Journal of Agriculture and Social Research (JASR) Vol. 12, No. 2, 2012

EFFECT OF DIFFERENT AQUEOUS MEDIA ON THE VIABILITY OF THE GERM CELLS OF CHICKEN EGGS

MEDUBARI B. NODU
Department of Livestock Production Technology,
Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria
Email: mb_nodu@yahoo.com

ABSTRACT
An experiment was conducted to evaluate effect of different aqueous media on the viability of the germ cells of chicken eggs. The aqueous media tested were Brackish water (BRW), Physiological saline solution (PSS), Deionized water (DW), and city tap water (CW). A total of 300 eggs were used for the study using a Completely randomized experimental design (CRD). The study revealed that brackish water had the highest number of clear eggs while the control had the lowest. There was no significant difference between CW and DW in respect to the number of clear eggs. The control had the highest number of early dead. There was no significant difference between treatments with regard to egg weight. Brackish water had the highest microbial load before storage while DW had the lowest. Deionized water had the highest microbial load after storage while PSS had the lowest. In ferric concentration (Fe^{3+}) concentration, DW had the highest before storage but the least after storage. The pH of all the media was neutral before storage except BRW which was near neutral (6.9). The pH of all treatments rose above neutral after storage ranging from 7.2 to 7.7.

Key words: aqueous media, storage technique, hatching eggs

INTRODUCTION
The importance of protein to human growth and development cannot be overemphasized. Protein is derived from both crops and livestock. Animal protein sources are varied and one of the commonest sources is poultry which produces eggs. There is no doubt that eggs provide means through which the animal protein of the populace can be met. It has various uses and contains many essential nutrients as it supports life during embryonic growth (Scott & Silversides, 2001) and one of the most nutritious and complete food known to man. However, egg quality characteristics, utilization for food, storage and other purposes have been studied mostly in chicken egg. Egg quality is composed of those characteristics of an egg that affect its acceptability to consumers, it is therefore important that attention is paid to the problems of preservation and marketing of eggs to maintain the quality (Adeogun & Amole, 2004; Song, Choi & Oh, 2000).

According to Newman (2004) eggs are potential reproductive bodies that contain the germ of an embryo. They have the potential of starting another generation. Long a symbol of fertility to the Chinese (and others), these oval balls of nutrition have four basic parts: Shell, shell membrane, white (also called the albumen), and the yolk. The first two are used medicinally, the others for food and folklore. Chicken eggs are the most commonly consumed eggs, worldwide.

Hatchable eggs are obtained from the mating of a cock to a hen. Such eggs are normally collected and stored prior to incubation under commercial conditions. Hatchability of fertile eggs depend to a great extent on favourable storage conditions. Brown (1980) observed that long and improper storage of hatchable eggs lower viability, hatchability and performance of chicks hatched from them. He further submitted that the length of time that fertile egg remains viable depends on the conditions under which they are stored. Byng and Nash (1962) storage period increases. Bohren et al (1961) noted that long storage of fertile

