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EVALUATION OF MICROBIAL STATUS OF BREEDER'S EGG

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BY

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Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Kedoktoran Veterinar dan Sains Peternakan untuk memenuhi sebahagian daripada keperluan kursus VPD 401 – Projek Perindustrian Ternakan.

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oleh

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1989

Penyelia : Professor Madya (Dr) Kassim Hamid

Satu kajian telah dijalankan di Ladang Ayam Pembiak Pedaging di Serting Tengah, Negeri Sembilan dari 6hb. November hingga 22hb. Disember. 1988. Tujuan kajian ini adalah untuk menentu dan menilai jenis dan populasi mikroorganisma pada permukaan luar kulit telur dari berbagai peringkat pengendalian telur di ladang sehingga

ke peringkat inkubasi di unit penetasan.

Dari kajian ini didapati populasi mikroorganisma bertambah pada setiap peringkat pengendalian telur terutamanya selepas penyimpanan-sejukan (Bakteria = 68.09% dan Kulat = 80.00%) dan juga selepas lapan belas hari tempoh inkubasi (Bakteria = 58.63%). Didapati juga jumlah bakteria adalah berkurangan selepas peringkat fumigasi.

Walaubagaimanapun, jumlah mikroorganisma yang dimusnahkan selepas fumigasi adalah kurang daripada 99 %, berkemungkinan disebabkan oleh ketidaksempurnaan fumigasi samada di ladang atau di unit penetasan. Jenis mikroorganisma yang dikenalpasti kebanyakannya adalah Mikrokokus, Stafilokokus, Streptokokus dan bakteria Kolifom dan juga lain-lain bakteria gram negatif dan kulat tetapi dalam jumlah yang sedikit.

Adalah didapati, bakteria gram positif adalah kurang patogenik kepada embrio berbanding dengan bakteria Kolifom dan lain-lain bakteria gram negatif atau kulat. Kebanyakan mikroorganisma yang patogenik seperti Salmonella, Pseudomonas dan bakteria Kolifom dimusnahkan melalui fumigasi yang sempurna dengan gas formaldehyde.

Walaubagaimanapun, langkah pengawalan perlulah diambil disebabkan telur boleh dikontaminasi melalui pemindahan vertikal oleh kerana organisma tersebut sukar untuk dimusnahkan.

Oleh itu, untuk mengatasi masalah pemindahan vertikal, telur-telur yang dipilih untuk penetasan mestilah datang daripada stok induk yang bebas daripada organisma patogenik yang boleh menyebabkan peratus penetasan yang rendah dan anak ayam yang lemah.

Sehubungan dengan itu, beberapa cadangan dari berbagai aspek pengurusan telah diberikan untuk mengurangkan kontaminasi telur yang akan meningkatkan peratusan penetasan dan jangkahayat anak ayam.

ABSTRACT

An abstact of the project paper presented to the Faculty of Veterinary Medicine and Aniamal Science in partial requirement of the course VPD. 401 - Animal Industry Project.

EVALUATION OF MICROBIAL STATUS OF BREEDER'S EGG

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Supervisor : Associate Professor (Dr) Kassim Hamid

A field study was carried out at the Broiler Breeder Farm in Serting Tengah, Negeri Sembilan from November 6 until December 22, 1988. The objectives of this study are to determine and evaluate the type and population of microorganisms on the outer surface of egg shells at the various stages of egg handling in the farm until incubation stage in the hatchery.

From this study, it was found that the

microorganisms population increased at every stage of egg handling especially after the cold-storage (Bacteria = 68.09% and Moulds = 80.00%) and after the 18th day of incubation (Bacteria = 58.63%) and the number of microorganisms were decreased after the fumigation stages. However, the destruction of microorganisms was less than 99%, probably due to inadequate fumigation either in the farm or hatchery. The types of microorganisms which were identified were mostly Micrococcus, Staphylococcus, Streptococcus and Coliform bacteria and less for other gram negative bacteria and moulds.

