Sudan University of Science and Technology
College of Graduate Studies

Effects of Flock age, Length of Storage Period and Frequency of Warming before and during Egg Storage on Hatchability of Layer Breeder Eggs

أثر عمر القطيع و طول فترة التخزين ومدة التدفئة قبل و اثناء التخزين على امكانية فقس بيض أمهات الدجاج البياض

A Thesis Submitted in Fulfillment of the Requirements for the degree for Ph.D. in Poultry Production

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August 2022

Dedication

To my father soul

My mother

My wife

My brothers

My sisters

My lovely children

With respect and love

Acknowledgement

First of all I thank **Allahwho** supported me in my life and being the giver of all good gifts. To **Allah** the glory and honour forever.I would like to express my deepest thank to soul of my supervisor **Dr. Osama Elsheik Yassin** for his support, encouragement, direction and valuable advice during the study. My sincere thanks and appreciation are extended to my co–supervisor, **Dr. ELfadil Ahmed Adam**for his valuable remarks, high support and continuous help. I would like to thank **Dr. Badr Hasabelrasoul Eljack** for the huge knowledge and skills he transferred to me. Deep thanks are also extended to Mr. **Ibrahim Hasabo** chairman of Coral Hatcheries and Feed Production Farms. My thanks are being extended to the staff of the hatchery unit for their assistance. My special thanks to my colleague **Abubakr Sayed Ali** for his great help in statistical analysis. Finally I am fully indebted to my mother for her faith and love.

ABSTRACT

The experiment was conducted to evaluate the effect of flock age, length of storage and warming time before and during storage on hatchability of layer breeder eggs. A total of 1620 clean free from abnormalities fertile eggs were randomly selected from 75, 80 and 85wks old layer breeder (540 / age), each group was divided into three treatments (180 each), each treatment was replicated three times (60/replicate) then warmed before storage for 0, 3 and 6 hrs at 37.5°C and 53% RH, each replicate was furthered subdivided into three subgroup (20/ each) and stored for 4, 9 and 14 days at 18°C and 75% RH. Thereafter, half of the stored eggs (810) were warmed daily during storage for 0, 1 and 2hrs at 37.5°C and 53% RH. All eggs were weighed before the commencement of the experiment and when transfer to the Hatcher for the calculation of egg weight loss. After 4, 9 and 14 days of storage eggs were set in a setter at 37.5°C and 53% RH. At day 18 of incubation, hatching eggs were candled and infertile eggs were removed and opened to determine macroscopically infertile or stage of embryonic mortality to calculate true fertility. After candling, hatching eggs with living embryos were transferred to the hatchery baskets and placed in Hatcher cabinets in which the temperature and relative humidity were adjusted at 36.6°C and 75% RH. At the end of hatching process, hatched chicks were counted and weighed, hatchability on total and on fertile were calculated, unhatched eggs and pipped chicks were removed and opened to determine the stages of embryonic mortality, egg weight loss and chick yield were calculated and classified as first or second grade chicks based on their external feature. The results revealed that egg weight loss and chick yield were significantly ($P \leq 0.05$) affected by warming time, breeders age and storage period. Warming eggs before storage for six hours then stored for fourteen days had higher egg weight loss 13.33%

and 14.67% when the breeder age 80 or 85weeks old. On the other hand, total weight losses and chick yield were significantly better 11.05 % and 68.12% when the breeders at 75 wks-old. Warming eggs before storage for six hours resulted in a significantly (P≤0.05) reduced the percentages of early 12.72%, mid 3.08% and late dead 4.57% compared to non-warmed eggs 26.05% ,4.07 and 8.40 or eggs warmed for three hours 18.27% 2.84% and 6.05% , Early. Eggs stored for 14 days had significantly (P≤0.05) higher early dead 27.53%, mid dead 3.95% and late dead 6.79% compared to those stored for 4 and 9 days(11.48%, 2.84%, 6.17%) (18.03%, 3.21%, 6.05%) respectively. On the Other hand, early 15.80%, 18.27%, 22.96%, mid 2.71%, 3.08%, 4.20% and late 4.81%, 5.19%, 9.01% embryonic mortality increased when the breeder age increased. Hatchability on total and on fertile were significantly (P≤0.05) affected by warming time before storage ,higher hatchability were recorded when the eggs warmed for 6 hrs (47.16%) and stored for 4 days(47.07%), meanwhile, hatchability on total and on fertile were decreased when the breeder age and storage period increased(50.87%,68.87%)(40.25%,59.67%)(25.18%,41.07%)(47.04%,69.43%)(40.25%, 58.72%) and (29.01%, 41.46%) respectively. Warming eggs before storage for 6 hrs significantly (P≤0.05) increase the (%) of first grade chicks (95.88%) and decrease the second grade (4.12%) compared to those warmed for 0.0 (67.4%)(32.55%) or 3hrs (90.84%)(9.16%).On the other hand, first grade chicks (%) were decreased when the flock age and storage period increased 75 , 80 to 85 wks old (91.14%, 86.52%, 76.51%), 4 , 9 and 14 days (90.52%,90.21%,73.45%). Egg weight loss significantly increased when warming time during storage (13.9%, 14.73%, 15.51 %), flock age (12.1% 15.59, 16.51) and storage period (13.65, 14.28%, 16.27%) increased. The best chick yield (66.82% and ‹%68.12) first grade chicks

(95.10%)(%91.33) (%90.72) , hatchability on total and on fertile (49.88% and 67.58%) were obtained when the eggs were warmed for 1 hr and the flock age was 75wks.

In conclusion, pre-storage warming of breeder's eggs for 6 hours or daily warming during storage for 1hr at 37.5°C and 53% RH and store for 4 days at 18ºC and 75%RH can be used by the poultry industry as a tool to improve hatchability results of late breeder eggs.

مخلص الدراسة

أجريت التجربة لتقييم أثرعمر القطيع ، طول فترة التخزبن و مدة تدفئة البيض قبل و اثناء التخزين على فقس بيض أمات البياض. تم اختيار 1620 بيضة مخصبه خالية من العيوب من قطيع امات بعمر 75، 80 و85 اسبوع عشوائيا (540 بيضة /عمر) تم وزن البيض قبل بداية التجربة ومن ثم تقسيمه الي ثلاثة مجموعات (180/ بيضة/مجموعة) وكل مجموعة تم تقسيمها الي ثلاث معاملات (60/ بيضة/معاملة) وكل معاملة قسمت الي ثلاث تكرارت (20 بيضة / تكرار) .تم تدفئة البيض علي درجة حرارة 37.5م ورطوبة نسبية 53% لفترات زمنية مختلفة (صفر ، 3 و6 ساعات) ومن ثم تخزينة علي درجة حرارة 18م ورطوبة نسبية 75% لمدة (4، 9 و 14 يوم) .بعد ذلك تم تدفئة نصف البيض المخزون (810 بيضة) يوميا لمدة صفر ، 1 و2 ساعة) و بعد انتهاء فترات التخزين تم وضع البيض في مفرخ درجة حرارتة 37.5م و رطوبة نسبية 53% . في اليوم 18 من التفريخ تم وزن البيض لحساب وزن البيض المفقود ومن ثم كشف البيض لاستبعاد البيض الغير مخصب وفتحة لتحديد مرحلة نفوق الجنين وذلك لحساب نسبة الخصوبة الحقيقية. بعد الكشف تم وضع البيض بالمفقس في درجة حرارة 36.5م ورطوبة نسبية 75% ، بعد نهاية عملية التفقيس تم حساب عدد الكتاكيت الفاقسة ووزنها وحساب نسبة الفقس من البيض الكلي ومن البيض المخصب، اما البيض الذي لم يفقس تم فتحة لتحديد مرحلة نفوق الاجنة (مبكر وسط او متأخر) كما تم حساب وزن البيض المفقود وتصنيف الكتاكيت الي درجة اولي اوثانية استنادا علي المظهر الخارجي للكتاكيت. اوضحت النتائج ان وزن البيض المفقود تأثر معنويا ($P\leq0.05$) بمدة التدفئة ، عمر القطيع وفترة التخزين حيث ان تدفئة البيض لمدة 6 ساعات ومن ثم تخزينة لفترة 14 يوم ادت الي ارتفاع نسبة الفقد في وزن البيض (13.33% و 14.67%) عندما يكون عمر القطيع 80 او 85 اسبوع ، ومن ناحية اخري كان الوزن المفقود واليلد افضل عندما كان عمر القطيع 75 اسبوع (11.05% و 68.12%).تدفئة البيض قبل التخزين ولمدة 6 ساعات ادي الي انخفاض معنوي ($P\leq0.05$) في نسبة النفوق المبكر 12.72%والمتوسط 3.08% والمتأخر4.57% مقارنتة مع البيض الذي لم تتم تدفئة (26.05%،4.07 ، 8.40)او الذي تمت تدفئة لمدة 3 ساعات (18.27%، 2.84% و 6.05%). تخزين البيض لمدة 14 يوم ادي الي زيادة معنوية ($P\leq0.05$) في نسبة النفوق المبكر 27.53%، المتوسط 3.95% والمتأخر 6.79% مقارنة مع البيض الذي تم تخزينة لمدة 4 او 9 ايام (6.17% ,2.84% ,11.48%) (6.05% ,3.21% ,18.03%) علي التوالي .

ومن ناحية اخري ارتفعت نسبة النفوق في كل المراحل مع تقدم عمر القطيع المبكر (15.80%، 18.27%، 22.96%) متوسط (2.71%، 3.08%، 4.20%) ومتأخر (4.81%، 5.19%، 9.01%). تأثرت نسبة الفقس من البيض الكلي ومن البيض المخصب معنويا ($P\leq0.05$) بمدة التدفئة قبل التخزين حيث ارتفعت النسبة عند تدفئة البيض لمدة 6 ساعات (47.16%) وتخزينة لمدة 4 ايام (47.07%) ومن ناحية اخري انخفضت النسبة بتقدم عمر القطيع وزيادة فترة التخزين (68.87%, 50.87%) (59.67%, 40.25%) (40.25%، 58.72%)، (41.07% 25.18%)، (69.43% 47.04%) (41.46%، 29.01%) علي التوالي. تدفئة البيض قبل التخزين ادت الي زيادة معنوية في نسبة كتاكيت الدرجة الاولي وانخفاض الدرجة الثانية ومن ناحية انخفضت نسبة كتاكيت الدرجة اولي مع تقدم عمر القطيع وزيادة فترة التخزين (73.45%, 90.21%, 90.52%) (76.51%، 86.54%، 91.14%) كما اوضحت الدراسة ان وزن البيض المفقود يزداد معنويا مع زيادة ساعات التدفئة خلال فترة التخزين (15.51%, 14.73%, 13.9%) ، عمر القطيع (16.51%، 15.59%، 12.10%) وزيادة فترة التخزين (16.27%, 14.28%, 13.65%) افضل عائد للكتاكيت (66.82%) و (68.12%) نسبة كتاكيت الدرجة الاولي (95.10%) (90.72%) (91.33%) و نسبة الفقس من العدد الكلي والبيض المخصب (49.88%، 67.58%) تم الحصول عليها عند تدفئة البيض لمدة ساعة وتخزينة لمدة 4 ايام عمر القطيع 75 اسبوع .

خلصت الدراسة الي ان تدفئة البيض المخصب قبل التخزين لمدة 6 ساعات او تدفئة لمدة ساعة اثناء التخزين في درجة حرارة 37.5م و53% رطوبة نسبية ومن ثم تخزينة لمدة 4 ايام في درجة حرارة 18م ورطوبة نسبية 75% يمكن استخدامة في صناعة الدواجن كوسيلة لتحسين نتائج الفقس لبيض الفقس المنتج من امات متقدمة في العمر.

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CHAPTER ONE

1. INTRODUCTION

Over the last few years, hatching percentages virtually stayed in the range of 79-82%. Limited improvement was made on hatchability which cost the poultry industry a lot of money each year. Improvements was made on feed conversion ratio, growth rate, meat yield, but not much on hatchability specially on broiler parents and heavy body line breeders (Schaal and Cherian, 2007).Storage of hatching eggs is a common practice in commercial breeder farms and hatcheries. The length of the egg storage period varies between a few days and several weeks. Many factors affecting storage duration such as hatching eggs supply, the hatchery capacity and changeable in one-day-old chicks market demand in poultry industry. In general, commercial hatcheries set their eggs after a short period of storage. In contrast, sometimes hatcheries need to extend the storage period exceeding 7 days. Egg storage period beyond 7 days could make a lag in embryonic development (Christensen *et al*., 2001), affecting negatively the hatchability and the livability (Christensen *et al*., 2002; Elibol *et al*., 2002; Van de Ven, 2004), and decline in hatchability (Tona *et al*., 2004; Petek and Dikmen, 2006; Yassin *et al*., 2008).Studies had shown that hatching eggs storage is detrimental to hatchability, embryonic development and mortality and the hatched chick quality especially storage length and conditions and especially for old age breeders and heavy body lines. The reproduction process can be divided into 3 periods, the pre-incubation, and the incubation and hatching periods. Embryonic chick mortality is also divided into 3 phases of early-mid and late deaths each with different and special causes, effects and outcomes. All these are reflected on the chick quality which is difficult to define through some qualitative and quantitative approaches used as

body weight, chick length, appearance, activity and quality of navel area (Deeming, 2000).For this many methods and approaches were made to improve hatchability percentages, reduce embryonic mortality and chick quality especially for old age breeders and heavy body lines. Warming hatching eggs was one of the methods adopted and indicated possible improvement on chick hatchability and quality (Marandure *et al*. 2012). There, warming eggs before or during storage and heating eggs just prior to setting were used. Warming could be administered prior to storage, during storage or for few hours immediately before egg setting. This study is an attempt to assess the multi factorial effect of age, storage time and duration on hatchability and chick quality of layers breeder' lines using egg warming technique using pre-storage warming and short period of incubation during egg storage.

