agri germ dipping, formalin dipping and hydrogen peroxide dipping, besides body weight was not influenced by sanitization methods as well. Mortality, body weight gain of chicks in experiment 1 or 4 were not statistically different between treatments at 8- day (tables 7 and 8).

Table (7): Eight – day mortality , body weight gain and daily gain in chicks hatched from nest – clean eggs exposed to different sanitizers treatment (Experiment 1)

Treatment				
variable	**Control**	**Agri-germ dipping**	**Formalin dipping**	**H2O2 dipping**
Mortality (%)	**0.00**	**2.5**	**0.00**	**2.5**
Body weight gain(%)	**87.1**	**90.75**	**97.3**	**95.25**
Daily gain (%)	**10.9**	**11.34**	**12.17**	**11.9**

Table (8): Eight – day mortality, body weight gain and daily gain in chicks hatched from nest – clean eggs exposed to different sanitizers treatment (Experiment 4).

Treatment		
Variable	**Control**	**H2o2 dipping**
Mortality (%)	**2.5**	**0.00**
Body weight gain(%)	**96.8**	**96.0**
Daily gain (%)	**12.10**	**12.0**

CHAPTER FIVE

DISCUSSION

Discussion

Fumigation is a dominant commercial tool for disinfection of hatching eggs in large commercial incubators. However the fumigation process can be hazardous to the producers and care-takers if not conducted carefully. In addition fumigation pose a threat to producers upon repeated or prolonged exposure to formaldehyde. Previous research (Scott and Swetnam, 1993) indicated that dipping in formalin solution was more efficient than fumigating in decontamination of hatching eggs. It has also been suggested that creating a temperature differential between (37C) and dipping formalin solution (at 4C) (Pardon ,1995) forces the formalin into the eggs pores, thus increasing the level of sanitation both inside and outside the shell. Similarly, in our study eggs were placed in warm water at 40 C for about 5 minutes and the immersed in agri-germ, formalin or hydrogen peroxide solutions. Early embryonic mortality is usually attributed to egg cleanness (Cox *et al.*, 2000), whereas hatchability of fertile eggs is usually indicates the success of the incubation and hatching process (Lesson and Summers, 2000). Therefore, more attention was paid to these two parameters when the different solution treatments in our research were compared. Although no significant differences were observed, in hatchability of fertile eggs and early dead embryos (table 1), they were better in the control and in those treated with agri-germ. These results disagree with those reported by (Sheldon *et al*., 1991). When was used 5% hydrogen peroxide solution as a substitute for formaldehyde, which in our experiment 3% hydrogen peroxide solution was used. It is not possible to explain the lower hatchability of fertile eggs (78.9 and 68.2 %) treated with formalin and hydrogen peroxide solution compared to those in

the control and agri-germ treated eggs. One possible explanation is that eggs treated with formalin or hydrogen peroxide were originally highly contaminated which contributed to the inactivation of disinfectants. The situation was different in experiment 2 (table 2) in which hatchability of fertile eggs was significantly higher for eggs dipped in hydrogen peroxide or in agri-germ solution compared to those dipped in formalin solution or in the control treatment. Our results agree with a previous research by (Cox *et al*., 1994a).

Percentages of early dead were significantly lower for embryos in eggs dipped in hydrogen peroxide and formalin solutions compared to those in the control and agri-germ treatments. It is worth noting that eggs in the control treatment were fumigated twice, the first in the farms storage room, and the second upon arrival to the hatchery. Our results indicated that disinfection of eggs as soon as they are collected could be satisfactory to ensure sanitation of nest clean hatching eggs. This procedure is expected to save time, effort and cost, and to reduce embryonic mortality associated with excessive handling and movement of hatching eggs. Both experiments indicated that hatchability and early embryonic mortality are affected not only by disinfection process, but also by immersion of eggs in warm water prior to dipping. Scott *et al.* (1993) indicated that washing hatching eggs by disinfectants which contained EDTA caused reductions in hatchability of 11 to 26%. These compounds caused below normal moisture loss during incubation ranging from 16 to 19% less than the formaldehyde treated standards. In our experiments (1and 4) percentage egg weight loss ranged from 12.2 to 14.8% in experiment 1 and from 13.4 to 18.43% in experiment