eggs under optimum conditions is desirable for better preservation of the germ cells, increased hatchability and the production of quality chicks. Since the poultry industry in any country is highly dependent on the supply of adequate and high quality chicks, efficient methods of preserving hatching eggs has become highly desirable. Several methods of egg storage are currently used within the industry for storage of hatching eggs. For example the storage method of placing the eggs on plastic or fibre creates with the broad ends uppermost in a storage room under controlled temperature (10^0c - 12.7^0c) and relative humidity (75% - 85%). This is the most widely used. Other methods of storing hatching eggs include the storage of eggs in open sided boxes and on trays, in cold rooms under low temperatures, refrigeration storage. In various cultures several preservation methods have been tried. For instance, Newman (2004) noted that in the Chinese culture the oldest and probably the most famous of all preserved eggs are salt cured, come from Zhejiang, and are loved there particularly at breakfast time. To make them, raw eggs are cracked, then layered between salt and wine residues. Then they are sealed in jars and kept that way for five or six months. Nowadays, chicken, duck, and quail eggs are preserved this way. Another preservation technique is to coat whole eggs with a thick layer of salt, soil, ash, and tea leaves. These are distilled or just ground together and made into a mud. Salt-cured eggs are called *hei dan*, and are stored at a room temperature of about 65 to 68 degrees F. They can be kept for thirty or more days, the coatings removed, and the eggs cooked. The mud-covered eggs stay longer, at least twice as long. Another way to preserve eggs is to immerse them in a pickling solution for about forty-five days, then wash and dry them and coat them with paraffin or mud or both. Some people who make salted or brined eggs flavor theirs with spices including cloves, anise, and black or Sichuan pepper. Another preservation technique is making the eggs taste sour. This is done by immersing them in vinegar and salt and keeping them in this solution until the shells soften. Some eggs made this way are kept so long that the shells dissolve.

Of all these methods of storing fertile eggs, none has proved to be totally problem free. Each method has its problem thereby lowering hatchability due to improper germ cell preservation. It has therefore become expedient to conduct more scientific investigations into better methods of storing fertile eggs. This will lead to improved hatchability and subsequent high production of quality chicks to boost poultry production.

This experiment was therefore designed to examine the prospects of egg storage using submersion under aqueous media. The purpose is to investigate the potential of the technique as a method for improving the preservation of germ cell viability of chicken eggs.

MATERIALS AND METHODS

This experiment was conducted at the poultry breeding and hatchery unit of the Rivers State ministry of Agriculture and Natural Resources located at Atali in Port Harcourt Local Government Area.

Eggs and treatment:

Three hundred (300) hatchable eggs were obtained from harco breeding hens maintained by the ministry outfit. They were selected from a batch laid within two days of collection.

Treatment consisted of four different aqueous media viz: brackish water (BRW), physiological saline solution (PSS), deionized water (DW) and tap or city water (CW) with a control group(Cont).

The brackish water was fetched from the University of Science and Technology, Port Harcourt fish pond (farm). The physiological saline solution was prepared in the Animal Science Laboratory. The deionized water was obtained from the chemistry laboratory in the university while the tap water was fetched from Diobu area in Port Harcourt. One thousand three hundred (1,300) mls of each solutions was poured into 2 liters volume plastic

containers. The eggs were divided into 15 groups of 20 eggs per group. Three groups were randomly assigned to each aqueous treatment in the plastic containers. All the eggs were completely covered by the solutions. The experimental design was a completely randomized arrangement. The control was represented by 3 replicated of 20 eggs stored on fibre filler flats with broad ends uppermost.

The eggs were stored for a total of 7 days. During storage, the p^H of each solution was determined using a pH meter. The room temperature was also taken on the first, fourth and last (7th) day using a thermometer. All eggs were weighed before and after storage.

Egg setting:

The eggs were weighed, trayed and set following storage on the seventh day. All cracked eggs which were observed after storage were discarded. Before moving the tray racks to the setter the eggs were fumigated in a fumigation chamber. Fumigation was by the action of 183ml of 36% formalin on 92g potassioum permanganate ($KMn0_4$) for 40 minutes. The 92g $KMn0_4$ was placed in a porcelain plate and the formalin added to it in the chamber. After 40 minutes the eggs rack was rolled into the setter. The walk—in type Western chick master incubator was used.

Egg Candling

Candling was done on the 10th and 19th day of incubation. Eggs observed to be 'clear' or dead were removed and recorded while the rest were returned to the incubator. All discarded eggs were broken and examined for signs of fertility or embryo development. 'Clears' and early dead were recorded according to treatments. On the 19th day of incubation, the eggs were transferred into the hatcher.

Determination of mineral content of media:

The ferric iron (Fe^{3+} concentration of each of the solution was examined before and after storage. This was done by the potassium thiocynate procedure by using the spectro 20 at 480 wave length,

Microbial determination:

The microbial profile of each solution was also determined both before and after storage of the eggs. The serial dilution procedure was used.