Study hypothesis:

Pre-storage warming and short period of incubation during egg storage improve of old light breed hatchability, embryogenesis and hatched chick quality.

Study Justification:

- Limited or lack of improvement on hatchability and storage effect of old breeder's eggs compared to other production factors.
- Negative effect of long storage on old breeder hatching eggs light body breeder lines.
- Economic loss of the industry from both reduction in hatchability and low hatched chick quality.

Study importance:

- Increased need for improved hatchability percentage and chick quality of old light body breeder lines.

- Decreasing embryonic mortality.
- Increasing first grade chicks for better profitability and returns to the poultry industry.

Objective of the study:

To assess the effect of breeder age, length of storage and frequency of warming eggs before and during storage on hatchability and chick quality of late layer breeder's eggs.

CHAPTER TWO

2. LITERATURE REVIEW

2.1: Incubation and Hatching

Hatchery as a segment of the poultry production chain is mainly to produce day old chicks safe to start the poultry production chain. It is said that the productivity of a hatchery is the total number of first-quality chicks produced. This is called saleable chicks. This number of saleable chicks expressed as a percentage of all eggs set to be incubated is normally referred to as hatchability (Cobb Hatchery Management Guide, (2008). Over the last 20 years, hatchability percentage had virtually stayed the same, ranging from 79-82%. The lack of improvement in hatchability is costing the poultry industry a lot of money each year. As improvements were made in meat yield, growth rate, and feed conversion ratio, a small amount of emphasis was placed on hatchability (Schaal and Cherian, 2007). One of the impressive aspects of commercial hatchery is the number of chicks that can be hatched with relative easiness from incubators equipped with sophisticated controls to maintain optimum conditions for hatchability (Neshiem and Leslie, 1972)**.** Chickens are grown from fertile eggs (hatching eggs). The process from the time of egg formation to hatching is very complex. Hatching is a process by which in the span of 21 days, a microscopic germ is changed into downy chick, capable of walking, eating and expressing its needs by voice and action (Sunil Kumar, 1993)**.** The reproduction process can be divided into pre-incubation, incubation and hatching periods. The pre-incubation period can be further divided, but will ultimately represent the time at which the egg is fertilized until it is set in the incubator. This includes hatching egg collection at the farm, transportation to the hatchery, and storage at the hatchery prior to setting. Incubation is the process of providing fertile eggs with optimum environmental

conditions (temperature, humidity and egg turning) to stimulate embryonic development until hatching, which can be natural or artificial (King'ori, 2011).

2.1.1: Natural incubation

The broody hen provides fertile eggs with optimum environmental conditions to stimulate embryonic development until hatching. The broody hen chosen for natural incubation should be large, healthy and preferably vaccinated and with a good brooding and mothering record. Signs of broodiness are that the hen stops lying, remains sitting on its eggs, ruffles its feathers, spreads its wings and makes a distinctive clucking sound. A maximum of 14–16 eggs may be brooded in one nest, but hatchability often declines with more than ten eggs, depending on the size of the hen. Feed and water provided in close proximity to the hen, will keep it in better condition and reduce embryonic mortality due to the cooling of the eggs if the hen has to leave the nest to scavenge for food (Olsen, 1930).

2.1.2: Artificial incubation

The modern incubator is a simulated artificial design that mimics or emulates the mother-hen role of providing fertile eggs with optimum environmental conditions to stimulate embryonic development until hatching (French, 1997). Due to the intensification of poultry production, the brooding hen was first replaced by a small still air incubator and then by a forced-draught incubator. The forced draught incubator was used as a multi-stage system in which eggs of different ages were setted in the incubator at the same time. Since the early nineties, it had been recognized that multi-stage incubators did not completely fulfill the embryonic requirements and did not optimize hatching quality (Hill, 2000). Therefore, single-stage incubation was introduced, in which only eggs of one age were set in an incubator. In a single-stage incubator, environmental conditions, such as temperature, relative humidity, and CO_2 concentration can be controlled, based on the changing embryonic requirements

during the different phases of embryonic development (French, 1997; Hulet *et al*., 2007; Bennett, 2010).The design of a modern incubator is essentially an engineering solution to the biological parameters of temperature, humidity, air supply and movement that have been obtained by research in incubation technique. The incubation requirements and practices had been summarized by Wilson (1991). These practices include setting eggs large end up, turning once per hour and providing a temperature of 37.5°C, a relative humidity of 53% during the first 18 days of incubation. During the last three days temperature should be decreased to 37.0 °C, and humidity increased to 75% without eggs turning.

2.2: Physical Conditions Required for Successful Incubation

Hatcheries act as a 'funnel', taking hatching eggs from very few breeder farms and producing day-old chicks to a much larger number of broiler and layer producers (International Hatchery Practice, 2015). A fertile egg is a self-contained life support system for the developing embryo. However, the HE depends on their environment, for heat, gas exchange and movement, to ensure that chick development continues. The modern broiler's embryonic period now composes 30 – 40% of its total lifespan, making it very important component of the production cycle (Ricks *et al*., 2003). The environmental conditions that result in the highest hatching percentage of fertile eggs were largely determined long ago. There are four factors that can be precisely controlled during incubation. These include temperature, humidity, ventilation and egg turning.

2.2.1: Temperature

Temperature is the most critical one among the four factors that affects hatchability. Incubation temperature and the optimum temperature ranges between 37.5-37.7°C at the development stage and 36-37°C during hatching period which is considered as a core determinant in the incubation process (Decuypere *et al.*, 2001; Meijerhof, 2009). The egg shell temperature seeks to

determine the embryo temperature and in turn is impacted by breeder age, embryonic development stage, heat generated by embryo, heat transfer between egg and environment, air temperature in setter and hatcher, air velocity and relative humidity (Hamidu *et al.,* 2007;Lourens *et al.,* 2007). High incubation temperatures at the beginning and at the end of incubation had reduced body weight when compared to normal incubation temperature (Lourens *et al*., 2005; Leksrisompong *et al*., 2007). Hill (2001) and Lourens *et al.* (2005) showed that environmental temperature is the most important factor in incubation efficiency. A constant incubation temperature of 37.8°C is the thermal homeostasis in the chick embryo and gives the best embryo development and hatchability (Wilson, 1991; Lourens, 2001; Lourens *et al.,* 2007). According to French (1997), embryos absorb heat from surrounding environment during the first period of incubation due to the fact that egg temperature being slightly lower than air temperature. As the embryo grows in size it produces more heat than it requires and may even need cooling. This why on day 18 eggs are transferred to the hatching baskets and into the hatchery that operates at a lower temperature of 37.0 °C (King'ori, 2011). Hatchability can also be impaired when the machine temperature fluctuates (Lourens *et al*., 2005). It was confirmed by Yalcin and Siegel (2003) that impaired lung development were noticed in embryos exposed to cold and heat during incubation. It had been well documented that chick embryos will develop and hatch in approximately 21days when conditions are optimal. Yalcin and Siegel, (2003) reported that many factors have been shown to affect the metabolism and growth of embryos during the incubation period; such as, turning, vital gas exchange, temperature control, and moisture loss. Temperature had been indicated to be the most important factor controlling embryo growth and development (Meijerhof, 2000)**.** Embryo body temperature was governed by incubation temperature as studies concerning thermogenesis in the chick embryo, had indicated that the embryo cannot properly regulate its body temperature until the hatching process is completed (Davisson, 1973).

2.2.2: Humidity

The relative humidity is another factor which has serious effect on the hatchling quality and incubation effect. Bruzual *et al*. (2000) reported that optimum relative humidity should range between 50-60% for optimum incubation results. During the incubation, there is an acceptable level of egg weight loss which should be within the range of 12-14% by transfer at 18days (Molenaar *et al.*,2010).When egg weight loss is between 6.5-13.5% until the time the neonate pips, it is not enough to get the right air cell size to begin lung respiration (Molenaar *et al.*,2010). At incubation, lower humidity levels cause the hatchling to be small, dehydrated and sticky (Deeming, 2000). Navels that are uncovered become a problem during higher relative humidity level. It inhibits utilization of yolk sac, induces yolk sac infections, and increases first week chick mortalities. The higher humidity seems to favour better growth and feed conversion (Winn and Godfrey, 1966). The relative humidity within an incubator affects the rate of evaporative water loss from the hatching egg. A relative humidity of 61% often gives the correct rate of water loss, but other variable factors such as shell porosity, air movement and differences between strains can influence it (Rose, 1997). Lundy (1969) concluded that maximum hatchability was associated with humidity which gave a weight loss of 300 mg per egg per day. Robertson (1961) found that the optimum relative humidity throughout the incubation period was 60%. However, he suggested that eggs of different weights might have different optima, where larger eggs need lower humidity. Moisture levels of 60 – 80%, relative humidity is important to stop excess moisture loss from the egg contents through the porous egg shell and membranes (King'ori, 2011).

2.2.3: Ventilation

The embryo depends on a supply of oxygen from the surrounding air. Embryos are more susceptible to low oxygen concentrations, and embryo

survivability is reduced when the oxygen concentration is below 15%. Inadequately ventilated incubators result in high Co_2 and low O_2 concentrations. The buildup of Co_2 often causes more hatchability problems than are caused by the lack of oxygen (Rose, 1997).Tullet and Deeming (1982) demonstrated that embryonic oxygen consumption is proportionally related to the egg shell porosity. Poor ventilation leads to the fluids collecting around the embryo which is caused by low levels of oxygen and high levels of carbon dioxide (Deeming, 2000). Carbon dioxide is needed in very small quantities (0.1-0.4%) while higher concentration (0.5-0.8%) reduces livability of chicks (Decuypere *et al.*, 2001). Although high carbon dioxide concentration serves as a stimulant to early embryonic development, it may also slightly increase the pH during these early embryonic stages (Decuypere *et al.*, 2001). During the last period of embryonic development, increase in carbon dioxide concentration can also stimulate hatching process (Willemsen *et al*., 2008).

2.2.4: Egg turning and egg position

Eggs are turned consistently during the time of incubation to prevent embryos from sticking into the membranes of the shell in the first week of incubation and help in development of the embryo (Cobb Hatchery Management Guide, 2015). During incubation eggs should be set large end up, so they can be turned around the short axis. Eggs are turned 24 times per day at a 45^{o} angle. Failure to turn will result in reduced hatchability due to adhesion of the embryo to the inner shell membrane (Wilson, 1991). He added that the adhesion causes embryonic death and can cause a rupture of the yolk's vitelline membrane. The most critical period for turning is during 3 -7 days of incubation, with little, if any benefit after day 13. Egg turning during incubation is important for successful hatching and influences hatchability. Ceasing turning of eggs during incubation resulted in low hatchability and delayed hatching by a few days (Van Schalkwyk *et al*., 2000; Yoshizaki and Saito, 2003). As embryo

grows, the heat increases alongside. Consistent turning is needed to help airflow and increase cooling.

2.3: Factors Affecting Hatchability

Despite following all the precise incubation requirements for the successful hatch of fertile eggs, it is necessary to know how some biological factors can limit the hatchability of eggs. They found that significant differences in hatchability among eggs from different breeder flocks were found. Hatchability was significantly related to the flock age, egg storage length, strain, feed company, season of the year, as well as hatchery (Yassin *et al.,* 2008). Improved management of eggs during incubation may therefore help to increase the hatchability. Some causes and problems associated with poor hatchability are early embryonic death, egg rotten, broken yolk, dead-in-shell chicks, prolonged pre-incubation storage, poor breeder nutrition, breeder age, contamination, incubator and hatchery malfunctions (Deeming, 1995; Van Schalkwyk *et al*., 2000; Chabassi *et al.*, 2004; Hassan *et al*., 2004; Ipek and Sahan, 2004; Malecki *et al*., 2005).

2.3.1: Breed and strain

Different breeds of birds have different genetic makeup which affects egg production, hatchability and chick quality (Al-Bashan and Al-Harbi, 2010). Infertility results in the inability of the eggs to hatch in some cases while in others, the zygote forms but do not develop and therefore die for a wide variety of reasons (Al-Bashan and Al-Harbi, 2010). In chickens, abnormal position has been estimated to cause 50-55% of mortality in the last 3 days of incubation and 25% of total embryo mortality (Kalita *et al.,* 2013). Other researches show that for chicken eggs with easily distinguishable large and small ends they have higher hatchability and a lower incidence of abnormal position than do eggs with indistinguishable ends (rounder shape) (Wilson, 1991). Wilson and Suarez

(1993) showed that slight variations in the incidence of malposition in chicken embryos can be attributed to genetic strain. It is suggested that genes are affected when young birds (pullet) gain some quality (albumen) from their maternal lineage to produce good albumen characteristics (Islam *et al.,* 2001). Egg quality is affected by selection on body weight, even though this effect may differ between experiments. The differences may originate from the breeding lines (Islam *et al.*, 2001). Although selection on egg production could increase yolk content, selection on egg quality traits has shown genetic variation for yolk content and yolk related characters (Manville and Oguz, 2002). In selecting birds for breeding, it is important to know the different genetic make-up which affects egg production, hatchability and chick quality **(**Al-Bashan and Al-Harbi, 2010). Other Parameters such as hatching time, chick quality characteristics, fertility , quality of egg (Tona *et al.,* 2002) first week chick mortality (Beaumont *et al.,* 1997) and eggshell conductance and embryonic metabolism (Hamidu *et al.,* 2007) have reflected difference in genetic strains. Characteristics of the parent flock are important to be understood because of their effect on the reproductive cycle, the physiological changes due to genetic selection can greatly affect the egg and embryo development. Coleman and Siegel (1965) found that populations of chickens selected for low body weight had more advanced embryonic development at oviposition. They also found an increased hatchability when compared to hens selected for high body weight. Breed has little effect on hatchability of poultry eggs (Islam *et al*., 2002). Management at the breeder farm as well as the hatchery should be adjusted according to the strains, because every strain responded differently to hatchability. Fertility of an egg and embryonic mortality during the hatching process are known to be differing for different strains. The effect of strain could be explained by egg weight and egg components like the yolk and albumen percentages, yolk: albumen ratio, shell thickness and incubation time (Suarez *et al.*, 1997; Joseph and Moran, 2005b).