It was also found that the gram positive bacteria are less pathogenic to embryo compared to Coliform bacteria and other gram negative bacteria or moulds. Most of the pathogenic organisms such as Salmonellae, Pseudomonas, Coliform bacteria were destroyed by proper fumigation with formaldehyde gas.

Since eggs can be contaminated by vertical transmission, precautions should be undertaken since the the organism is difficult to be destroyed. To overcome this problem, hatching eggs should be selected from the parent stock which are free from pathogenic bacteria. This is important because of the negative effect of the pathogenic bacteria on the percentage hatchability and production of

day-old chicks.

Therefore, some recommendations on various aspects of management were made to reduce contamination of eggs which will increase the percentage of hatchability and livability of chicks.

INTRODUCTION

Microbial contamination, and the subsequent results of this contamination of eggs intended especially for hatching purposes and also for human consumption, have attracted attention of several workers. As early as 1939, Haines observed that the egg is equipped with physical and chemical defences against microbial infection and it has been suggested that these defences have been developed to protect the developing embryo. Contamination and rotting of eggs take place when physical and chemical defences become overloaded or disrupted.

When egg has been laid, it is usually moistened and become soiled at the same time. The presence of dirt in the surrounding environment adds to the number of contaminating organisms. The only pathway through which bacteria can get into the interior part of the egg is via the pores because the humid condition promotes growth of mould and facilitates the enlargement of pores, which helps the entry of bacteria into the egg.

The major source of egg contamination is bacteria and fungi on the shell surface. Bacterial and fungal contamination on egg's shell frequently originate either from the farm egg holding or after the eggs are placed in the setters. This contamination is usually occurs due to

"exploders" or "leakers" which contaminate the egg.

The degree of egg contamination appears to be a function of the cleanliness of the surface onto which they are laid, and the manner in which the eggs are handled after laying, which produces the greatest effect when the egg become cracked.

The level of contamination on the shell of eggs collected with gloved hands was less than that when the eggs were handled normally (without glove). It also varies with the standard of hygiene of the farm. Environmental conditions such as temperature and length of storage as well as any treatment they received such as washing, buffing, sanding, fumigation also exert an effect. Handling, storage, incubation and hatching procedures may have a direct bearing on the rate of bacterial penetration which could cause embryo contamination.

The normal microflora of the egg shell is dominated by gram positive bacteria which may originate from dust, soil or faeces while the rotten and unhatched eggs normally contain a mixed infection of gram positive and gram negative bacteria.

The objectives of this study are

i) to determine the type and population of microorganisms which are present on the shell of eggs following farm sanitation, traying at

hatchery and after the 18th day of incubation

ii) to determine the operational stages of bacterial contamination and infection as a cause of embryonic death and early chick mortality

iii) to develop procedures which would prevent extraneous contamination of the eggs from the time of collection through hatching

iv) to evaluate the general management practices in the farm

v) to provide some recommendation to improve this farm operation.

LITERATURE REVIEW

Bacteria on egg shells have been implicated as a source of contamination of broken-out eggs. Bacteria on shells may also, under certain conditions, penetrate through the shells into the interior and cause spoilage (Board, 1968). An indirect cause of contaminated or dirty egg is due to faulty or insufficient ventilation in the laying house. Drops of water formed by condensation in a poorly ventilated house fall from an uninsulated roof on to the litter which become damp. The birds get their feet dirty in the damp litter and transfer the dirt to the eggs by walking on them.