4. Our results are in agreement with those reported by (Padron 1995, Sandars *et al*., 1999).

None of the disinfectants used in our study had negatively affected egg weight loss during incubation. However, floor eggs in experiment 4 that were rubbed by sand-paper had relatively the highest egg weight loss during incubation. This explains why these eggs had numerically low hatchability compared to those that were dipped in hydrogen peroxide following cleaning by sand-paper. Scott *et al*. (1993) reported that hydrogen peroxide caused an increased loss of moisture from the eggs during incubation but did not affect hatchability. In our study (experiment 2) eggs which were dipped in hydrogen peroxide had significantly higher hatchability compared to those in the control or those that were dipped in the formaldehyde solution (table 2). Wilson. (2003) concluded that the time lapsed from contamination of hatching eggs to treatment with a disinfectant is vital to the success of the disinfection. The author also reported that the results of disinfection are influenced the timing of treatment and the type of disinfection.

Our results are in disagreement with the above conclusions in that eggs that were treated with hydrogen peroxide 1 hr, 3hrs or 6 hrs following collection did not differ significantly. However out results do agree with those of by (Buhr *et al*., 1993) who found that the effects of disinfection of nest clean eggs or dirty eggs ranged from no effect on hatchability to increase of 2 percentage points for sanitized dirty eggs. The lack of differences in hatchability of eggs treated at different time intervals in experiment 3 of our study can be explained by the fact that nest clean eggs

that where used are probably not heavily contaminated. Also the time lapsed from collection (6hrs) may not be enough to cause severe contamination of nest clean eggs.

Limited numbers of research have dealt with the effect disinfection. process on broiler production parameters. Sanders and Wilson. (1991) and Cox *et al*. (2002) concluded that the use of hydrogen peroxide as a hatchery sanitizers did not affect broiler livability, body weight, or feed conversion but did not reduce the incidence of retained yolk sacs in 42-day-old chickens. These authors exposed hatching eggs to either distilled water or 3% hydrogen peroxide fogged into the incubators during the incubation period. Our results agree with those of (Sander and Wilson, 1999). By 8 days of age, body weight, mortality and daily gain of chicks hatched from nest – cleans eggs (table 7) were not significantly different among treatments. Similar results were found when dirty eggs were treated with hydrogen peroxide compared to those in the control treatment. However that body weight gain was higher for chicks from hatching eggs that were dipped in formalin solution or hydrogen peroxide. Sander and Wilson. (1999) concluded that the use of hydrogen peroxide in commercial poultry hatcheries may be a reasonable alternative to formaldehyde. Our findings also indicated that dipping practice in hydrogen peroxide is appropriate and may replace the current practice of fumigating hatching eggs in the in farm storage room and in the hatchery. These finding are supported by (Wilson, 2003) who reported that immersion of the egg in the disinfectant was more effective than a spray which in turn was more effective than foam application.

Conclusions and Recommendations

In conclusion, on-farm dipping of hatching eggs (nest – clean or dirty) followed by immersion in worm water may be a reasonable practice to decontaminate hatching eggs prior to setting.

Hydrogen peroxide is a reasonable disinfectant due to its relative user- and environmental friendliness. One – twice dipping procedure could be used successfully to replace the common two – step fumigation procedure.

Hatchability and early chick mortality were not adversely effected by any of the incubation treatments.

Because of hazardous associated with fumigation or formalin dipping , it would probably requires to adapt alternatives , especially hydrogen peroxide dipping , to be used in a commercial hatcheries.