2.3.2: Breeder's nutrition

Breeder nutrition according to Waller (2007) is that right amount of nutrient given to the breeder which is made up of two parts, nutrient composition of the diet and amount of feed given to breeder birds. Both composition need to be in the right proportion to ensure correct daily nutrient allocation. When feed is not given in the right amount and quality it has a negative influence on the later stages of embryonic development when parent stock have an early production period. For good development of embryo, it is essential that nutrients are deposited in the eggs. And this is when the nutrition of the breeder stock becomes a matter of importance (Qiao, 2008). According to Kenny and Kemp (2005) chicks hatched and the embryo formed all depend on nutrients embedded in the eggs for their survival i.e., their growth and development. The physiological state of the chick during hatching is primarily due to how the breeder flocks was fed which will then have an influence on size of chick, strength and how well its immune system is built. For success in broiler production, a chick must have right body weight with excellent nutrition reserves at day old, especially; essential amino acids are needed for cell membrane building, immune strength and embryonic development which affect chick quality (Qiao, 2008). The diet of poultry breeders should be adequate in both quality and quantity to meet the recommended levels set out in the feed standards for the type. In the management of poultry breeders, feed is regulated to prevent excessive weight gain, a major cause of poor quality ejaculate and ovulation. This will ensure production of good quality and number of eggs and semen (Brillard, 2007). The estimated dietary requirement of protein for laying chicken is in a range of 14% to 18% for light and medium sized exotic birds (Harms *et al*., 1966). Javanka *et al*., (2010) reported improved egg fertility and hatchability of fertile eggs of breeding layers fed brewery by-products. Supplementation of laying hen diets with organic selenium increased fertility

and improved hatchability of fertile eggs (Cantor and Scott, 1974; Davtyan *et al*., 2006; Osman *et al*., 2010).

2.3.3: Breeder's age

The age of the breeders affects hatchability, because it is related to the quality of the H E, such as the internal egg composition or ratio, egg weight, and shell quality, where by the incubation condition and the development of the chick embryo is also influenced (Yassin *et al*., 2008). As breeders age, egg weight increase (Khursid *et al*., 2003), shell thickness reduces (Peebles *et al.,* 2000) and yolk weight increases (Suarez *et al.,* 1997). Eggs laid by young breeder stocks have better albumen quality and hence produces better chicks (Tona *et al.,* 2004). Old breeder stocks produce a large number of heavier chicks (Suarez *et al.,* 1997; O'Dea *et al.,* 2004). At oviposition the proteins of the albumen possess various anti-microbial defenses against organisms that may invade immediately after oviposition, before the drying of the cuticle, and before structural changes in the shell membranes have been completed (Brake *et al*., 1997). As an egg weight increases with age, due to an increase in yolk deposition, the albumen quality or the Haugh Unit value (HU) significantly decreases (Tona *et al.,* 2004). Older breeder lays an egg developmentally more advanced and the embryo may be going through a more active stage of development therefore, reducing its resistance to storage. As flock age increases, the size of the egg increases, due to increased yolk deposition, which causes the decrease in shell thickness. The albumen quality decreases causing the blastoderm to be positioned closer to the egg shell which may result in embryonic mortality (Tona *et al.,* 2004). Most likely the development of chick is affected by combinations of these factors, and that strongly influences the outcome of the embryo is egg storage (Fasenko, 2007). Chicks that hatch from older breeder flocks are usually larger, and of higher quality because they are naturally more resistant to dehydration up to hatching

as compared to smaller chicks from young breeder flocks (Sinclair *et al*., 1990). Factors affecting fertility which originate from the male include sperm quality traits like sperm metabolism, semen concentration, sperm motility and the percentage of abnormal or dead sperm cells (Brammel *et al*., 1996). Fertility factors originating from the female include egg sperm storage tubules (Siegel, 1965).There was also a significant interaction between flock age and age at first delivery, egg storage length at hatchery, strain, feed company, and season. The variation in hatchability was larger among the breeder farms than within breeder farms (Yassin *et al.,* 2008). They added that the average estimated difference in hatchability among the hatcheries was 8%. The average estimated hatchability at 25 week of age was 66% and it increased to 86% between 31 and 36 week and decreased to 50% at 65 week of age.

2.3.4: Egg size and egg weight

Under normal conditions, a fertile egg contains all the nutrients necessary for the development of embryo up to hatching. However, there are certain physical and chemical conditions that may lower hatchability. Effect of egg weight on hatchability is one of the important economic traits used in poultry industries. Egg weight has a function to play in egg hatchability and it is a prerequisite for successful poultry production. According to Farooq *et al*. (2001) egg weight has positive correlation with hatching chick weight and has significant influence on hatchability (Farooq *et al*, .2000). According to Khurshid *et al*., (2004) smaller chick size at hatch is as a result of smaller egg size set for hatching. Gonzalez *et al*. (1999) and Nahm (2001) also stated that pre-incubation egg weight has strong positive correlation and the performance of the bird. Chick weight is 62% -72% of the initial egg weight (Wilson, 1991; Murad *et al*., 2001). Eggs which are large and are heavy normally have poor chick quality compared to small size average weight eggs. Wilson (1991) and Kalita (1994) stated that medium size eggs (51-55g) gives highest hatchability

than small size (< 52g) or large eggs (>65g) (Abiola 1999; Senapati *et al*., 1996). Asuquo and Okon (1993) also reported that intermediate egg size which ranges from 45g-56 hatch better than eggs that are small, but this range falls outside the recommended range for commercial incubation (<52 -65g). Research has proven that egg weight and size increase as the hen ages and egg weight is strongly related to chick weight at hatch. These may be due to the hen or some environmental factors. The physical characteristics of the egg play an important role in the processes of the embryo development and a successful hatching (Narushin and Romanov, 2002). The most important factors that influence egg parameters are egg weight, shell thickness and porosity, shape index and the consistency of the contents. Thin egg shell increases the rate of water loss and egg shell conductance compared with thick egg shell (Joseph and Moran, 2005a). Low egg shell porosity and decreased oxygen availability can be a major limiting factor on embryonic growth (Burton and Tullet, 1983)**.** This can be explained by the associated increase in egg weight, as larger eggs have less shell area per unit of interior egg weight than smaller eggs (Kirk *et al*., 1980; North and Bell, 1990; Reis *et al*., 1997; Roque and Soares, 1994). Secondly, as egg size increases, yolk size increases more than the quantity of albumen (North and Bell, 1990; Zakaria *et al*., 2005). As one might assume, larger eggs produce larger chicks (Lourens *et al*., 2006). However, these larger eggs require a longer hatching time, compared to other eggs produced by the same flock, and may take about 12 hours longer to hatch than smaller ones. This is true even across species; chicken eggs require an incubation period of 21 days, while larger eggs from larger birds such as turkey and peafowl require 28 days (Parkhurst and Mountney, 1988).

2.3.5: Breeder flock management

Temperature and photoperiod are the main factors that influence fertility and hatchability. The optimum temperature ranges for poultry is 18–26°C. Feed

intake in heat-stressed chickens associated with high ambient temperature and relative humidity was reduced by 20%. Heat stress reduced the external and internal egg qualities. Heat stress affects all phases of semen production in breeder cocks (Banks *et al.*, 2005).

2.3.6: Egg storage

Egg storage is a common and important practice in the poultry industry. Knowledge of the effects of storage on HE, the embryo and incubation yield is important for planning incubation by hatcheries. Recommendations for storage environmental conditions depend mainly on the breeder age and storage time. Storage for seven days or more alters the characteristics of albumen, reduces incubation yield, increases incubation period and can damage embryonic development. When working with long storage periods, the adoption of management practices such as storing the egg with the small end down, egg turning during storage and pre-storage incubation should be considered to reduce the negative effects on the incubation yield. Hatching eggs are held at temperature that causes developmental arrest. The temperature where embryogenesis ceases is called physiological zero (Rocha *et al.*, 2013)**.** Normally eggs are stored either at the hatchery or at the breeder farm. In most farms, the hatchery and the breeder farms are considerably separated from each other. The distance between them, coupled with the small number of daily egg collection, which are normally insufficient to be set for incubation forces unintentional storage of eggs before incubation. The hatching eggs are therefore stored in the barn or farm at the prevailing temperature. Heier and Jarp (2001) reported that quality of fresh egg stored in a refrigerator was higher than that of eggs stored at ambient temperature. Sometimes, hatching eggs are also stored at the hatchery because there is insufficient incubator space available. Generally, if eggs are stored for a number of days their quality and hatchability is affected (Petek *et al.*, 2003). Butler (1991) reviewed that the exact temperature had been

widely debated for many years. He concluded that physiological zero lies between 25 and 27°C, farm coolers are typically set between 17 and 20°C but lower storage temperature are recommended if length of storage is increased. Ideally, hatching eggs should be set immediately after they are laid to reduce storage problems and optimize hatchability. This rarely practical, and some storage is always necessary. The main reason for on-farm storage is to minimize transportation costs incurred by the hatcheries, which would be high with daily egg pick-up (Fasenko *et al*., 2001b). After careful collection of fresh eggs, they are stored in a cooler on the farm at a temperature of 18.3°C (North and Bell, 1990). Brake *et al.* (1997) suggested that eggs from older hens should be quickly placed in a cooler place to maintain good hatching quality. There is a period of time during which the contents of the egg reaches equilibrium with respect to ambient temperature after having been placed in the cooler. This period of cooling is largely dependent on the type of storage containers being used. In sealed egg cases, the eggs take four to five days to cool completely; in cases with holes in the sides, only two days are required. In incubator egg trays, eggs take 18 hours to cool completely (North and Bell, 1990).It was suggested that an increase in egg storage duration could activate mechanisms of apoptotic cell death at the blastodermal level. This maybe one of the molecular mechanisms that leads to reduced daily embryonic weight during incubation. Though, experimental controls capable of reducing the cellular and molecular mechanisms of egg storage should be used to increase embryo quality (Hamidu *et al*., 2010). Water is lost through evaporation during storage, and it is influenced by relative humidity (RH), temperature, and shell porosity. Mayes and Takeballi (1984) concluded that attempts should be made to prevent water loss because it negatively affects hatchability. The recommended relative humidity during cooling and storage of HE is 75% (North and Bell, 1990). Prior to incubation, the duration of egg storage affects chick quality (Tona *et al.*, 2003). Storage before incubation may have both the pros-and-cons implications

on chick quality which is dependent on storage time (Reijrink *et al.,* 2009). A lot of investigation has been conducted on the effect of pre-storage incubation to reduce the negative effect of egg storage on hatchability (Fasenko *et al.,* 200l a, b). It was generally concluded that pre-storage time from day zero to day-6 had no effect on hatchability, however, when the time was increased beyond that it could be both beneficial or detrimental (Reijrink *et al*., 2008).Studies had shown that egg storage length is detrimental to the embryo and hatchability, especially when eggs were stored for longer than seven days. Hatchability of eggs from older flocks decreased more with increasing storage time (Kirk *et al.,* 1980). Because of this, it had been suggested that if eggs have to be stored, eggs from younger breeders should be stored rather than those from older breeders (Tona *et al*., 2004). As reviewed by Meijerhof (1992), several studies had shown that hatchability may be reduced by 0.5% per day of storage. Albumen height was significantly decreased with storage time, while albumen pH was increased (Lapao *et al*., 1999). Long egg storage increased incubation length and adversely affects day-old chick quality (Tona *et al*., 2003), and increased embryonic mortality (Kuurman *et al*., 2002). Long egg storage periods affect the pH of the albumen due to loss of carbon dioxide (Dawes, 1975), which is important in maintaining embryonic viability and result in decreased hatchability (Kirk *et al*., 1980; Deeming, 2000; Heier and Jarp, 2001). Overall embryo mortality increased from 10.7% in embryos from eggs stored for 4 days to 27.7% in embryos from eggs stored for14 days (Fasenko *et al*., 2001a).

2.3.7: Storage temperature

Many researchers investigated the effect of storage temperature on the hatchability of fertile eggs**.** Wilson (1991) suggested that the optimum temperature ranges from 20-25°C when storing eggs for less than four days; 16-17°C for four to seven days; and 10-12°C for storage for more than seven days. A study by Ruiz and Lunam (2002) revealed an improvement in hatchability of

fertile eggs from older hens by reducing early embryonic death. This was accomplished by reducing storage temperature from 16.5°C to 10°C during prolonged storage. However, an increase in storage temperature to 20°C for short duration (1-3 days) did not affect hatchability of fertile eggs. Bourassa *et al.* (2003) found that holding broiler eggs for one to four days at 23°C did not alter hatchability or incidence of embryo or chick abnormalities compared to 19°C. However, prolonged storage may have adverse effects on fertile eggs, such as delaying the initiation of development of the embryos following storage (Mather and Laughlin, 1979). Christensen *et al.* (2003) stated that a delay in embryonic development may be compensated by increasing machine temperature during the first periods of incubation. If the ambient temperature is higher, delayed cooling may be a problem. In this case eggs should be collected more frequently to assure that the temperature of the embryo is brought down from 40°C (body temperature) to 26 – 27°C within six hours. A temperature in the range of 27 – 37°C leads to unbalanced development and hence early embryonic mortality. Too quick cooling may also weaken the embryo (Schulte and Svensson, 2011).