The composition of the bacterial flora of surface in the environment of the egg also, could be as important factor influencing the incidence of rots as the degree of spoilage of the shells, so that in certain cases superficially clean egg could have a high incidence of rots on storage (Miller et al,1950). Shell contamination due to the penetration of bacteria of the dirty or soilage shells will eventually cause the eggs to rot and could not be hatched. However, Quarles et al (1970) has stated that there were no significant correlations between bacteria in the air and hatchability or bacteria on the egg shells and hatchability. A possible reason for this could be due to

a relatively few of the total bacteria involved. Only a small percentage that penetrate the egg was lethal to the embryos where the coliform bacteria are common but vary greatly in their pathogenicity for embryos and chicks (Gentry, 1968). If possible, eggs for hatcheries should be produced in as clean a condition to prevent the entry of bacteria through shell contamination (Watson, 1969).

Since 1961, there has been a growing interest in better sanitary practices and standard for the production of hatching eggs. Attention has been directed to the microbial contaminants that reach the egg surface from the air, dirty nest and floor litter, and unclean collection and storage equipment. The bacterial flora on the surface of the egg can infect and kill the developing embryo before hatching or the chick after hatching or they may lower hatchability (Chute and Gershman, 1961; Gentry et al, 1962; and Magwood, 1964).

The number and types of bacteria on the shell depends on season and degree of sanitation on the farm and in the hatchery (Board, 1966). Bacteria in the air were directly correlated with bacteria on eggs. When number of bacteria in the air increased there was an increase in the number of bacteria on the eggs (Quarles et al, 1970). Normally, eggs from flocks on wire routinely has less gross contamination than eggs from conventional litter floor

houses. Bacterial counts on the eggs produced in litter floor houses were 20 to 30 times greater than on eggs produced in wire floor houses (Quarles et al, 1970).

Floor and slat eggs, if possible should be separated from the nest clean eggs. Floor eggs, even though their appearance may be identical to nest eggs, can carry as many as 10,000 times the number of microoganisms as nest eggs. Dirty eggs, especially floor eggs will carry a large number of microorganisms into the hatching environment where condition are ideal for the multiplication of bacteria. Dirty eggs also, have potential for exploding in the incubator and contaminating all the surrounding eggs (Anon, 1978a, 1978b. 1980 and 1983).

Eggs which have become rotten during storage have been the subject of many investigations and it has been shown that gram negative bacteria are the principle contaminants. The presence of coliform bacteria in dead embryo and cull chicks has been suggested that their concentration in the air of the poultry houses may be an important contributing factor. The frequent isolation of coliform bacteria indicates that egg contamination and penetration of the shell must have taken place (Quarles et al, 1970). The shell thickness also, has a major effect on the ability of bacteria to pass through the egg shell. The specific gravity of the egg was used as a criterion for

classifying the eggs either the egg is poor, average or good because it is directly related to the shell thickness or shell quality Only 21 % of good quality shells had been penetrated after 24 hours, while for poor quality shell about 34 % has been penetrated and less than 30 minutes (Table i; Sauter and Peterson, 1974).

Table i : The relationship between shell quality and bacterial penetration.

Specific gravity of eggs	Shell quality	Bacterial penetration of shell (%) After 20 min	After 60 min	After 24 hour
1.070	poor	34	41	54
1.080	average	18	25	27
1.090	good	11	16	21

Formaldehyde fumigation was demonstrated to be very effective in destroying most of the normal bacterial flora to which chicken hatching eggs are exposed after being laid. Only clean eggs were fumigated because formaldehyde gas cannot adequately penetrate to kill bacteria which are under dirt, manure or egg smears on the shell. It is more

successful if done using the high temperature between 21.1 - 32.2 degree Celcius and 90 % humidity (Beesley, 1980).

Furthermore, fumigation is done only on newly clean hatched egg, shortly after being layed and before the eggs has time to cool. This is a further safeguard against penetration of harmful bacteria such as Salmonellae, Escherichia Coli, Pseudomonas etc and fungi from contaminated eggs shells' into the newly clean hatched eggs via shell-pores during the cooling process. Therefore, care must be taken not to reinfect the hatching egg through the use of dirty, contaminated egg filler flats, cages and/or incubator trays because there is little or no residual action against subsequent shell contamination. Dust and employees unwashed hands can also be vehicle for recontamination (Beesley, 1980 and Hunton, 1982). In addition, it is worthwhile to note that the contamination of eggs is not only cause by bacteria penetrating into the egg shells but also can be caused by the hen itself which is known as vertical transmission.