More attention should be given to produce clean eggs , since this will make the decontamination easier. In addition more work is required to deal safely with the dirty eggs. More research is needed on the best means of reducing the number of floor eggs in – farm and to sanitize dirty hatching eggs.

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APPENDICIES

Appendix (1)

Data of the effects of chemical sanitizers on hatchability of nest- clean eggs and embryo mortality (experiment 1).

Treatment	Replicate	Number of eggs	Hatch of fertile eggs	Hatch of all eggs	Non hatching eggs	Non fertile eggs	Early dead embryos	Med. dead embryos	Late dead embryos
Control	1	60	57	51	9	3	2	1	3
Control	2	60	55	50	10	5	2	2	1
Control	3	60	57	48	12	3	4	3	1
Agri-germ	1	60	55	51	9	5	2	2	0
Agri-germ	2	60	58	53	7	2	0	0	5
Agri-germ	3	60	57	45	15	3	3	2	3
Formalin 1%	1	60	54	44	16	6	3	0	5
Formalin 1%	2	60	50	32	28	10	9	6	4
Formalin 1%	3	60	45	41	19	15	3	0	1
Hydrogen peroxide 3%	1	60	56	48	14	4	4	4	3
Hydrogen peroxide 3%	2	60	56	47	13	4	4	2	2
Hydrogen peroxide 3%	3	60	55	49	11	5	2	4	1

Appendix (2)

Data of the effects of chemical sanitizers on hatchability of nest- clean eggs and embryo mortality (experiment 2).

Treatment	Replicate	Number of eggs	Hatch of fertile eggs	Hatch of all eggs	Non hatching eggs	Non fertile eggs	Early dead embryos	Med. dead embryos	Late dead embryos
Control	1	60	58	47	13	2	5	4	2
Control	2	60	56	47	13	4	6	3	0
Control	3	60	56	51	9	4	3	2	0
Agri-germ	1	60	54	53	7	4	2	1	0
Agri-germ	2	60	55	47	13	5	6	1	1
Agri-germ	3	60	59	51	9	1	4	3	1
Formalin 1%	1	60	57	55	5	3	1	1	0
Formalin 1%	2	60	56	48	12	4	2	3	2
Formalin 1%	3	60	55	47	13	5	1	4	2
Hydrogen peroxide 3%	1	60	60	59	1	0	0	1	0
Hydrogen peroxide 3%	2	60	60	60	0	0	0	0	0
Hydrogen peroxide 3%	3	60	60	60	0	0	0	0	0

Appendix (3)

Data of the effects of chemical sanitizers on hatchability of nest- clean eggs and embryo mortality (experiment 3).

Treatment	Replicate	Number of eggs	Hatch of fertile eggs	Hatch of all eggs	Non hatching eggs	Non fertile eggs	Early dead embryos	Med. dead embryos	Late dead embryos
Hydrogen peroxide (1 hr)	1	30	26	21	9	4	2	1	2
Hydrogen peroxide (1hr)	2	30	26	21	9	4	3	1	1
Hydrogen peroxide (1 hr)	3	30	30	25	5	0	4	0	1
Hydrogen peroxide (3 hrs)	1	30	28	24	6	2	0	2	2
Hydrogen peroxide (3 hrs)	2	30	29	28	2	1	0	0	1
Hydrogen peroxide (3 hrs)	3	30	29	28	2	1	0	1	1
Hydrogen peroxide (6 hrs)	1	30	29	25	5	1	2	1	1
Hydrogen peroxide (6 hrs)	2	30	29	24	6	1	3	0	1
Hydrogen peroxide (6 hrs)	3	30	28	26	4	2	1	0	1

Appendix (4)

Data of the effects of chemical sanitizers on hatchability of floor eggs and embryo mortality (experiment 4).