2.4: Embryonic Mortality

There are three periods of embryonic mortality; early, mid and late embryonic mortality. The early dead embryo mortality period represents embryos that die during the first seven days of incubation. The death is usually a result of failure of the embryo to resume development after having been stored and placed in the setter. The mid-dead embryo mortality period represents the embryos that die between day eight and 14 of incubation. The death is usually related to nutritional deficiencies inlayer breeder diet or embryonic abnormalities. The late dead embryonic mortality peak represents the embryos that die during the last week of incubation. In this case, death is often due to abnormal positioning, complication in physiological changes, and lethal genes

(North and Bell, 1990). There are many factors contributing to the failure of fertile eggs to hatch which is known as embryonic mortality and these factors include strain and age of the flock, nutrition, egg size and egg weight, egg storage duration and condition (Tona *et al*., 2005).Egg shell porosity must also be appropriate to accommodate the respiratory needs of the embryo, allowing for adequate gas exchange but also prevention of desiccation (Westmoreland, 2003). It had been reported that eggs from early production usually have thicker albumen and egg shell, which can contribute to reduced moisture loss and vital gas exchange (Brake *et al*., 1997), and nutrient availability (Benton and Brake, 1996).Romanoff (1960) indicated that high incubation temperature caused inhibition of embryo growth due to underutilization of albumen. This might be due to the interference of temperature with albumen transfer from the egg into the amniotic cavity, i.e. due an induced nutritional energy deficiency. In a review of the effects of incubator design on embryonic development, French (1997) suggested that machine temperature is to be reduced when incubating larger eggs, since metabolic heat production is not constant throughout incubation. Although, eggs are endothermic during the first half of incubation, they become exothermic as embryonic development proceeds. Consequently, larger eggs had been observed to produce more heat leading to a decline in hatchability as a result of increased embryonic mortality (French, 1997).

2.5: Measurement of Chick Quality

Chick quality is a term that many breeders, hatchery operators and farmers still have difficulty defining. Almost every poultry farmer can identify a quality chick but every one of them has a different way of defining chick quality (Fairchild, 2005). According to Deeming (2000) and Decuypere *et al*. (2001) a good quality day old chick should be clean, dry and free from dirt and contamination. The eyes should be clear and bright, free from deformities and the navel should be sealed with no yolk sac bulging out from the navel. The

chick should have normal body and leg conformity with no sign of respiratory disease. It should also be alert and be interested in its environment with beak well-formed and toes firm and straight. The quality of day old chick is determined by all the process that come into play from egg handling to egg hatching. These factors include pre-incubation factors and incubation factors. Pre- incubation factors are strain of bird, age of hen, health status of the hen, egg quality, egg handling and storage conditions. Incubation factor are incubation temperature, humidity, turning frequency and ventilation (Peebles *et al*., 2001; Tona *et al*., 2003; Decuypere *et al.*, 2001). Different methods both quantitative and qualitative for assessing chick quality have been developed. The first quantitative method for describing chick quality is the body weight of one-day-old chick (Deeming, 2000). A second quantitative method for assessing chick quality is chick length (Hill, 2001; Wolanski *et al.*, 2004, Meijerhof, 2006; Molenaar *et al*., 2008). In addition to quantitative method for assessing chick quality, qualitative measurements had been developed (Decuypere *et al.*, 2001; Tona *et al*., 2003; Boerjan, 2010)**.** Each developed a scoring system based on several qualitative parameters such as appearance, activity, quality of the navel area, free of any abnormalities (Tona *et al.*, 2003). Reijrink *et al.*, (2009) who suggested that pre-storage incubation can be positive or negative for chick quality in dependence of pre-storage incubation time. Also, Marandure *et al*. (2012) found that pre-incubation of broiler breeder HE significantly improved hatchability and post hatch chick uniformity.

2.6: Warming of Hatching Eggs

Older breeders have lower hatchability which could be due to the stage of the embryo development at oviposition. A young breeder will lay a fertile egg containing an embryo that has developed to the gastrulation stage. There are particular embryonic developmental stages that are better able to survive storage. Embryos that have completed hypoblast formation may be at a

relatively inactive stage and may better withstand developmental arrest Fasenko (2007). Eggs of hens with poor hatching records are most likely to profit from pre-incubation warming (Lancaster and Jones, 1986).The modern broiler breeder is a product of high body weight selection (Pollock, 1999; Schaal and Cherian, 2007), thus the modern broiler may benefit from pre-incubation warming. In fact, heating eggs just prior to setting is reported to improve hatchability (Meijerhof, 1992). Pre-incubation warming can be administered prior to storage (Fasenko *et al*., 2001a, 2001b), during storage (North and Bell, 1990), or for a few hours immediately before setting (Proudfoot, 1970).

2.6.1 Warming eggs before storage (WEBS)

Pre-warming eggs before hatching before incubation prevents condensation and also reduces changes that occur within the environment of the egg temperature. This process affects embryo viability, as it affects cell death especially when cell viability is reduced after prolonged storage (Reijrink *et al.,* 2008)**.** In nature, each hen heats their eggs through direct contact with her brood patch and turns the eggs frequently at the beginning of incubation. Therefore, the two major things that a hen has control over are turning frequency and egg temperature. Elibol and Brake (2006) reported that hens keep turning or shift their laid eggs in the natural environment about 96 times in the day. In his review, Wilson (1991) reported turning eggs 96 times daily to be the optimum rate. However, due to maintenance costs associated with the machines and relatively small differences in hatchability, most companies turn the eggs 24 times daily (Elibol and Brake, 2006). Temperature on the other hand, has also been considered to be one of the most influential factors on embryonic growth and development during all stages of incubation. Fasenko *et al.,* (2001a) demonstrated that the hatchability of long stored eggs exhibited a greater percentage improvement when preheated prior to standard incubation than those eggs that were stored for only a short time. Hodgetts (1999) suggested that eggs

could be in a state of shock if warming is not done slowly while Wilson (1991) suggested that it was favorable to mildly heat eggs rapidly to incubation temperature. Yuan *et al., (*2009) indicated that the chicken egg at the time of lay was in the process of active hypoblast formation. Due to the fact that eggs have been found to be in different developmental stages at the time of oviposition, preheating has become a part of hatchery management as preheating has provided a means to incrementally increase the temperature of eggs just prior to incubation. This has been found to be beneficial for eggs that need to be transformed into a state more ready for incubation. Increasing egg temperature to an intermediate range, the eggs were made to achieve temperature more easily when set. This has been suggested to promote early embryonic growth (Güçbilmez *et al.*, 2009). Embryos from broilers strains meant for commercial purposes worldwide are usually intolerant of temperature variations with abnormality and death of the embryo being the extreme of case of exceeding the range of the temperature has been thought to be optimum for incubation (Wilson, 1991). Brannan (2008) mentioned that preheating allowed embryos to more safe and adequately adjust to the dramatic increase in temperature between an egg cooler and an incubator. Eggs being preheated experienced high air velocities were warmed rapidly, while eggs at a low air velocity took several hours to warm (Elibol and Brake, 2008). Wilson (1991) and Lourens *et al.* (2005) suggested that it was favorable to mildly heat eggs rapidly to incubation temperature. Fasenko *et al.* (2001a) reported significantly improved hatchability of turkey breeder eggs that were pre-incubated for 12 hours and then stored for 14 days. Subsequently, Fasenko *et al.* (2001b) observed similar results with broiler breeder eggs. They concluded that although their experiment yielded best results with a pre-incubation treatment of six hours and 14 day storage period, the actual optimum pre-storage incubation treatment may be somewhere between zero and 12 hours. Other studies done by Laurens (2002) indicated that hatchability percentages improved by pre-storage incubation warming eggs.

Also, Petek and Dikmen (2004) found that hatchability percentage of total quail eggs (82.6%) significantly improved by exposure egg to a pre-storage incubation of 8 hours compared to the control (79.7%). Abdel Azeem (2009) concluded that warming quail eggs for seven hours before storage improved hatchability percentage of eggs stored for four days. Lotfi *et al*. (2011) who found that warming quail eggs for short-term before storage increased total hatchability and decreased incubation length without any negative effect on chick quality. Warming older breeder eggs during storage may increase the development stage to an active stage helping withstand storage (Fasenko, 2007).It was concluded that heating eggs for six hour before storage improves incubation results as it decreases incubation length and late embryonic mortality, therefore its use can be indicated in commercial operations (Silva *et al.,* 2008). Embryos of eggs stored for long-term can be affected in such that after proper incubation temperatures are provided; they initiate growth, but they grow at a slower rate than eggs stored for short term (Fasenko, 2007)**.** In both turkey and chicken eggs, this technique was successful in improving the hatchability of long-term stored eggs. It was hypothesized that particular embryonic developmental stages are better able to survive long-term storage. It was indicated that storing fertile eggs below physiological zero inhibits embryonic development (Fasenko and O'Dea, 2009). Gamble *et al*. (2010) concluded that a pre-storage warming protocol might increase hatchability in the commercial industry.

2.6.2: Warming eggs during storage (WEDS)

It is common practice for hatching eggs to be stored for several days before starting incubation. If temperature (18-20°C) and humidity (75%) in storage rooms are controlled properly, eggs can be stored for more than seven days with adverse drop in hatchability. Longer periods of storage however do affect the viability of the embryo (Pas Reforms, 2015). Earlier, Decuypere

(1992) showed that hatchability increased when eggs were incubated for short periods before being stored. At the turn of the century (Fasenko *et al*., 2001a; Fasenko, 2007) reported that after six hours of storage before incubation, chicken embryos reach the more storage resistant hypoblast stage of embryonic development. Even though Dymond *et al.* (2013) suggested alternatively that eggs introduced to short periods(less than 6 hours) of incubation at consistent intervals during a longer time of storage would allow the embryo to repair its cells and also minimize death. In the broiler industry, embryonic temperature stimulation during pre-storage incubation has been adapted still further to deliver multiple periods of stimulation. Dymond *et al.* (2013) have shown that three-to-four 'Short Periods of Incubation during Egg Storage' – or 'SPIDES' - of 21 days increased hatchability and reduced hatching time. This depicts the natural settings where hens sit on the eggs to bring to lay, rewarms the eggs laid initially and then keeps coming back to sit to lay more eggs **(**World Poultry, 2014). According to data from Pas Reform (2015) when practicing WEDS, eggs are transferred from the storage room to a pre-warmed or running incubator and cooled again to storage as soon as eggshell temperature reaches a maximum of 32°C. The time needed to reach 32°C (90°F) varies with incubator type, but is typically after 3-6 hours incubation at 37.8-38 °C (100.0-100.4 °F). To prevent embryos from developing beyond the storage resistant stage, care must be taken that, during the complete or multiple WEDS treatments, the cumulative time that eggshell temperature rises above 32°C (90°F) does not exceed 12 hours. One treatment of pre-storage incubation or multiple treatments (WEDS) tends to improve hatchability and internal chick condition if eggs are stored for seven days or more. During SPIDES the interval between incubation treatments is typically 5-6 days (Pas Reforms, 2015). SPIDES increase hatchability by about 2-3% especially for eggs stored for about one to two weeks. WEDS is not a short cut to recovery from poor hatchability but it minimizes the rate of decrease of hatchability caused by the long period of egg stored (Aviagen,

2014).The pre-incubation warming profile is the time and curve used to increase the internal egg temperature from the storage temperature to the incubation temperature. Warming eggs before incubation (pre-incubation warming) has been shown to affect the hatchability of eggs from both chickens and turkeys. Slow pre-incubation warming prevents condensation on eggs at the onset of incubation, but the effects of the pre-incubation warming profile on embryo viability are unknown. Some authors have suggested that it was beneficial to warm eggs quickly to the incubation temperature because a prolonged time at temperatures below 35°C may increase embryonic mortality and / or abnormal embryonic development (Wilson, 1991; Renema *et al*., 2006). Proudfoot (1970) stored eggs for seven and 14 days in coolers at temperature ranging from 11-23°C as a way to emulate transportation conditions when shipped by air. The pre-incubation warming treatments were carried out 18 hours prior to setting. Although the results showed an improvement in hatchability of egg receiving the pre-warming treatments, the standard control group of eggs ultimately had the best reported hatchability. Christensen *et al*., (2003) noted that long term storage effects can be alleviated by exposure to higher incubation temperature.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1: Experimental Site and Duration

The experiment was conducted at a hatchery unit of Coral Company for Feed and Chicks Production. This farm is located north of Khartoum State. This experiment was undertaken during the year 2016(August to November) to study the effect of breeder age, length of storage and frequency of warming eggs before and during storage on hatchability, chick quality and embryonic mortality of late layer breeder's eggs.

3.2: Egg Collection

Hatching eggs (HE) were collected from late DeKalb breeder's flock of 75, 80 and 85 week of age. The flock was raised in closed system of housing. Natural mating was practiced and the ratio of males to females was 1:10. The eggs were collected three times a day and were immediately transported to the hatchery to be stored there.

3.3: Experiments Lay Out

3.3.1: Warming eggs before storage (WEBS)

A total of 810 clean, normal and fertile eggs from DeKalb White layer breeder flock at different ages were randomly selected and transported to the hatchery in three groups (270 eggs each). Each group was distributed in a 3 x 3 x 3 factorial arrangement in a complete randomized design with three warming eggs before storage times (0, 3 and 6 h at 37.5°C and 53% RH), age (75, 80, and 85 weeks) and three storage periods (4, 9 and 14 days at 18°C and 75%) summing up to twenty-seven treatments with three replicates ten eggs each placed in setting trays.

3.3.2: Warming eggs during storage (WEDS)

A total of 810 fertile eggs, clean and without shell abnormalities from DeKalb White layer breeder flock eggs at different ages(75, 80, and 85 weeks) was randomly selected and transported to the hatchery in three groups (270 eggs each). Each group was distributed in a 3 x 3 x 3 factorial arrangement in a complete randomized design, with three daily warming durations (WEDS) (0, 1 and 2hours) at 37.5°C and 53% RH), age (75, 80, and 85 weeks) and three storage periods (4, 9 and 14 days 18 °C and 75% RH) summing up twenty seven treatments with three replicates ten eggs each placed in setting trays.