All data analysed by analysis of variance according to the procedure of the statistical tools of SAS(Ref).

RESULTS

Mean egg weights before and after storage for each treatment are presented in Table 1. Significant differences in egg weight before storage among the various treatment groups was observed. These differences disappeared after storage. The data show that there were increases in egg weight after storage in all the aqueous media. The weight gains were not significantly different. There was weight loss after storage in the control groups. Four (0.6%) crack eggs were recorded in both the BRW and CW while six eggs (1.8%) were cracked in each of the PSS and DW.

Table 1: Mean weight of eggs before and after storage

Treatment	Wt of eggs before storage (g)	Wt of eggs after storage (g)	Weigh gain or loss (g)	(%)
Cont.	54.67^{a} ($\pm$ 0.47)	54.35^{a} ($\pm$ 1.22)	-0.32	-0.59
BRW	53.50^{b} ($\pm$ 0.54)	54.15^{a} ($\pm$ 0.63)	0.65	1.21
PSS	52.67^{b} ($\pm$ 0.94)	52.92^{a} ($\pm$ 0.77)	0.25	0.47
CW	53.75^{ab} ($\pm$ 0.24)	53.87^{a} ($\pm$ 0.47)	0.57	1.07
DW	53.75^{b} ($\pm$ 0.57)	54.33^{a} ($\pm$ 0.74)	0.66	1.23

^{a,b}Means bearing different superscript within the same column are statistically different (P<0.05)

The fertility performance of the eggs on treatment basis is presented in table 2. The control group has the highest number of early dead (11.33) while there were no significant difference among the treatment groups. Brackish water has the highest number of clear eggs with statistical differences among the treated and the control in terms of clear eggs.

Table 2: Egg fertility

Treatment	No. of eggs incubated	Total clear	Early dead	Mean clear	Mean early dead
Cont.	60	26	34	8.67^{c}	11.33^{a}
BRW	59	52	7	17.33^{a}	3.50^{b}
PSS	57	41	16	13.67^{b}	5.33^{b}
CW	59	46	13	15.33^{ab}	4.33^{b}
DW	47	49	8	16.33^{ab}	2.67^{b}

$^{a, b C,}$ Means bearing different superscript within the same column are statistically different (P<0.05)

The microbial profile, p^{H} and $Fe3^{+}$ concentration of the various media are presented in Table 3. The data shows that the solutions had microbial load varying from 5.1 x to 2.2 x 1011 Control group contained the highest number (2.2×10^{11}) cfu/ml of colony forming units with a pH of 6.9 and lowest in Fe3+ concentration.The PSS, CW and DW were neutral p^{H} 7.0. DW contains the lowest number of colony forming units with the highest concentration of Fe3+ concentration. Fe^{3+} concentrations ranged from 192.65 to 543.96.

Table 3: Water quality (micro organism, p^{H}, f_e concentration) profile of the various aqueous media before storage.

Treatment	Microbial profile (cfu/m1)	p^{H}	Fe^{3+} concentration (mg/litre)
Cont.	2.20×10^{11}	6.9	192.65
PSS	1.27×10^{11}	7.0	284.07
CW	1.39×10^{11}	7.0	426.86
DW	5.10×10^{8}	7.0	543.96

The microbial profile, and Fe3 concentration of the various media after storage are presented in Table 4. The data shows that the solution after storage of eggs had microbial load ranging from 5.1 x 10^{8} to 3.3 x 10^{10} . p^{H} ranged from 7.2 to 7.7 while $Fe3^{+}$ ranged from 49.11 to 169.99. The DW and BRW contained the highest colony forming units with the lowest Fe^{3+} concentration (49.11 and 98.22). The PSS had the lowest colony forming units (5.1 x 108) and concentration (169.99) followed by the CW with p^{H} of near neutral (7.2).