Treatment	Replicate	Number of eggs	Hatch of fertile eggs	Hatch of all eggs	Non hatching eggs	Non fertile eggs	Early dead embryos	Med. dead embryos	Late dead embryos
Control	1	30	26	22	8	4	3	0	1
Control)	2	30	26	17	13	4	4	2	3
Hydrogen peroxide 3%	1	30	29	22	8	1	6	0	1
Hydrogen peroxide 3%	2	30	28	25	5	2	2	1	2

Appendix (5)

Data of the effects of chemical sanitizers on egg weight and percentage weight loss of nest clean eggs (Experiment 1)

Treatment	Replicate	Egg weight loss (gm) Day 0 - day 8	Egg weight loss (gm) Day 8 - day 15	Egg weight loss (gm) Day 15 - day 18	Egg weight loss (gm) Day 15 - day 18
Control	1	66	61.7	57.1	53.6
Control	2	62.2	59.6	56.9	55
Control	3	69.9	66.4	63.1	60.8
Control	4	64.3	61	57.7	55.2
Control	5	59.5	56.3	53.1	50.6
Control	6	67.6	63	60.2	57.6
Control	7	66	62.7	59.3	56.6
Control	8	67.2	63.7	60.7	58.3
Control	9	67.9	63.5	60.2	57.8
Control	10	67	63.3	60	57.6
Agri-germ	1	64.7	61.9	59.4	57.5
Agri-germ	2	68.3	65.5	52.6	60.2
Agri-germ	3	72.6	69.2	65.8	63
Agri-germ	4	68.2	64.8	61.1	58.3
Agri-germ	5	67.8	64.3	62	59.9
Agri-germ	6	68.7	63.7	61	58.6
Agri-germ	7	66.8	65	61.8	59.1
Agri-germ	8	67.1	64.4	61.9	59.9
Agri-germ	9	66.5	63.4	60.2	57.9
Agri-germ	10	68.8	66.3	64.2	62.3
Formalin 1%	1	64.2	61.1	57.6	55
Formalin 1%	2	63.1	60.5	57.7	55.6

Treatment	Replicate	Egg weight loss (gm) Day 0 - day 8	Egg weight loss (gm) Day 8 - day 15	Egg weight loss (gm) Day 15 - day 18	Egg weight loss (gm) Day 15 - day 18
Formalin 1%	3	69.1	66	62.9	60.5
Formalin 1%	4	60.7	57.1	53.4	50.6
Formalin 1%	5	66.9	63.4	59.7	56.5
Formalin 1%	6	61.8	58.7	55.4	52.8
Formalin 1%	7	66.6	60.9	55.2	50.5
Formalin 1%	8	74.8	71	67.1	63.8
Formalin 1%	9	66.8	63.3	59.8	57
Formalin 1%	10	67.5	64.5	61.6	59.2
Hydrogen peroxide 3%	1	65	61.7	58.7	56.4
Hydrogen peroxide 3%	2	65.2	61.7	58.4	55.9
Hydrogen peroxide 3%	3	57.3	53.4	49.5	46.3
Hydrogen peroxide 3%	4	68.8	65.7	61.1	58.4
Hydrogen peroxide 3%	5	65	61.1	57.9	55.1
Hydrogen peroxide 3%	6	68	65.2	62.4	60.2
Hydrogen peroxide 3%	7	70.4	67.1	63.7	61.1
Hydrogen peroxide 3%	8	66.7	62.8	59	59.9
Hydrogen peroxide 3%	9	70	66.5	63.3	60.5
Hydrogen peroxide 3%	10	62.3	66.3	63.8	61.6

Appendix (6)

Data of the effects of chemical sanitizers on egg weight and percentage weight loss of floor eggs (Experiment 4)