3.4: Incubation Management

Eggs were collected three times a day and transported to the hatchery and immediately disinfected by simple fumigation with 3.2g paraformaldehyde⁄m3 area for 20 minutes heated in an electric pan to 105°C in the fumigation room at 25°C and relative humidity 70%. After disinfection the room was ventilated with fresh air for 1.5 hrs to remove the fumigation residues. Hatching eggs in the control (0 minutes) were kept in the cooler at 18°C and relative humidity 75% during the entire storage period. The four treatments were placed in a setter (Pas Reform, type Corridor 57, 2002, Zeddam) operating at 37.5°C and 53% RH, removed after 3,6hours(WEBS) and 1, 2 hours (WEDS) respectively and transferred to the egg storage room. This protocol was repeated on the three storage periods (4, 9 and 14 days). After 4, 9 and 14 days of storage period the eggs were set in a setter (Pas Reform, type Corridor 57, 2002, Zeddam) at 37.5°C average temperature and 53% RH, eggs were hourly turned for 18 days using single stage incubation program of layer eggs. At day 18 of incubation, hatching eggs (HE) were candled and consequently the clear eggs were removed and opened to determine macroscopically infertile or stage of embryonic mortality to calculate true fertility.

3.5: Hatching

After candling, HE with living embryos were transferred to the hatchery baskets and were placed in the hatcher cabinets (Pas Reform, Tiros, 2002, Zeddam) in which the temperature and relative humidity were adjusted at 36.6°C and 75% RH.

3.6: Measured Parameters

At the end of the hatching process hatched chicks and pipped eggs were removed and counted. All chicks were classified as first or second grade chicks based on the physical parameters. A chick was classified as a first grade chick if it was clean, dry, and free of deformities or lesions and had bright eyes. The other chicks were classified as second grade chicks. The remaining unhatched eggs were broken to determine the late stage of embryonic mortality. At 18 and 21 day of incubation the following periods and phases of embryonic mortality were used to classify the dead embryos. The main characteristics observed in the current study based on description of Tona *et al.*, (2004) who reported them as follows:

Days 1 – 7 (white membrane over the yolk, blood ring).

Days 8 – 14 (black eye visible, embryo without down).

Days 15 – 21 (small embryos with down, full grown embryos with yolk out or full grown dead embryos).

The fertility, hatchability and mortality records were reported according to (Erensayin, 2000) as follows:

True Fertility (%) = Number of fertile eggs ⁄ total number of eggs set X100

Hatchability eggs set (%) = Number of chicks hatched ⁄ total number of eggs set X 100

Middle phase mortality (%) = Number of embryos dead in middle phase ⁄ number of unhatched eggs X100

Late phase mortality (%) = Number of embryos dead in late phase ⁄ number of unhatched eggs ×100

First grade chicks (%) = Number of first grade chicks ⁄number of chicks hatched×100

Second grade chicks (%) = Number of second grade chicks ⁄number of chicks hatched×100

3.7: Statistical Analysis

Data were subjected to ANOVA using the General Linear Model procedure of SPSS (2008). Duncan's multiple range test used to assess the significant differences among treatment means according to the method described by (Steel *et al.*, 1996).

CHAPTER FOUR

4. RESULTS

4.1 Effect of Breeder Age, Length of Storage and Frequency of Warming Eggs before Storage on Egg Weight Loss and Chick Yield:

4.1.1 Fresh egg weight:

The main effects of pre-storage warming duration, egg storage period and breeder's age on fresh egg weight are presented in Table (1). No significant differences were found in fresh egg weights among the different warming eggs before storage frequency treatments, breeder' age and storage period. Also all main factors interactions effects resulted in no significant effect on fresh egg weights.

4:1:2 Egg weight loss during storage:

Effect of breeder age, length of storage and frequency of warming eggs before storage on egg weight loss percentage during storage is shown in table (1). All factors and interactions were highly significantly ($P \leq 0.01$) the egg weight losses percentage during storage. The result showed eggs warmed for three hours WEBS duration had the highest weight loss percentage during storage followed by six hours and non-warmed eggs. On the other hand, the weight loss percentage during storage was significantly ($P \leq 0.01$) better for 75-weeks-old breeders compared to 80- and 85-weeks old breeders. However, storage period (days) was significantly ($P \leq 0.01$) affected the egg weight loss during storage. Four days storage had the lowest egg weight loss percentages during the storage period followed by nine and fourteen days. The results indicated that there were significant interactions between storage period and WEBS duration on egg weight loss percentages during storage (Table 2). Egg

weight loss percentage during storage increased as a function of storage period at any WEBS duration. However, eggs warmed for three or six hours and stored for fourteen days had higher weight loss percentage, during storage, as compared to non-heated eggs at 80 or 85-weeks-old breeders.

4:1:3 Egg weight loss (%) during incubation:

Eggs warmed for three hours WEBS times showed a significantly ($P \leq 0.01$) lower weight loss percentage during incubation compared to the six hours and non-heated eggs. No significant differences in egg weight losses percentage during incubation between eggs warmed for zero and 6 hrs. On the other hand, the weight loss percentage during incubation was significantly ($P \leq 0.01$) better for 75-weeks-old breeders compared to 80- and 85-weeks old breeders. However, storage period for nine days storage period significantly ($P \leq 0.01$) improved egg weight loss percentages during incubation followed by four and fourteen day's storage period, respectively. The results indicated that there were significant interactions between storage period and WEBS duration on egg weight loss percentages during incubation (Table 2). eggs warmed for three or six hours and stored for fourteen days had higher weight loss percentage, during incubation, as compared to non-warmed eggs at 80 or 85-weeks-old breeders.

4:1:4 Total egg weight loss and chick yield (%):

Table 1 shows that Total eggs' weight loss and chick yield were significantly ($P \leq 0.01$) affected by breeder age, length of storage and frequency of WEBS eggs warmed for three hours WEBS times showed a significantly ($P \leq 0.01$) lower weight loss percentage in total weight losses compared to the six hours and non-warmed eggs. WEBS for three hours significantly ($P < 0.01$) improved chick yield percentage followed by six hours and non-warmed eggs. On the other hand, total weight losses and chick yield percentage were significantly ($P \leq 0.01$) better for 75-weeks-old breeders compared to 80- and 85-weeks old breeders. However, storage period (days) was significantly ($P \leq 0.01$)

affected the total weight loss and chick yield. Four days storage significantly ($P \leq 0.01$) had the lowest chick yield followed by nine and fourteen days, while nine days storage period significantly ($P \leq 0.01$) improved total weight loss followed by four and fourteen days storage period, respectively. The results indicated that there were significant interactions between storage period and WEBS times on total egg weight loss percentage (Table 2). Total egg weight loss percentage indicated that eggs stored for 14 days were influenced by WEBS times, eggs warmed for six or three hours had higher weight loss percentage as compared to non- warmed eggs at 75, 80 and 85-weeks-old breeders. These results were observed because exposure to long-time storage and WEBS treatment would increase the opportunity for water evaporation from the eggs.

4:2 Effect of Breeder Age, Length of Storage and Frequency of Warming Eggs before Storage (WEBS) on Embryonic Mortality:

Early, mid and late death of embryos and unhatched egg percentages were significantly influenced by the experimental treatments (Table 3). WEBS for six hours resulted in significantly lower percentages of early, late and total unhatched eggs when compared to the non- warmed eggs or warmed for three hours. Early, mid and late death and unhatched eggs were increased as breeder's age increased. Late death was not influenced by the storage periods. Higher percentages of early death and unhatched eggs were associated with longer egg storage period. When eggs were stored for 14 days, they had significantly ($P \leq 0.01$) increased early, mid death and total embryonic mortality when compared to the other storage period groups (9 and 4 days). Table 4 shows that regardless of the storage period, WEBS for six hours resulted in significantly ($P \leq 0.01$) lower percentages of early, late and total unhatched eggs at all ages when compared to the non- warmed eggs or warmed for three hours. The results indicated that WEBS for 6 h significantly decreased early embryonic mortality

when eggs were stored for four, nine and fourteen days at 75, 80 and 85 weeks of age breeder's eggs. When eggs were stored for more than four days, total embryonic mortality rates were significantly ($P \leq 0.01$) lower when eggs were WEBS for six hours, as compared to those not warmed or warmed for nine hours at 75 as compared to 80 or 85-weeks-old breeder's eggs. Eggs stored for 4, 9 and 14 days and were warmed for six hours prior to storage presented significantly lower total embryonic mortality and as compared to those not warmed. The improvement in the incubation yield in WEBS for six hours, as compared to those not warmed may be related to the embryos stage and the total number of viable embryonic cells, prior to storage.

4:3 Effect of Breeder Age, Length of Storage and WEBS on Fertility and Hatchability Percentage:

4:3:1 Fertility:

The result of the true fertility is shown in Table 5. There were no significant effects of the WEBS times (0, 3 and 6 h), storage period (4, 9 and 14 days) on the true fertility percentage. On the other hand, true fertility percentage was significantly affected due to the breeder's age. Fertility decreased as the age of the breeder stock advanced ($P \leq 0.01$). Table 6 shows that the true fertility percentage was not affected by the interaction of WEBS times, breeder's age and storage period. Storage heating eggs did not affect apparent fertility. Fertility should not have been affected by the two main treatments because fertilization would or would not have occurred before the eggs were exposed to the treatments.

4:3:2 Hatchability:

The results of the hatchability of total and fertile eggs are shown in Table5. Hatchability decreased as the age of the breeder stock advanced ($P \leq 0.01$). Hatchability was improved when the period of the WEBS times increased ($P \leq 0.01$). Deterioration in hatchability has been reported when the

period of storage increased (P≤0.01). In all ages of the breeder stock, the best hatchability was observed with 6 h WEBS and 4 days of storage period. The highest values for the two parameters obtained from eggs produced by 75 weeks old breeders followed by those produced by 80 weeks old breeders and the lowest values obtained from eggs produced from 85 weeks old breeders. Longer period of egg storage resulted in a linear significant decrease in the hatchability of fertile and total eggs. The current results revealed that egg storage for more than four or nine days markedly impaired incubation results due to higher egg weight loss, as shown by the lower hatchability, higher total embryonic mortality percentage. {WEBS times x age (week)} interaction resulted in no significant differences in hatchability from total eggs and hatchability from fertile eggs. {WEBS times x storage period (days)} and {Age (week) x storage period (days)} interactions resulted in highly significant (P≤0.01) effect on hatchability from total eggs and hatchability from fertile eggs percentage. {Pre-warming (time) x Age (week) x storage period (days)} interactions resulted in no significant differences.

4:4 Effect of Breeder Age, Length of Storage and WEBS on Chick Quality:

Commercial chick quality grades were used for measuring chick quality. Chick quality grades were significantly affected by effect of breeder age, length of storage and WEBS times (Table 7). WEBS for six hours resulted in significant (P≤0.01) improvement in both chick quality grades followed by WEBS for three hours, as compared to non-warmed eggs. Egg produced from 75-weeks-old breeders resulted in significant (P≤0.01) improvement in the chick quality grades compared to those produced from 80 and 85-weeks-old breeders. First-grade chick's percentage was significantly (P≤0.01) decreased by the increased storage period, whereas second-grade chick's percentage was significantly increased. The deleterious effects of long-term egg storage on

chick quality could be due to the reduction of embryo weight. There were significant interactions between the storage period and WEBS times for chicks' grade (Table 8). The obtained data indicated that the chicks produced from warmed eggs for six hours and stored for 4to 14 days at 75, 80 and 85 weeks of age breeder's eggs, respectively had higher percentages of grade A chicks. The significant improvement in grade A chicks' percentage in the six hours warming group, as compared to three hours warming group was observed, when eggs were stored for four, nine or fourteen days at 75, 80- and 85-weeks old breeder's eggs, respectively.

4:5 Effect of Breeder Age, Length of Storage and Frequency of Warming Eggs During Storage (WEDS) on Egg Weight Loss and Chick Yield:

Data of WEDS (hours), breeder' age (weeks) and storage period (days) on fresh egg weight, egg weight loss and chick yield percentage are shown in Table (9). The results revealed that warming time (hours) had no significant effect on fresh egg weight (g). On the other hand highly significantly ($P \leq 0.01$) increase in egg weight losses during storage and total egg weight losses were observed by increasing warming time (0, 1and 2 hours). Two hours as warming time highly significantly ($P \leq 0.01$) increased the egg weight losses during incubation compared to those warmed for zero or one hour. No significant differences between eggs warmed for zero and one hour were observed. Significant ($P \leq 0.01$) improvement in chick yield was observed by increasing warming time (0, 1and 2 hours). Significant ($P \leq 0.01$) improvement in egg weight losses during storage, incubation, total losses percentage and chick yield (%) was observed due to breeder age. Seventy five week old breeders eggs were the best followed by 80 week old breeder eggs and the poorest result was obtained for the eggs produced 85 week old breeders. No significant differences were observed on fresh egg weight. significant ($P \leq 0.01$) improvement were observed

for eggs stored for 4 days followed by those stored for 9 days while the poorest results were obtained from eggs stored for 14 days for egg weight losses during storage, total egg losses and chick yield percentage. Significant ($P \leq 0.01$) increase in egg weight losses during incubation was observed for eggs stored for 14 days compared to other periods, but no significant differences in fresh egg weight. Also all factors studied resulted in no significant effect on fresh egg weight and similar tendency was observed on the main interactions effects. The main factors interaction effect resulted in a highly significant ($P \leq 0.01$) effect on all parameters except the fresh egg weight as stated above.