Table 4: Water quality (micro_organisms p^H concentration) profile of the various aqueous media after storage.

Treatment	Microbial profile (cfu/m1)	p^H	Fe^{3+} concentration (mg/litre)
Cont.	$3.3x10^{10}$	7.4	98.22
PSS	$5.1x10^{8}$	7.6	169.99
CW	$6.0x10^{9}$	7.2	128.44
DW	$5.0x10^{10}$	7.7	47.11

DISCUSSION

The data on egg weight indicated only slight increases in egg weights after storage in the various media. This may be due to the effectiveness of the egg cuticle in preventing flooding of the egg content through the pores on the egg shell. It is however possible that some water did penetrate the egg content and hence the slightest increase.

A slight decrease in weight of - 0.59% was however observed in the control group after storage. This might have been due to evaporative loss. Low relative humidity and air movement in the store also contributed to this loss in weight. From the fertility result, it was observed that a greater proportion of the eggs were infertile or 'clear'. This situation could be caused by several factors including feed, male to female ratio, age and health of the birds, and management.(Ref) It was observed that the birds were not adequately fed. The diets were also poor in quality. The importance of balanced ration in the diet of breeding bird can never be overemphasized.

It was also observed that due to the paucity of cocks on the Ministry farm, the male to female ratio was 1 to 30. (Ref) reported that maximum fertility was recorded with male to female ratios of 1 to 12 broiler - type breeder and 1 to 18 with egg production breeders. They indicated that a male to female ratio of 1 to 36 was too large for maximum fertility of broiler breeders.

The age of the flock also contributed to this low fertility rate. The parent stock on the farm were old and this lowered their fertility level. Other factors which contributed to this poor fertility level are poor health condition of the flock and improper management which include lack of medication due to shortage of drugs, improper disinfection of the environment and other routine management practices. It was observed that brackish water (BW) contained the highest microbial load before storage with a fairly low Fe3+ concentration and a p^H of near neutral (6.9). This condition favoured microbial growth especially organisms that thrive under such an environment. This growth was observed on some shell of the eggs stored in this medium as slimy brownish coating. It is possible that these microbes might have penetrated the egg shell into the egg to cause varying degrees of damage to the eggs.Water provides a good environmental condition for rapid bacterial multiplication leading to bacterial attack of eggs and there is no doubt that water is necessary for the translocation of organisms across the shell.

Tap water (CW) and physiological saline solution (PSS) also showed a high microbial count. This was probably caused by a favourable environment well fortified with useable nutrient in these media. When discarded eggs from these media were broken, they emitted a foul odour. It was also observed that Fe^{3+} concentration of the various storage media decreased after storage while the microbes- increased (particularly in deionized water). The organisms must have utilized Fe^{3+} for rapid multiplication causing a decrease in its concentration after storage. This enables the bacteria to grow rapidly and may cause heavy contamination of the albumen and the addling of the affected eggs. This decrease in Fe^{3+} concentration traction therefore showed that the bacteria used up the Fe^{3+} for growth which led to their increase in number. This rapid growth (multiplication) of the microbes and the

resultant rottening of the albumen is as a result of the fact that natural or artificial contamination of water with iron salts increases the rate and incidence of rottening during the storage of washed eggs. When there is an addition of iron to an infected shell membrane it is likely to promote extensive bacterial multiplication which rendered the albumen to be heavily infected.

Moulds were also observed in the media after storage of the eggs. This must have been introduced into the media by contaminated eggs. The shells were probably contaminated with mould by contact with the floor of the poultry house. The high value of Fe^{3+} concentration however observed in this study might be due to the method used in its determination which must have converted some iron in the ferrous state (Fe^{2+})to the ferric state (Fe^{3+}) in the media, this is particularly suspected in deionized water which is suppose to be free of these ions.