Buffing and sanding of dirty hatching eggs should be avoided because it will remove the hatching eggs' protective cuticle and can result in the dirt and microorganisms being grinded into the pores of the shells. The washing of hatching egg also, will remove the egg's protective cuticle, thereby making the egg more susceptible

to microbial penetration from the outer surface and lead to further contamination and deterioration of egg quality (Warfield, 1984).

Prior to incubation, formaldehyde fumigation of hatching eggs on the farm are very useful as a routine procedure in order to lower the normal bacterial contamination on the surface of egg shell. In general, there was a decrease in the bacterial population on the shell as the concentration of formaldehyde fumigant increases. According to Williams (1970), destruction of bacteria was greater on brown-shell eggs even though bacterial population were consistently higher on unfumigated brown eggs. This factor is believed to be due to cuticular differences between brown- and white-shell egg which contribute to higher absorption levels of the fumigants on brown eggs.

Lastly, the most effective method of controlling eggshell-borne infection is the production of eggs free of shell contamination. Close attention to the eggs at the breeder house offers the primary means of breaking the natural transmission cycle of the infection. For example, the use of Salmonella-free eggs from Salmonella-free flocks will reduce the risk of introducing the infection into the hatchery (Halvorson, 1974).

MATERIALS AND METHODS

Breed of breeder bird

The farm reared Arbor Acres breed of chicken that produce white shell eggs. This breed was imported from Thailand and Holland. Age of birds in this study, is around 53 weeks. The birds were kept on the deep litter system and nest boxes are lined with wood shavings.

Collection of egg

Eggs were collected 4 times a day, at 8.00 a.m, 10.00 a.m, 1.00 p.m, and 3.00 p.m. This is done in the effort to reduce dirty or soiled eggs and cracked eggs. During collection of the eggs, the workers wore gloves to reduce eggs' contamination. Floor or dirty eggs and nest eggs that were collected were placed in a separate flat at the time of collection of the eggs.

Management of the breeders' egg

Fumigation was done immediately after all the eggs were collected. Before that, the eggs were sprayed

with 1 % of Chicgard solution (disinfectant). The eggs were fumigated in a wooden box with formaldehyde gas concentration) from a mixture of 60 g of potassium permanganate and 120 ml formalin for 20 minutes (3 X concentration). The wooden box measured 0.9 cubic meter and has a capacity of 1440 eggs. and located outside the breeder house.

Immediately after the eggs were fumigated, the eggs were brought to the hatchery unit and the second fumigation was done with formaldehyde gas, using 85 g Alphagen Standard (Paraformaldehyde) in a 25.5 cubic meter fumigation room for 20 minutes.

Grading of the eggs was done either manually or automatically 50 minutes later. Then the eggs were stored in a storing room (cold-room) up to 3 days at 19.4 to 20.5 degree Celcius with 79 - 90 % relative humidity.

The eggs were transferred to the incubator on Mondays and Thursdays of every week. Apart from that, the eggs were kept in the incubator until the 18th days under the temperature of 36.7 to 37.8 degree Celcius and 50 to 60 % of relative humidity. Fumigation was also done in the incubator using 350 g of potassium permanganate and 700 ml of formalin for 30 minutes. The incubator measured about 175 cubic meter.

However, fumigation is only done on Mondays and

not on Thursday since by Thursday only 72 hours has lapsed since incubation. If fumigation is carried out between 24 to 96 hours of incubation, formaldehyde gas will destroy the embryos.

Collection of sample

In this study, 31 nest eggs were selected from this farm. A simplified method of measuring egg-shells contamination utilizing pre-moistened sterile cotton bud was developed and used to evaluate the effects of various stages of egg handling and incubation on egg-shells bacterial contamination.