4:6 Effect of Breeder Age, Length of Storage and WEDS on Embryonic Mortality:

Early death, mid death, late death and total embryonic mortality percentages were significantly influenced by the experimental treatments (Table 10). WEDS treatment for (1 hour) resulted in significantly ($P \leq 0.01$) the lowest percentages of early, late and total embryonic mortality when compared with the other short period incubation (2 hours) or (zero hour) group, while mid death was not influenced by the WEDS treatment. Early death, late death and total embryonic mortality percentages were significantly ($P \leq 0.01$) increased by breeder's age, while no significant effect in mid death. Four days of storage period resulted in significantly ($P \leq 0.01$) reduction in early death and total embryonic mortality followed by nine. Mid death results showed a significant ($P \leq 0.05$) reduction for eggs stored for four days compared to nine and fourteen days of storage period. Late death was not influenced by the storage period. Significant interactions were also detected between the WEDS treatment duration and storage period on all embryonic mortality rates (Table 2). The results indicated that WEDS treatment for 1 hour significantly decreased embryonic mortality within all storage periods as compared to those not warmed or warmed for 2 hours at 75, 80 and 85 week of age breeder's eggs respectively except for mid death which showed no significant differences.

When eggs were stored for more than four days, total embryonic mortality rates were significantly lower when eggs were exposed to WEDS treatment for 1 hour, as compared to those not warmed or warmed for 2 hours at 75, 80 and 85 weeks old breeder's eggs respectively.

4:7 Effect of Breeder Age, Length of Storage and WEDS on Fertility and Hatchability (%):

The results of the true fertility, hatchability of total and fertile eggs were shown in Table 11.There were no significant effects of WEDS treatment for (0, 1 and 2 hours), storage period (4, 9 and 14 days) on the true fertility percentage. Storage warming eggs did not affect apparent fertility. Fertility should not have been affected by the two main treatments because fertilization would or would not have occurred before the eggs were exposed to the treatments. On the other hand, true fertility percentage was significantly ($P \leq 0.01$) affected due to breeder's age. The highest values obtained from eggs produced by 75 weeks old breeders followed by eggs produced from 80 weeks old breeders and the lowest values obtained from eggs produced from 85 week old breeders. True fertility percentage was not affected by the all interactions between factors. Hatchability of fertile and total eggs was significantly ($P \leq 0.01$) affected by the experimental factors. The results showed that higher percentages of both hatchability of fertile or total eggs set were observed for groups exposed to WEDS treatment for 1 hour followed by those WEDS for 2 hours and the poorest values observed for control group (0 hour). The highest values for the two parameters obtained from eggs produced by 75 weeks old breeders followed by those produced by 80 weeks old breeders and the lowest values obtained from eggs produced from 85 week old breeders. Longer period of egg storage resulted in a linear significant decrease in the hatchability of fertile and total eggs. A significant ($P \leq 0.01$) improvement in hatchability from total eggs and hatchability from fertile eggs was observed for eggs stored for 4 days followed by those eggs

stored for 9 days and the lowest values stand for eggs stored for 14 days. The interaction between WEDS treatment (hours) and storage period days showed a highly significant (P≤0.01) effect on both hatchability on fertile and total eggs. WEDS treatment for 1 hour significantly increased hatchability in eggs stored for more than 4 days as compared to those not warmed or warmed for 2 hours at 75, 80 and 85 week of old breeder's eggs respectively (Table 12).

4:8: Effect of Breeder Age, Length of Storage and WEDS on Chick Quality:

Commercial chick quality grading was used for measuring the chick quality. Chick quality grade studied were significantly (P≤0.01) affected by WEDS treatment for (0, 1 and 2 hours), breeder's age (75, 80 and 85week) and storage period (4, 9 and 14 days) (Table 13). WEDS treatment for 1 hour resulted in significant (P≤0.01) improvement in both chick quality grades followed by WEDS treatment for 2 hours, as compared to non-warmed eggs. Breeder's age significantly (P≤0.01) affected the chick quality. Chicks produced from 85 weeks of age breeders were significantly (P≤0.01) lower in quality (lower percentage of first grade chicks and higher percentage of second grade chicks) compared to those chicks hatched from 75 and 80 week of age of breeders. No significant differences between chicks hatched from 75 and 80 weeks of age breeders. Long storage period 14 days resulted in significant (P≤0.01) lower quality hatched chicks compared to those hatched from eggs stored for 4 or 9 days. No significant differences in chick's quality between chicks hatched from eggs stored for 4 or 9 days. There were significant interactions between the storage period and WEDS treatment duration for chicks' grade (Table 14). The obtained data indicated that the chicks produced from WEDS treatment for 1 hour and stored for 4, 9 and14 days had significantly higher percentages of grade (A) chicks, as compared to non-warmed eggs at 75, 80 and 85 week old breeder's eggs respectively.

Table 1. Effect of Breeder Age, Length of Storage and WEBS on Egg Weight Loss and Chick Yield.

Main factors	Fresh egg weight (g)	Egg wt. loss during storage (%)	Egg wt. loss during incubation (%)	Total wt. loss (%)	Chick yield (%)
Overall mean	65.00	1.78	11.44	13.21	64.34
±SEM	0.104	0.01	0.019	0.023	0.125
Pre-heating (hours)					
0	64.98	1.67^{c}	11.59^{a}	13.26^{a}	60.51^{c}
3	65.07	1.91^{a}	11.13^{b}	13.04^{b}	66.82^{a}
6	65.21	1.75^{b}	11.59^{a}	13.33^{a}	65.68^{b}
±SEM	0.181	0.017	0.034	0.040	0.217
Significant	NS	**	**	**	**
Age (weeks)					
75	64.78	1.37^{b}	9.68^{b}	11.05^{b}	68.12^{a}
80	64.88	1.99^{a}	12.33^{a}	14.31^{a}	64.43^{b}
85	65.02	1.97^{a}	12.30^{a}	14.27^{a}	60.46^{c}
±SEM	0.181	0.017	0.034	0.040	0.217
Significant	NS	**	**	**	**
Storage (days)					
4	65.10	1.10^{c}	11.74^{a}	12.84^{b}	67.59^{a}
9	64.93	1.33^{b}	10.79^{b}	12.12^{c}	66.78^{b}
14	65.12	2.89^{a}	11.78^{a}	14.67^{a}	58.64^{c}
±SEM	0.181	0.017	0.034	0.040	0.217
Significant	NS	**	**	**	**

N=27/treatment, SEM=standard error of mean

Different superscript letters under the same factor in the same column means significant differences

NS=No significant differences, **=significant difference at P<0.01, *=significant difference at P<0.05

Table 2. Interaction Effect of Breeder Age, length of Storage and WEBS on Egg Weight Loss and Chick Yield

	breeder's age (week)														
	Fresh egg weight (g)			Egg wt. loss during storage (%)			Egg wt. loss during incubation (%)			Total wt. loss (%)			Chick yield (%)		
	75	80	85	75	80	85	75	80	85	75	80	85	75	80	85
0hr × 4 days	64.9	65.0	65.3	0.9^{e}	0.8^{f}	0.8^{d}	11.2^{b}	12.5^{bc}	13.1^{bc}	12.1^{b}	13.3^{d}	13.8^{c}	68.1^{abc}	64.7^{b}	70.6^{a}
3hrs × 4 days	65.3	64.9	65.0	1.8^{a}	1.2^{e}	0.8^{d}	8.5^{f}	12.5^{bc}	13.8^{a}	10.3^{e}	13.7^{b}	14.6^{b}	68.1^{abc}	68.5^{a}	67.2^{b}
6hrs × 4 days	65.1	65.3	64.9	1.1^{de}	1.2^{e}	1.4^{b}	7.9^{g}	12.8^{b}	13.4^{b}	9.0^{f}	14.1^{b}	14.8^{b}	68.7^{abc}	64.8^{b}	70.8^{a}
0hr × 9 days	65.1	65.2	64.9	1.2^{cd}	1.6^{c}	1.4^{b}	9.8^{e}	13.9^{a}	10.4^{d}	11.0^{d}	15.5^{a}	11.8^{d}	69.3^{ab}	67.7^{a}	68.1^{b}
3hrs× 9 days	65.3	64.8	65.0	1.5^{b}	1.4^{d}	1.3^{c}	7.6^{h}	11.9^{d}	10.4^{d}	9.1^{f}	13.3^{d}	11.7^{d}	70.0^{a}	67.4^{a}	69.2^{ab}
6hrs× 9 days	64.8	65.0	65.2	1.1^{de}	1.2^{e}	1.2^{c}	10.1^{d}	12.4^{c}	10.6^{d}	11.2^{cd}	13.7^{c}	11.8^{d}	68.6^{abc}	64.1^{b}	66.5^{c}
0hr × 14 days	64.9	64.8	64.8	1.4^{bc}	3.3^{b}	3.4^{a}	10.0^{e}	10.1^{e}	13.1^{bc}	11.3^{c}	13.6^{cd}	16.5^{a}	65.6^{d}	61.5^{c}	0.0^{d}
3hrs× 14 days	65.0	65.1	65.1	1.9^{a}	3.5^{a}	3.9^{a}	10.5^{c}	12.3^{c}	12.7^{c}	12.4^{b}	15.8^{a}	16.5^{a}	66.9^{cd}	62.0^{c}	68.0^{b}
6hrs× 14 days	65.4	65.3	65.3	1.5^{b}	3.6^{a}	3.6^{a}	11.6^{a}	12.5^{bc}	13.4^{b}	13.1^{a}	15.8^{a}	16.7^{a}	67.7^{bc}	59.2^{d}	67.7^{b}
SEM	0.247	0.268	0.227	0.063	0.210	0.236	0.259	0.188	0.264	0.261	0.201	0.399	0.297	0.863	4.219
Significant	NS	NS	NS	**	**	**	**	**	**	**	**	**	**	**	**

N=27/treatment, SEM=standard error of mean

Different superscript letters under the same factor in the same column means significant differences

NS=No significant differences, **=significant difference at P<0.01, *=significant difference at P<0.05

Table 3. Effect of Breeder Age, Length of Storage and WEBS Times on Embryonic Mortality.

Main factors	Early Death (%)	Mid Death (%)	Late Death (%)	Unhatched (%)
Overall mean	19.01	3.33	6.34	28.68
SEM	0.319	0.170	0.226	0.334
Pre-heating (hours)				
0	26.05[a]	4.07[a]	8.40[a]	38.52[a]
3	18.27[b]	2.84[b]	6.05[b]	27.16[b]
6	12.72[c]	3.08[b]	4.57[c]	20.37[c]
SEM	0.552	0.294	0.391	0.579
Significant	**	*	**	**
Age (weeks)				
75	15.80[c]	2.71[c]	4.81[c]	23.33[c]
80	18.27[b]	3.08[b]	5.19[b]	26.54[b]
85	22.96[a]	4.20[a]	9.01a	36.17[a]
SEM	0.552	0.294	0.391	0.579
Significant	**	**	**	**
Storage (days)				
4	11.48[c]	2.84[b]	6.17	20.49[c]
9	18.03[b]	3.21[ab]	6.05	27.28[b]
14	27.53[a]	3.95[a]	6.79	38.27[a]
SEM	0.552	0.294	0.391	0.579
Significant	**	*	NS	**

N=27/treatment, SEM=standard error of mean

Different superscript letters under the same factor in the same column means significant differences

NS=No significant differences ,**=significant difference at P<0.01, *=significant difference at P<0.05

Table 4. Interaction Effect of Breeder Age, Length of Storage and WEBS Times on Embryonic Mortality Percentage.

	Embryonic mortality (%) at different breeder's age (week)											
	Early death %			Mid death %			Late death (%)			Un-hatched (%)		
	75	80	85	75	80	85	75	80	85	75	80	85
0hr × 4 days	13.3^{d}	16.7^{cd}	15.6^{e}	3.3	3.3	3.3^{bc}	6.7	5.6^{abc}	10.0^{ab}	23.3^{cd}	25.6^{c}	28.9^{d}
3hrs × 4 days	10.0^{de}	12.2^{de}	12.2^{ef}	2.2	2.2	3.3^{bc}	6.7	3.3^{c}	10.0^{ab}	18.9^{de}	17.8^{de}	25.6^{d}
6hrs × 4 days	5.6^{e}	8.9^{e}	8.9^{f}	2.2	2.2	3.3^{bc}	3.3	2.2^{c}	7.8^{bc}	11.1^{f}	13.3^{e}	20.0^{e}
0hr × 9 days	26.7^{b}	20.0^{bc}	31.1^{bc}	3.3	3.3	5.6^{ab}	5.6	8.9^{a}	12.2^{a}	35.6^{b}	32.2^{b}	48.9^{b}
3hrs× 9 days	13.3^{d}	16.7^{cd}	22.2^{d}	2.2	3.3	2.2^{c}	4.4	5.6^{abc}	5.6^{c}	20.0^{cde}	25.6^{c}	30.0^{d}
6hrs× 9 days	7.8^{de}	11.1^{e}	13.3^{e}	3.3	3.3	2.2^{c}	2.22	4.4^{bc}	5.6^{c}	13.3^{ef}	18.9^{d}	21.1^{e}
0hr × 14 days	33.3^{a}	36.7^{a}	41.1^{a}	3.3	4.4	6.7^{a}	6.7	7.8^{ab}	12.2^{a}	43.3^{a}	48.9^{a}	60.0^{a}
3hrs× 14 days	20.0^{c}	23.3^{b}	34.4^{b}	2.2	2.2	5.6^{ab}	4.4	5.6^{abc}	8.9^{b}	26.7^{c}	31.1^{b}	48.9^{b}
6hrs× 14 days	12.2^{d}	18.9^{bc}	27.8^{c}	2.2	3.3	5.6^{ab}	3.3	3.3^{c}	8.9^{b}	17.8^{def}	25.6^{c}	42.2^{c}
SEM	1.775	1.595	2.115	0.309	0.247	0.382	0.482	0.514	0.528	2.220	1.969	2.661
Significant	***	***	***	NS	NS	**	NS	*	**	**	**	**