Treatment	Replicate	Egg weight loss (gm) Day 0 - day 8	Egg weight loss (gm) Day 8 - day 15	Egg weight loss (gm) Day 15 - day 18	Egg weight loss (gm) Day 15 - day 18
Control	1	71.3	66.4	61.8	58.2
Control	2	70.7	66.1	63.9	61.2
Control	3	66.2	61.4	41.5	29.6
Control	4	73.3	68.9	66.6	63.7
Control	5	70.3	65.9	64.1	61.5
Control	6	63.5	62.4	53.8	50.6
Control	7	66.7	65.9	59.2	56
Control	8	66.8	63.6	58.7	57.1
Control	9	67.7	64.8	62.3	59.9
Control	10	69	63.7	59.8	51.6
Control	11	65.7	62.3	60.1	57.7
Control	12	62.7	58.2	54.9	51.8
Control	13	60.1	59.9	49.4	46.1
Control	14	72.3	69.3	65.6	62.9
Control	15	65.8	61.9	58.7	56.1
Control	16	66.2	64.5	53.4	50.8
Control	17	60	58.2	51.8	47.2
Control	18	64.4	58.7	57.4	54.5
Control	19	68.4	66.1	55.9	53.1
Control	20	63.3	62.3	63.7	60.2
Hydrogen peroxide 3%	1	70	66.9	62.5	60
Hydrogen peroxide 3%	2	69.1	67.4	58.8	55.4

Treatment	Replicate	Egg weight loss (gm) Day 0 - day 8	Egg weight loss (gm) Day 8 - day 15	Egg weight loss (gm) Day 15 - day 18	Egg weight loss (gm) Day 15 - day 18
Hydrogen peroxide 3%	3	63.4	62.4	59.2	57.5
Hydrogen peroxide 3%	4	71.8	71.3	65.8	63.4
Hydrogen peroxide 3%	5	67.7	67.5	62	58.8
Hydrogen peroxide 3%	6	68.5	67.7	59.8	57.7
Hydrogen peroxide 3%	7	66.4	63	58.7	55.4
Hydrogen peroxide 3%	8	62.5	60.7	60.1	57.2
Hydrogen peroxide 3%	9	66.5	65	59.1	57
Hydrogen peroxide 3%	10	68	66.5	56.9	54.7
Hydrogen peroxide 3%	11	64.1	62.9	56.8	53.1
Hydrogen peroxide 3%	12	68.1	66.2	61.3	59.3
Hydrogen peroxide 3%	13	61.7	54.8	53.7	52.8
Hydrogen peroxide 3%	14	71.3	69.1	67.4	65.9
Hydrogen peroxide 3%	15	63	62.4	61.4	60.9
Hydrogen peroxide 3%	16	66.8	65.9	61.7	60
Hydrogen peroxide 3%	17	67	64	55.5	53.3
Hydrogen peroxide 3%	18	61.3	61.1	55.8	53.7
Hydrogen peroxide 3%	19	66	65.4	63.2	60.8
Hydrogen peroxide 3%	20	69.8	67.8	59	56.5

Appendix (7)

Data of the effects of chemical sanitizers on chick performance of nest-clean eggs and embryo mortality (Experiment 1).

Treatment	Replicate	Chick weight day (0)	Chick weight day (8)	Mortality percentage
Control	1	39	122.5	0
Control	2	39	120	0
Control	3	39.5	130	0
Control	4	38.5	132	0
Agri- germ	1	39	131	0
Agri-germ	2	38.5	122	10
Agri- germ	3	38.5	132	0
Agri- germ	4	41	135	0
Formalin 1%	1	38.5	132	0
Formalin 1%	2	41	123	0
Formalin 1%	3	37.5	150	0
Formalin 1%	4	38.5	132	0
Hydrogen peroxide 3%	1	38.5	120	10
Hydrogen peroxide 3%	2	38.5	132	0
Hydrogen peroxide 3%	3	38	141	0
Hydrogen peroxide 3%	4	37	140	0

Appendix (8)

Data of the effects of chemical sanitizers on chick performance of floor eggs and embryo mortality (Experiment 4).