To collect the bacteria from the egg-shells, the surface of the egg shell was swabbed with a pre-moistened sterile swab. Each egg was swabbed on an area half of the egg's shell surface. The swab is then placed in a bottle of Stuart's Transport Medium. One swab per bottle.

Sampling was done according to these periods

(1) immediately after the eggs laid

(2) after the collection of egg (15 minutes after the egg was laid)

(3) immediately after the first fumigation (at the farm)

(4) after the second fumigation (at the hatchery unit) (30 minutes after the second fumigation was done)

(5) immediately after the grading of the eggs

(6) after the cold-storage (3 days after the eggs were stored)

(7) after the 18th day of incubation

Culture

The cotton bud was then directly streaked on the Blood Agar and Mac Conkey Agar. The swab was rotated on the medium, so that all surfaces of the swab came in contact with a small portion of the medium. The remainder of plate will be streaked out with a sterile wire loop. The plates were incubated at 37 degree Celcius for 24 hours. Plates were examined to count the number of bacterial and mould colonies which were grown on the both plates.

Identification of bacteria and mould

Identification of bacteria was done based on colonial morphologic (such as size, shape, structure, colour and texture), gram staining and biochemical test, Observation was also done on Blood Agar plate whether there was any evidence of hemolysis and also on Mac Conkey Agar plate for lactose fermentation processes. Gram staining was done to differentiate between gram positive and gram negative bacteria.

Confirmation of species of gram negative bacteria was done with biochemical test and various tests were involved :

a. Triple Sugar Iron (TSI)

b. Sulfide-Indole-Motility (SIM)

c. Simmons Citrate

d. Urease

e. Methyl Red-Voges Proskauer (MR/VP)

f. Oxidase

Catalase test was done to differentiate between Staphylococci and Streptococci group, while the Slide Agglutination Coagulase Test to differentiate between Staphylococcus aureus and Staphylococcus epidermidis.

For mould/fungus, one colony of mould from Blood Agar plate agar was inoculated into Sabauroud's Dextrose Agar. After 14 days of incubation, identification of mould species was done using Lactophenol Cotton Blue Wet Mount Method and seen under the microscope.

Observation was also done on the litter floor system, nest's litter, the procedure of the eggs collection and grading and on the standard of sanitation in the cold-room which may influenced the bacterial population on the eggs' shells.

RESULT AND DISCUSSION

Table 1 shows the microbial counts on the egg-shells at the various stages of egg handling. From this table, bacteria and mould counts were increased at the various stages of egg handling except after the first and second fumigation, where the bacteria and mould counts were decreased.

Table 1 : Bacterial and mould colony counts at various stages of egg handling

Stages	Number of egg tested	Total of bacteria	Total of mould
Immediately after the eggs laid	31	10273	72
After the collection of eggs	31	14818	164
After the first fumigation	31	1881	62
After the second fumigation	31	313	39
After the grading of the eggs	31	360	49
After the cold-storage	31	707	245
After the 18th day of incubation	31	1709	0

The number of microbial counts on the egg shells were increased after the collection of eggs (Bacterial = 30.67 % and Mould = 56.10 %) (Table 2). Bacterial population on the eggs' shells will be increased if the eggs were kept a few hours or longer in the nests especially in dirty nest. The longer the eggs stay in the nest and in the breeder house environment, the greater is its risk for contamination. Therefore, the breeder nests should be kept in good condition and the depletion of wood shaving in the nest should be avoided (Mauldin, 1981).