N=27/treatment, SEM=standard error of mean

Different superscript letters under the same factor in the same column means significant differences

NS=No significant differences **=significant difference at P<0.01, *=significant difference at P<0.05

Table 5. Effect of Breeder Age, Length of Storage and WEBS Times on Fertility and Hatchability Percentage

Main factors	fertility and hatchability %		
	True fertility	Hatchability from total eggs	Hatchability from fertile eggs
Overall mean	67.46	38.77	56.54
±SEM	0.436	0.393	0.426
Pre-heating (hours)			
0	67.07	28.15[c]	41.05[c]
3	67.78	40.99[b]	59.39[b]
6	67.53	47.16[a]	69.17[a]
±SEM	0.755	0.680	0.738
Significant	NS	**	**
Age (weeks)			
75	73.83[a]	50.87[a]	68.87[a]
80	67.16[b]	40.25[b]	59.67[b]
85	61.40[c]	25.18[c]	41.07[c]
±SEM	0.755	0.680	0.738
Significant	**	**	**
Storage (days)			
4	67.16	47.04[a]	69.43[a]
9	67.90	40.25[b]	58.72[b]
14	67.32	29.01[c]	41.46[c]
±SEM	0.755	0.680	0.738
Significant	NS	**	**

N=27/treatment, SEM=standard error of mean

Different superscript letters under the same factor in the same column means significant differences **=significant difference at P<0.01, NS=No significant differences

Table 6. Interaction Effect of Breeder Age, Length of Storage and WEBS Times on Fertility and Hatchability Percentage

	fertility and hatchability percentage at different breeders' age(week)								
	True fertility (%)			Hatchability from total eggs (%)			Hatchability from fertile eggs (%)		
	75	80	85	75	80	85	75	80	85
0hr × 4 days	73.3	67.8	60.0	50.0^{b}	42.2^{bcd}	31.1^{b}	68.1^{cd}	62.3^{c}	51.9^{c}
3hrs × 4 days	74.4	66.7	60.0	58.9^{a}	48.9^{ab}	34.4^{b}	79.5^{ab}	73.3^{b}	57.4^{b}
6hrs × 4 days	73.3	67.8	61.1	62.2^{a}	54.4a	41.1^{a}	84.8^{a}	80.2^{a}	67.4^{a}
0hr × 9 days	71.1	70.0	63.3	35.6^{c}	34.4e	14.4^{d}	50.2^{e}	49.2^{d}	22.8^{e}
3hrs× 9 days	75.6	67.8	61.1	55.6^{ab}	42.2^{bcd}	31.1^{b}	73.6^{bcd}	62.3^{c}	51.0^{c}
6hrs× 9 days	73.3	66.7	62.2	60.0^{a}	47.8^{abc}	41.1^{a}	81.9^{ab}	71.5^{b}	66.1^{a}
0hr × 14 days	73.3	64.4	60.3	30.0^{c}	15.6^{f}	0.0^{e}	41.0^{f}	24.1^{e}	0.0^{f}
3hrs× 14 days	76.7	66.7	61.1	50.0^{b}	35.6^{de}	12.2^{d}	65.1^{d}	52.5^{d}	19.8^{e}
6hrs× 14 days	73.3	66.7	63.3	55.6^{ab}	41.1^{cde}	21.1^{c}	75.7^{abc}	61.7^{c}	33.3^{d}
SEM	0.748	0.634	0.663	2.170	2.163	2.634	2.841	3.119	4.324
Significant	NS	NS	NS	**	**	**	**	**	**

N=27/treatment, SEM=standard error of mean

Different superscript letters under the same factor in the same column means significant differences

NS=No significant differences **=significant difference at P<0.01, *=significant difference at P<0.05

Table 7. Effect of Breeder Age, Length of Storage and WEBS Times on Chick Quality

	Chick quality %	
Main factors	1st Grade	2nd Grade
Overall mean	84.72	11.57
±SEM	0.817	0.817
Pre-warming (hours)		
0	67.45[c]	21.44[a]
3	90.84[b]	9.16[b]
6	95.88[a]	4.12[c]
±SEM	1.414	1.414
Significant	**	**
Age (weeks)		
75	91.14[a]	8.86[b]
80	86.52[b]	13.48[a]
85	76.51[c]	12.38[ab]
±SEM	1.414	1.414
Significant	**	*
Storage period (day)		
4	90.52[a]	9.48[b]
9	90.21[a]	9.79[b]
14	73.45[b]	15.44[a]
±SEM	1.414	1.414
Significant	**	**

N=27/treatment, SEM=standard error of mean
Different superscript letters under the same factor in the same column means significant differences
**=significant difference at P<0.01

Table 8. Interaction Effect of Breeder Age, Length of Storage and WEBS Times on Chick Quality.

	chick quality percentage at different breeders' age (week)					
	1st Grade			2nd Grade		
	75	80	85	75	80	85
0hr × 4 days	87.0^{bcd}	86.9^{b}	71.1^{b}	13.0^{abc}	12.5^{bc}	13.1^{bc}
3hrs × 4 days	96.3^{ab}	95.5^{ab}	87.0^{ab}	3.7^{cd}	12.5^{bc}	13.8^{a}
6hrs × 4 days	98.0^{a}	98.1^{a}	94.7^{a}	2.0^{d}	12.8^{b}	13.4^{b}
0hr × 9 days	84.2^{cd}	73.9^{c}	83.3a^{b}	15.8^{ab}	13.9^{a}	10.4^{d}
3hrs× 9 days	94.0^{abc}	94.8^{ab}	92.6^{a}	6.0^{bcd}	11.9^{d}	10.4^{d}
6hrs× 9 days	96.4^{ab}	97.9^{a}	94.7^{a}	3.6^{cd}	12.4^{c}	10.6^{d}
0hr × 14 days	77.2^{d}	43.3^{d}	0.0^{c}	22.8^{a}	10.1^{e}	13.4^{b}
3hrs× 14 days	90.9^{abc}	90.9^{ab}	75.6^{ab}	9.1^{bcd}	12.3^{c}	12.7^{c}
6hrs× 14 days	96.2^{ab}	97.2^{a}	89.7^{ab}	3.8^{cd}	12.5^{bc}	13.1^{bc}
SEM	1.564	3.427	5.755	1.564	0.188	0.264
Significant	**	**	**	**	**	**

N=27/treatment, SEM=standard error of mean
Different superscript letters under the same factor in the same column means significant differences
**=significant difference at P<0.01

Table 9. Effect of Breeder Age, Length of Storage and WEBS Times on Egg Weight Loss and Chick Yield (%)

Main factors	Fresh egg weight (g)	Egg wt. loss during storage (%)	Egg wt. loss during incubation (%)	Total wt. loss (%)	Chick yield (%)
Overall mean	64.93	2.30	12.44	14.73	64.34
SEM	0.176	0.008	0.025	0.030	0.125
Pre-warming (hours)					
0	64.99	1.68^{c}	12.27^{b}	13.96^{c}	60.51^{c}
1	64.79	2.54^{b}	12.20^{b}	14.73^{b}	66.82^{a}
2	65.00	2.67^{a}	12.84^{a}	15.51^{a}	65.68^{b}
±SEM	0.305	0.014	0.044	0.052	0.217
Significant	NS	**	**	**	**
Age (weeks)					
75	65.07	1.57^{c}	10.53^{c}	12.10^{c}	68.12^{a}
80	64.89	2.58^{b}	13.00^{b}	15.59^{b}	64.43^{b}
85	64.82	2.74^{a}	13.77^{a}	16.51^{a}	60.46^{c}
±SEM	0.305	0.014	0.044	0.052	0.217
Significant	NS	**	**	**	**
Storage (days)					
4	65.43	1.22^{c}	12.44^{b}	13.65^{c}	67.59^{a}
9	64.59	1.98^{b}	12.29^{c}	14.28^{b}	66.78^{b}
14	64.75	3.70^{a}	12.58^{a}	16.27^{a}	58.64^{c}
±SEM	0.305	0.014	0.044	0.052	0.217
Significant	NS	**	**	**	**
Pre-warming × Age					
±SEM	0.528	0.024	0.076	0.090	0.376
Significant	NS	**	**	**	**
Pre-warming × Storage					
±SEM	0.528	0.024	0.076	0.090	0.376
Significant	NS	**	**	**	**
Age × Storage					
±SEM	0.528	0.024	0.076	0.090	0.376
Significant	NS	**	**	**	**
Pre-warming × Age × Storage					
±SEM	0.914	0.042	0.132	0.155	0.651
Significant	NS	**	**	**	**

N=27/treatment, SEM=standard error of mean

Different superscript letters under the same factor in the same column means significant differences. **=significant difference at P<0.01, NS=No significant differences

Table 10. Effect of Breeder Age, Length of Storage and WEDS Times on Embryonic Mortality

Main factors	Embryonic mortality (%)			
	Early death	Mid death	Late death	Total mortality
Overall mean	19.42	3.41	7.20	30.04
SEM	0.359	0.148	0.236	0.434
Pre-warming (hours)				
0	25.19[a]	3.58	8.77[a]	37.53[a]
1	14.32[b]	2.96	5.93[c]	23.21[c]
2	18.77[c]	3.70	6.92[b]	29.38[b]
SEM	0.621	0.257	0.41	0.751
Significant	**	NS	**	**
Age (week)				
75	14.32[c]	3.83	5.93[c]	24.07[c]
80	19.01[b]	2.96	7.16[b]	29.14[b]
85	24.94[a]	3.45	8.52[a]	36.91[a]
SEM	0.621	0.257	0.41	0.751
Significant	**	NS	**	**
Storage (days)				
4	11.36[c]	2.84[b]	6.79	20.99[c]
9	18.89[b]	3.45[a]	6.79	29.13[b]
14	28.03[a]	3.95[a]	8.03	40.00[a]
SEM	0.621	0.257	0.41	0.751
Significant	**	*	NS	**

N=27/treatment, SEM=standard error of mean

Different superscript letters under the same factor in the same column means significant differences

**=significant difference at P<0.01, *=significant difference at P<0.05, NS=No significant differences

Table 11. Interaction. Effect of Breeder Age, Length of Storage and WEDS Times on Embryonic Mortality (%)

	Embryonic mortality (%) at different breeder's age (week)											
	Early death %			Mid death %			Late death (%)			Total mortality (%)		
Pre-warming × Storage	75	80	85	75	80	85	75	80	85	75	80	85
0hr × 4 days	14.82^{def}	15.56^{d}	15.56^{ef}	2.59^{b}	1.11^{b}	3.33	8.15^{ab}	8.89	8.89^{b}	25.55^{de}	25.56^{cde}	27.78^{e}
1hr × 4 days	7.78^{g}	7.78^{f}	7.78^{g}	2.59^{b}	1.11^{b}	3.33	5.56^{b}	6.67	7.78b^{c}	15.93^{f}	15.56^{f}	18.89^{f}
2hrs × 4 days	11.48^{fg}	11.11^{ef}	12.22^{fg}	3.33^{ab}	3.33^{a}	3.33	6.67^{b}	7.78	7.78^{bc}	21.48^{ef}	22.22^{e}	23.33^{ef}
0hr × 9 days	24.07^{bc}	20.00^{c}	28.89^{cd}	3.7^{ab}	3.33^{a}	4.44	7.78^{b}	7.78	10.00^{ab}	35.55^{bc}	31.11^{c}	43.33^{c}
1hr × 9 days	14.07^{efg}	13.33^{de}	20.00^{e}	3.33^{ab}	3.33^{a}	3.33	5.56^{b}	6.67	5.56^{c}	22.96^{ef}	23.33^{de}	28.89^{e}
2hrs × 9 days	18.52^{cde}	15.56^{d}	26.67^{d}	3.33^{ab}	3.33^{a}	3.33	7.04^{b}	8.89	5.56^{c}	28.89^{cde}	27.78^{cde}	35.55^{d}
0hr × 14 days	36.67^{a}	37.78^{a}	43.33^{a}	4.44^{a}	4.44^{a}	4.44	10.37^{a}	8.89	12.22^{a}	51.48^{a}	51.11^{a}	60.00^{a}
1hr × 14 days	21.11^{bcd}	21.11^{c}	33.33^{bc}	2.96^{ab}	3.33^{a}	2.22	6.67^{b}	4.44	8.89^{b}	30.74^{bcd}	28.88cd	44.44^{bc}
2hrs × 14 days	26.30^{b}	28.89^{b}	36.67^{b}	4.44^{a}	3.33^{a}	3.33	7.04^{b}	4.45	10.00^{ab}	37.78^{b}	36.67^{b}	50.00^{b}
SEM	1.166	1.769	2.253	0.165	0.272	0.217	0.312	0.494	0.482	1.358	1.946	2.559
Significant	**	**	**	*	*	NS	**	NS	**	**	**	**

N=27/treatment, SEM=standard error of mean
Different superscript letters under the same factor in the same column means significant differences
NS=No significant differences **=significant difference at P<0.01, *=significant difference at P<0.05

Table 12. Effect of Breeder Age, Length of Storage and WEDS Times on Fertility and Hatchability Percentage

Main factors	%		
	True fertility	Hatchability from total	Hatchability from fertile
Overall mean	67.41	37.24	54.50
SEM	0.485	0.414	0.495
Pre-warming (hours)			
0	67.04	29.26[c]	42.87[c]
1	67.16	43.95[a]	64.76[a]
2	68.03	38.52[b]	55.88[b]
SEM	0.840	0.716	0.857
Significant	NS	**	**
Age (weeks)			
75	73.83[a]	49.88[a]	67.58[a]
80	66.54[b]	37.04[b]	55.70[b]
85	61.85[c]	24.82[c]	40.23[c]
SEM	0.840	0.716	0.857
Significant	**	**	**
Storage (days)			
4	66.91	45.93[a]	68.31[a]
9	67.41	37.78[b]	55.34[b]
14	67.90	28.02[c]	39.86[c]
SEM	0.840	0.716	0.857
Significant	NS	**	**