Treatment	replicate	Chick weight day (0)	Chick weight day (8)	Mortality percentage
Control	1	38	152	0
Control	2	40	130	0
Control	3	38	129	0
Control	4	39.5	132	0
Hydrogen peroxide 3%	1	38.5	132	0
Hydrogen peroxide 3%	2	38	140	0
Hydrogen peroxide 3%	3	38.5	142	0
Hydrogen peroxide 3%	4	39	124	0

Appendix (9)

Chemical composition of agri-germ.

A combination of 4 different active materials :

1- Didecyl Dimethyl Ammonium Chloride (100g/L)

2- Formaldehyde (31.5g/L)

3- Glutaraldehyde (40g/L)

4- Glyoxal (32g/L)

Appendix (10)

Properties of disinfectants.

Property	Chlorine	Iodine	Phenol	Quaternary Ammonium Compounds	Formaldehyde
Bactericidal	+	+	+	+	+
Bacteriostatic	_	_	+	+	+
Fungicidal	_	+	+	±	+
Virucidal	±	+	+	±	+
Toxicity	+	_	+	+	+
Activity with organic matter	+ + + +	+ +	+	+ + +	+

Source: Canadian Dept. Agr., Hatchery Sanitation, 1970.

جامعة النجاح الوطنية
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فراس طلال محمد بليه

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قدمت هذه الأطروحة استكمالاً لمتطلبات درجة الماجستير الإنتاج الحيواني بكليـــة الدراســـات العليا في جامعة النجاح الوطنية/ نابلس فلسطين.
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الملخص

يستخدم التبخير بواسطة الفورمالدهايد بشكل روتيني لتطهير بيض التفقيس في مـــزارع أمهات دجاج اللحم والفقاسات. محليا لم يستخدم تغطيس بيض التفقيس في محاليـــل مطهـــرات متنوعة كوسيلة لتطهير بيض التفقيس. ولأن تطهير بيض التفقيس مسألة هامه فقد أجريت هـــذه الدراسة لتحديد تأثير طرق تطهير مختلفة على نسبة التفقـــيس و نقـــص وزن البـــيض أثنـــاء الحضانة وموت الأجنة المبكر وأداء الأفراخ بعد التفقيس. أستخدم في هذه الدراسة بيض تفقيس أعشاش نظيف وبيض متسخ (وضع على أرضية المز رعه) حيث تم تطهير هذا البيض بواسطة التبخير بالفورمالدهايد (الشاهد) أو بالتغطيس في ماء دافئ (حرارة 40 درجه مئويه) لمدة خمس دقائق ثم بالتغطيس في أحد المطهرات التاليه: 1% فورمالين أو أجري _جيرم (مطهر تجاري) أو 3 % فوق أكسيد الهيدروجين. تبين أن نسبة نفوق الأجنة ونسبة التفقيس لم يتأثرا بمعـــاملات التطهير المختلفة الا أنهما كانا أفضل لبيض التفقيس الذي تم تغطيسه في محلول فـــوق أكســـيد الهيدروجين. لم تؤثر آي من معاملات التطهير المختلفة على مقدار الـــنقص فـــي وزن بـــيض التفقيس على مدار الثمانية عشر يوما الأولى من حضانة البيض. أظهرت نتـــائج الدراســـة أن تغطيس بيض التفقيس في خطوة واحده في المزرعة عوضًا عن تبخير البيض في المزرعة ثـــم في الفقاسه يشكل بديلاً ملائماً. تبين ايضاً أن أي من معاملات التطهير المختلفة لم تـــؤثر علـــى أداء الأفراخ ونسبة النفوق فيها حتى عمر ثمانية أيام. خلصت الدراسة إلى أن تطهيـــر بـــيض التفقيس بواسطة التغطيس في محلول مطهر داخل المزرعة ولمرة واحده قد يكون بديلاً جيـــداً لطريقة التطهير المتبعة تجارياً وهي تبخير البيض في المزرعة ثم في الفقاسه قبـــل الحضـــانة وأنه من الممكن استخدام فوق أو كسيد الهيدروجين بديلاً عن الفورمالين الذي يستخدم حالياً.

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