Table 2 Percentage increase in bacteria and mould counts

Stages	Bacteria	Mould
After the collection of eggs	30.67	56.10
After the grading of eggs	13.06	20.41
After the cold-storage	68.09	80.00
After 18th day of incubation	58.63	

- no mould growth on SDA

Furthermore, Mauldin (1981) reported that it is very important for the litter to be kept dry. The nest also,

should be cleaned to produce clean egg. So, shaving or other nesting materials should be stored in a dry area to prevent mould growth. Dropping, broken eggs, wet shaving and foreign materials should be removed from the nest daily and replaced with fresh, clean nest material. Wet litter will attach to the feet and feathers of the hens and the bacterial of the litter are presumably transferred into the nest causing hatching egg contamination. The deep litter also could be as rich source of bacterial of the egg contamination or spoilage.

After the first and second fumigation, the microbial counts decreases (Table 3). Fumigation with formaldehyde gas will destroy the disease organisms on the

Table 3 Percentage reduction in bacteria and mould counts

Stages	Bacteria	Mould
After first fumigation (3x)	81.69	13.89
After second fumigation (1x)	96.95	45.83

1x 1 times concentration (20 g of ptassium permanganate and 40 ml formalin)

3x - 3 times concentration (60 g of potassium permanganate and 120 ml formalin)

shell surfaces such as Salmonella, Coliforms and some moulds, which are common inhabitant in the chicken environment, when used immediately after the eggs are laid. These organisms are major cause of reduced hatchability and chick livability. The routine preincubation fumigation of hatching eggs in the farm is highly recommended, in order to reduce egg shell-borne infection from poultry flocks. It is needed because the egg is laid through the same body opening in the hen where faeces is excreted resulting in contamination of the shell as soon as the egg is laid. Nesting material may also contain faeces which can contaminate the egg after it is laid (Beesley, 1980). Williams (1981) stated that on-farm fumigation of hatching eggs will improve about 3 % of hatchability and 5 % of livability.

From this study, reduction of microbial counts after the first and second fumigation are still low (Table 3). It indicates that the fumigation programme used in this farm did not completely eliminate the microflora. Actually, after the first fumigation (3 times concentration of fumigants) destruction of microbial should be 99 %. Beesley (1980) stated that formaldehyde fumigation can kill 99 % of organism on the egg shell if the process is done properly. However, the type of egg's shell also affect the elimination of microflora on the shell surface. Destruction

of bacteria is less on white-shell, due to lower absorption levels of the fumigant on white-shell. Williams (1970) reported that the destruction of bacteria was greater on brown-shell egg, even though bacterial population were consistently higher on unfumigated brown-egg.

The main purpose of fumigation is to reduce the number of shell surface microorganisms to as near zero as possible, thereby reducing the likelihood of subsequent penetration into the egg's interior. Therefore, it is important that hatching eggs are fumigated immediately after lay before the egg cools. As the egg cools, the interior or contents contract creating a partial vacuum which may draw the microorganisms into the eggs. Once the contaminants have penetrated the shell, they are unaffected by external treatment (Beesley, 1980). Destruction of moulds (eg : Aspergillus fumigatus) was low because they are resistant to formaldehyde.

After the grading of the eggs, bacterial and mould colonies were increased (Bacteria = 68.09 % and Mould = 80.00 %) (Table 2). Increased microbial counts might be due to the tranfer of contaminants from workers' hand during grading of the eggs. Too much handling of the hatching eggs not only increase the possibility of contaminating the eggs but also increase the chances of cracking some of the eggs. These condition will reduce

hatchability of the eggs(Jones, 1976).

Bacterial counts on the egg's shell increased after the 3 days in the cold-storage and after the 18th day of incubation (Table 2). The bacterial counts should decreases in both stages. Gentry and Quarles (1972) and Furuta and Maruyama (1981) reported that bacterial counts on the shell decreases during incubation or storage and then increases during hatching. Increased bacterial counts after cold-storage might be influenced by temperature and relative humidity. The temperature and relative humidity in the cold-room of this farm is about 19.4 to 20.5 degree Celcius and 79 to 90 % respectively. In these ranges, bacterial activity slightly increases, because most of the saprophyte isolated from eggs grow better at 20 to 25 degree Celcius than 37 degree Celcius (Forsythe et al, 1953). However, most of the bacterial in these catogeries are non-pathogenic and doesn't produce any harmful effect. Eggs that are stored at 15.5 to 18.0 degree Celcius and 85 % relative humidity, have low bacteria counts, indicating death of some of the bacteria on the shells during storage.