The p^H value of these media was neutral before storage but all rose above neutral after storage being highest (7.7) in deionized water. This was probably due to the depliction of the minerals from the media by the micro organisms. Nevertheless, these values could still be dangerous to the germ cell as they would still be conducive to microbes. For successful hatchability the storage temperature and incubation temperature must be optimal and stable. A stable optimal storage temperature is required to maintain the dormant germ cell in a viable state until time for incubation. Similarly a stable optimal incubation temperature is also required for eggs in the incubator for progressive embryonic development. This means that for successful hatchability, the micro environment round each egg must be exactly correct. The fundamental elements of this environment are temperature, humidity, ventilation and the turning of the eggs. In this experiment however, there was fluctuations in temperatures and relative humidity both at storage and incubation periods of the experiment. This was due to breakdown in the air conditioner in the storage room and inconsistent power (electricity) supply from the farm plant respectively. These conditions led to the massive damage (death) to the germ cells of all the eggs. Also, the eggs in this experiment were stored under ambient temperature which was fluctuating depending on the weather and time or period of the day.

This temperature was sometimes between the range of 21^0c to 35°c which was against the 10.00 to 12.7°C which is reported as been satisfactory for holding (storing) fertile eggs(Ref). This condition was not favourable to the germ cells of the stored eggs leading to their death. It is common knowledge that the germ cell of chicken eggs divided continuously under a favourable condition when laid. In effect the germinal disc has grown as far as the blastula stage, but the development stops when the egg cools after laying. It can stay in this dormant stage for quite some time. It is known that at temperatures above 21^OC, growth recommences very slowly but this growth is weak and if prolonged, the embryo either dies or is so weakened that it does not survive one of the major developmental changes in its later growth. This was the condition faced by the eggs in this study and consequently they all died because they could not survive the major developmental changes in the growth phase. This was due to the fluctuation in temperature and relative humidity in the incubator.

From the foregoing, it is evident that temperature is an important factor in hatchability of fertile eggs. Therefore for a successful hatchability of fertile eggs, a steady supply of power (electricity) is necessary to maintain the constant and optimum temperature and relative humidity needed for hatching of eggs both at the storage and incubation stages.

CONCLUSION AND RECOMMENDATIONS

From the results obtained it is demonstrated that a large proportion of the eggs used in this experiment were ‘clear’ (infertile). This could be due to fertility problems on the farm from where they were obtained. The result however points to the fact that these aqueous media could be used to store hatching eggs for a short time prior to incubation. Sequel to power

(electricity) interruption and subsequent spoilage of all the eggs, a definite conclusion could therefore not be made in respect to the effect of these selected aqueous media on the germ cell preservation.

Also, this experiment explains the necessity for constant and steady optimum temperature and relative humidity during storage and incubation stages of fertile eggs.

REFERENCES

Adeogun I.O. & Amole F.O., (2004) Some quality parameters of exotic chicken eggs under different storage conditions. *Bull. Animal. Hlth*. Prod. Africa, 52, 43-47.

Bohren, B. B., L. B. Crittenden and R. T. King. (1961). Hatching time and hatchability in the fowl. *Poultry Sci*, 40: 640-643.

Brown, A. A. F. (1980). *The incubator book.* 36-66. Saiga. Publishing Co. Limited. London England.

Byng, A. J. and D. Nash. (1962). The effects of eggs storage on hatchability. *Br. Poult. Sci.*, 3: 81 - 87.

Newman, J. M. (2004) Eggs: Fresh and Preserved, Chinese Style. Eggs. *Winter Volume, 11 (4), 27, 29, 30, and 32*

Scott T.A. & Silversides F.G., (2001) Effect of storage and layer age on quality of eggs from two lines of hens. *Poult. Sci*. 80, 1245-1248.

Song K.T., Choi S.H. & Oh H.R., (2000) A comparison of egg quality of pheasant, chukar, quail and guinea fowl. *Asian- Aus. J. Animal. Sci.* **13**, 7, 986-990.