The bacterial counts increases during incubation because some bacteria such as Micrococcus, Staphylococcus, Streptococcus and Bacillus can survive in high temperature 42 to 50 degree Celcius. Mould counts increase after the cold-storage because they grow better at low temperature

and high relative humidity. Certain fungi grow bette at 37 degree Celcius and this temperature is needed to convert the mould form of dimorphic form ti the yeast form (Soltys, 1979). However, there was no evidence of mould growth during incubation (Table 1).

High number of microorganisms either during the cold-storage or during incubation might be due to residual contamination during handling or grading or the eggs. The workers should be careful not to handle eggs after fumigation without washing hands and cleaning finger nails.

Table 4 shows the type of microflora on the egg's. Micrococcus, Staphylococcus, Streptococcus and Bacillus are common organisms which are found on the egg's shell. However, these organisms are ess pathogenic to the embryo as compared to Salmonellae, Pseudomonas and Coliform bacteria like *E. coli*, Klebsiella and Citrobacter. *Aspergillus fumigatus* and *Mucor sp* are two type of moulds found in this study.

Most of the pathogenic organisms such as Salmonella, Pseudomonas and Coliform bacteria were destroyed with formaldehyde fumigation. However, care must be taken because eggs can be contaminated by vertical transmission and by penetration of the egg-shell via pores.

Penetration of microorganisms through the egg-shell can be prevented by proper fumigation with formaldehyde

Table 4 : The microflora of the egg's shell

Type of organisms	Frequency of occurence on the egg's shell at various stages of egg handling						
	1	2	3	4	5	6	7
Micrococcus spp	+++	++++	++	++	++	+	++
Staphylococcus spp	++	+++	++	+	++	++	+++
Streptococcus spp	+	+	+	+	+	+	++
Bacillus spp	–	+	+	++	+	+++	+
E. Coli +	+	–	–	–	–	–	
Salmonella spp	+	+	–	–	–	–	–
Pseudomonas spp	–	++	+	–	–	–	–
Corynebacterium spp	–	+	+	–	–	–	–
Proteus spp	–	+	+	–	–	–	–
Citrobacter spp	–	+	+	–	–	–	–
Acinetobacter spp	–	++	+	–	–	–	–
Aeromonas spp	–	+	+	+	+	–	–
Alcaligenes spp	+	+	–	–	–	–	–
Klebsiella spp	–	+	–	–	–	–	–
Mould	+	++	+	+	+	++	–

+ The more plus signs, the more frequent the occurence
– no microorganisms

1 – immediately after the eggs laid
2 – after the collection of eggs
3 – after the first fumigation
4 – after the second fumigation
5 – after the grading of eggs
6 – after the cold-storage
7 – after the 18th day of incubation

gas. If contamination of the eggs is by vertical transmission, it is difficult to destroyed the organism.

Therefore, to solve the above problem, the eggs selected for hatching should come from parent stock which are free from any pathogenic organisms which would cause low percentage of hatchability and poor baby chicks.

CONCLUSIONS

The ultimate aim of the breeder farm is to produce high percentage of hatchability or maximum number of healthy chick which would bring more profit.

Good egg management is one of the most important areas in producing good-day-old chick or healthy chick. Egg management includes a number of areas such as nest management, gathering and grading as well as egg room management. In other words, the quantity and quality of the chicks hatched depends a great deal on how the eggs are handled shortly after they are laid and in the subsequent period prior to incubation. The quality of the baby chicks is directly related to the quality of the egg from which it is collected.

RECOMMENDATIONS

(1) The nests should be closed at night to prevent roosting and also to reduce the amount of faecal material in the nest. The nest should only be reopened when the lights come on in the morning.

(2) To reduce contamination of the eggs, two/third slat, one/third litter floor is one of the recommended designs in modern breeder housing.

(3) Egg collection vehicles should be thoroughly cleaned and washed at weekly intervals and should be subjected to regular fumigation (Shane, 1981).

(4) The brooders should be prevented from bnrooding because it will contaminate the egg and also increase the risk of spoilage by warming the eggs before storage. Brooders in a nest also restrict the laying space thus increase the risk that the egg will soiled or broken by the laying birds (Cook,1969).

(5) Do not wash hatching egg. If the eggs are needed to sanitise with sanitizers, it is very important to do before the eggs cool off, preferably within one hour after gathering. The solution used should be warmer than the eggs, or at about 48.9 degree Celcius. Egg should not remain in the solution more than two minutes.

(6) Check nest weekly. Replace dirty nest litter with fresh shavings. Floor litter should be kept dry and remove wet litter immediately.

(7) Floor eggs, dirty eggs, cracked and punctured eggs should be culled and placed in a separate flat at the time of collection. These egg should never be placed in a flat with clean nest eggs.

(8) The egg room must be cleaned and free from rodents, insects, dust and cobwebs. The room should be mopped thoroughly twice a week or as often as necessary. No water should remain standing on the egg room floor. If the egg are stored in a dirty environment at the wrong temperature and/or humidity, all the efforts previous expended to properly manage the nests, gather and grade the eggs are negated.

(9) Reduce the incidence or minimize the number of floor eggs. Therefore, the nest should be available at least two weeks or more earlier before the first egg is laid in order to train the hens to lay in the nest (Anon, 1978b).

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APPENDIX 1

INTERPRETATION OF BIOCHEMICAL TESTS

(1) Triple Sugar Iron (TSI)

Orange-red	uninoculated
Yellow (acid)	glucose and/or sucrose and/or lactose fermented
Red (alkaline)	glucose, sucrose and lactose not fermented or exhausted
Blackening in butt	Hydrogen sulfide produced
Gas bubbles in butt	Aerogenic organism

(2) Sulfide-Indole-Motility (SIM)

<u>Sulfide</u>

Positive	The presence of hidrogen sulfide is manifested by blackening wherever growth has occured due to reaction with iron to form black

<u>Indole</u>

Positive	red in color (presence of indole)

<u>Motility</u>

Positive	evidenced by the spread of turbidity from the site of inoculation to all parts of the medium. If a motile

organism is hidrogen sulfide positive, the whole tube will eventually be black

(3) Simmons Citrate

Determines the ability of an organism to use citrate as the sole source of carbon. Useful in identification of Enterobacteriaceace and certain other gram negative bacteria.

Growth	blue color
No growth	green color (unchanged)

(4) Urease

Determine the presence of urease activity in microoganisms. When urea is hydrolyzed, ammonia is liberated and causes the medium to turn alkaline.

Positive	red color
Negative	yellow (no change)

(5) Methyl red – Voges Proskauer (MRVP)

MR test

Red	positive (pH less than 4.4)
Red-orange	weakly positive (pH less than 5.3 but above 4.4)

Yellow — negative (pH above 5.3)

VP test

Positive — pink to red (due to a condesation reaction between diacetyl, a final oxidation product of glucose fermentation produced in the presence of KOH and Oxygen, and certain ingredients in the medium creatine)

Negative — no significant color change

(6) Oxidase

Positive — dark purple within 10 second

Negative — yellowish reaction or delayed reaction of purple (more than 10 second)

APPENDIX 2

Catalase Test

Positive bubbles (presence of Oxygen)

Negative no bubbles

Slide Agglutination Coagulase Test

Actually this test was done to detect clumping factor which was found in the organism.

Positive clumps (reaction between organism and rabbit plasma)

Negative no clumps