N=27/treatment, SEM=standard error of mean
Different superscript letters under the same factor in the same column means significant differences
**=significant difference at P<0.01, NS=No significant differences

Table 13. Interaction Effect of Breeder Age, Length of Storage and WEDS Times on Fertility and Hatchability Percentage.

	fertility and hatchability percentage at different breeders' age (week)								
	True fertility (%)			Hatchability from total eggs (%)			Hatchability from fertile eggs (%)		
Pre-warming × Storage	75	80	85	75	80	85	75	80	85
0hr × 4 days	67.04	66.67	62.22	41.48^{abcd}	41.11^{bc}	34.45^{bc}	61.54^{b}	61.69^{bc}	55.28^{c}
1hr × 4 days	66.67	66.67	60.00	50.74^{a}	51.11^{a}	41.11^{a}	75.77^{a}	76.88^{a}	68.56^{a}
2hr × 4 days	67.04	65.56	61.11	45.56^{ab}	43.33^{b}	37.78^{ab}	67.64^{ab}	66.11^{b}	61.94^{b}
0hr × 9 days	67.41	66.67	63.33	31.11^{d}	35.55^{c}	17.78^{ef}	45.62	53.42^{d}	28.03^{e}
1hr × 9 days	67.04	66.67	61.11	43.70^{abc}	42.22^{b}	32.22^{c}	64.50^{ab}	63.44^{bc}	52.65^{c}
2hrs × 9 days	67.78	66.67	61.11	38.52^{abcd}	37.78^{bc}	25.55^{d}	55.89^{bc}	56.76^{cd}	41.69^{d}
0hr × 14 days	66.67	66.67	60.00	15.19^{e}	15.56^{e}	0.00^{g}	21.44^{d}	23.28^{f}	0.00^{g}
1hr × 14 days	67.78	66.67	63.33	37.41^{bcd}	37.78^{bc}	20.00^{e}	54.02^{bc}	56.59^{cd}	31.54^{e}
2hr × 14 days	69.26	66.67	64.45	31.48^{cd}	28.89^{d}	14.44^{f}	44.12^{c}	43.15^{e}	22.41^{f}
SEM	0.693	0.576	0.782	1.662	1.927	2.468	2.223	2.910	4.061
Significant	NS	NS	NS	**	**	**	**	**	**

N=27/treatment, SEM=standard error of mean

Different superscript letters under the same factor in the same column means significant differences

NS=No significant differences **=significant difference at P<0.01, *=significant difference at P<0.05

Table 14. Effect of Breeder Age, Length of Storage and WEDS Times on Chick Quality

Main factors	1st Grade (%)	2nd Grade (%)
Overall mean	85.21	14.79
SEM	0.703	0.703
Pre-warming (hours)		
0	69.66[c]	30.34[a]
1	95.10[a]	4.90[c]
2	90.88[b]	9.12[b]
SEM	1.217	1.217
Significant	**	**
Age (weeks)		
75	91.33[a]	8.67[b]
80	89.05[a]	10.96[b]
85	75.27[b]	24.73[a]
SEM	1.217	1.217
Significant	**	**
Storage period (days)		
4	90.72[a]	9.28[b]
9	87.96[a]	12.04[b]
14	76.96[b]	23.04[a]
SEM	1.217	1.217
Significant	**	**

N=27/treatment, SEM=standard error of mean

Different superscript letters under the same factor in the same column means significant differences

NS=No significant differences **=significant difference at P<0.01, *=significant difference at P<0.05

Table 15. Interaction Effect of Breeder Age, Length of Storage and WEDS Times on Chick Quality

Pre-warming × Storage	chick quality percentage at different breeders' age (week)					
	1st Grade			2nd Grade		
	75	80	85	75	80	85
0hr × 4 days	83.11^{ab}	89.32^{a}	73.74^{c}	16.89^{ab}	10.68^{b}	26.26^{a}
1hr × 4 days	95.97^{a}	97.78^{a}	91.88^{a}	4.03^{c}	2.22^{b}	8.12^{d}
2hrs × 4 days	93.08^{a}	95.06^{a}	88.13^{abc}	6.92^{c}	4.94^{b}	11.87^{abc}
0hr × 9 days	77.08^{b}	75.00^{b}	75.56^{bc}	22.92^{a}	25.00^{a}	24.44^{ab}
1hr × 9 days	94.92^{a}	94.87^{a}	93.94^{a}	5.08^{c}	5.13^{b}	6.06^{d}
2hrs × 9 days	91.88^{a}	94.19^{a}	87.83^{abc}	8.12^{c}	5.81^{b}	12.17^{abc}
0hr × 14 days	48.80^{c}	65.00^{b}	0.00^{d}	17.87^{ab}	35.00^{a}	0.00^{d}
1hr × 14 days	94.40^{a}	97.22^{a}	89.68^{ab}	5.60^{c}	2.78^{b}	10.32^{bc}
2hrs× 14 days	87.69^{ab}	92.96^{a}	76.67^{bc}	12.31^{bc}	7.04^{b}	23.33^{ab}
SEM	2.183	2.302	5.542	1.156	2.302	2.091
Significant	**	**	**	**	**	**

N=27/treatment, SEM=standard error of mean
Different superscript letters under the same factor in the same column means significant differences
**=significant difference at P<0.01

CHAPTER FIVE

5. DISCUSSION

In the present study, the results revealed that for all studied groups egg weight and chick yield parameters were significantly affected by warming duration, breeder' age and storage period and the interaction between them. Egg weight loss percentage during storage increased as a function of storage period at any warming duration. However, eggs WEBS for three or six hours and stored for fourteen days had higher weight loss percentage, during storage, as compared to non-heated eggs at 80 or 85-weeks-old breeders. On the other hand, the weight loss percentage during storage, weight loss percentage during incubation, total weight losses and chick yield percentage parameters were significantly better for 75-weeks-old breeders compared to 80- and 85-weeks old breeders. However, storage period (days) was significantly affected the egg weight loss during storage, incubation, total weight loss and chick yield. Four days storage significantly had the lowest egg weight loss percentages during the storage period and chick yield followed by nine and fourteen days, while nine days storage period significantly improved egg weight loss percentages during incubation and total weight loss followed by four and fourteen days storage period, respectively. Results of total egg weight loss percentage indicated that eggs stored for 14 days were influenced by heating duration, eggs warmed for six or three hours had higher weight loss percentage as compared to non-warmed eggs at 75, 80 and 85-weeks-old breeders. These results were observed because exposure to long-time storage and heat treatment would increase the opportunity for water evaporation from the eggs. These findings are in agreement with that of Silva *et al*. (2008) and Reijrink *et al*. (2010). Moreover, Fasenko and O'Dea (2009) reported that pre-heating eggs for long periods increased weight loss. They later attributed this weight loss to the evaporation of moisture from eggs. The moisture loss is progressively

enhanced by continued exposure of eggs to high temperatures. Similar results were observed by Petek and Dikmen (2004), who reported that egg weight losses during the storage were significantly increased by main effects of pre-storage incubation treatment and the length of egg storage. This result could be justified, since exposure to PRESI and along time of storage would increase water evaporation from the eggs.

Early, mid and late death of embryos and unhatched egg percentages were significantly influenced by the experimental treatments. WEBS for six hours resulted in significantly lower percentages of early, late and total unhatched eggs when compared to the non-warmed eggs or warmed for three hours. Early, mid and late death on shell and unhatched eggs were increased as breeder's age increased. Higher percentages of early death and unhatched eggs were associated with longer egg storage period. When eggs were stored for 14 days, they had significantly increased early, mid death and total embryonic mortality when compared to the other storage period groups (9 and 4 days).The results indicated that WEBS for 6 h significantly decreased early embryonic mortality when eggs were stored for four, nine and fourteen days at 75, 80 and 85 weeks of age breeder's eggs. The improvement in the incubation yield in WEBS for six hours, as compared to those not warmed may be related to the embryos stage and the total number of viable embryonic cells, prior to storage.

The results related to WEBS for long period stored eggs were in agreement with previous reports. Reijrink *et al*. (2010) reported that pre-storage heating eggs for seven hours increased the stage of embryonic development, the total number of embryonic cells, and the total number of viable embryonic cells. The stage of embryonic development depends on warming duration and temperature. Reijrink *et al.* (2009) showed that the ability of an embryo to survive prolonged egg storage may depend on the cell activity at a particular stage of development but may also depend on the number of viable embryonic cells. When the number of viable embryonic cells is low, at the onset of

incubation, due to cell death during storage, particular steps in the embryo development may be impeded. This may result in abnormal development or embryonic death. Therefore, WEBS of eggs for 6 h may be considered as a good practice to improve incubation results. The storage immediately after egg collection increased early embryonic mortality and reduced hatchability, probably due to the higher number of embryos in a pre- gastrula stage, which would be more sensitive to cool temperature and storage stress than the embryos at gastrula stage (Fiuza *et al*., 2006). In a study by Fasenko *et al*. (2001a)**,** after their pre-storage heating treatment of 6 h, 76.7% of the embryos were at developmental stage EG13 (hypoblast stage). Hypoblast formation is the initial stage of gastrulation, ensuring their survival during prolonged storage. They hypothesized that embryos at developmental stage EG12 or EG13 are less sensitive to prolonged egg storage than embryos that are less or further advanced. At EG13 stage, the embryo has completed hypoblast formation, and cell migration and differentiation are minimal **(**Bellairs, 1986). Petek and Dikmen (2004) observed that pre-storage warming of poultry eggs resulted in more live chicks and lower level of embryonic mortality. In quail eggs, 7 h of pre-storage warming for two days stored eggs as a short-term storage period, improved hatchability percentage as it decreased embryonic mortality rate (Abdel-Azeem, 2009). These findings were consistent with the findings of Petek and Dikmen (2006) who indicated that total embryonic mortality rate during incubation was significantly affected by pre-storage incubation warming and egg storage periods. They found that embryonic mortality of eggs of 5 h pre-storage incubation warming was lower compared to the control group (0 h). Atif *et al*. (2015) showed that warming hatching eggs of White Hisex breeders at 37.5°C for four hours before storage improved hatchability reduced embryonic mortality and increased the percentage of first-grade chicks.

The present results revealed that hatchability of fertile and total eggs was significantly affected by the triple experimental factors and the interaction between them. There were no significant effects of the WEBS duration (0, 3 and 6 h), storage period (4, 9 and 14 days) on the true fertility percentage. On the other hand, true fertility percentage was significantly affected due to the breeder's age. Fertility and hatchability decreased as the age of the breeder stock advanced. Hatchability was improved when the period of the WEBS period increased. Deterioration in hatchability has been reported when the period of storage increased. True fertility percentage was not affected by the interaction of WEBS duration, breeder's age and storage period. Storage warming eggs did not affect apparent fertility. Fertility should not have been affected by the two main treatments because fertilization would or would not have occurred before the eggs were exposed to the treatments. Similar suggestions were reported by Fasenko *et al*. (2001a) who showed that fertility of broiler breeder eggs was not affected by the interaction as fertilization. The lower percentage fertility of the eggs stored for 14 days and pre-storage incubation for 18 h occurred as a result of an underestimation of fertility, germinal discs that were fertile, but had died very early during development were likely misclassified as infertile. This overestimation of infertility occurred because of the difficulty in distinguishing between fertile germ and embryos that died at very early stages of development. Petek and Dikmen (2004) found that the pre-storage incubation treatments or the interaction with the duration of the storage period did not significantly affect apparent fertility. In all ages of the breeder stock, the best hatchability was observed with 6 h WEBS and 4 days of storage period. The highest values for the two parameters obtained from eggs produced by 75 weeks old breeders followed by those produced by 80 weeks old breeders and the lowest values obtained from eggs produced from 85 weeks old breeders. Longer period of egg storage resulted in a linear significant decrease in the hatchability of fertile and total eggs. The current

results revealed that egg storage for more than four or nine days markedly impaired incubation results due to higher egg weight loss, as shown by the lower hatchability; higher total embryonic mortality percentage. These results are in agreement with previous reports on broiler breeder's eggs (Fasenko, 2007; Silva *et al*., 2008). They observed lower hatchability and higher embryonic mortality percentage of embryos stored for 14 days as compared to 4 days of storage. These results may be due to that some embryos, from eggs stored for a long period, and could not start developing immediately after normal incubation temperatures were provided or they develop at a slower rate (Fasenko *et al*., 2001a). Haque *et al*. (1996) observed lower embryo metabolic rate, particularly during the last stage of embryo development, as well as changes in the circulatory system during embryogenesis as the storage period increased. Heating the eggs for six hours before storage may be considered as a good practice to improve incubation results of eggs stored for short, intermediate and long periods. Also, pre-heating the eggs for six hours resulted in the highest average hatchability when stored for four or nine days as compared to eggs stored for 14 days at 75 as compared to 80 or 85-weeks-old breeder’s eggs. These results agree with Lotfi *et al*. (2011) who found that warming quail eggs for short-term before storage increased total hatchability and decreased incubation length without any negative effect on chick quality. These reports indicated that hatchability was improved by pre-storage warming of hatching eggs. Lourens *et al*. (2006) confirmed a positive effect of pre-storage warming time on the hatchability of broiler breeder eggs. In quail eggs, seven hours of pre-storage warming for two days stored hatching eggs as a short-term storage period, improved hatchability percentage as it decreased embryonic mortality rate (Abdel-Azeem, 2009).

Commercial chick quality grades were used for measuring chick quality. Chick quality grades were significantly affected by pre-storage heating duration, breeder’s age and storage period. WEBS for six hours resulted in significant

improvement in both chick quality grades followed by WEBS for three hours, as compared to non-warmed eggs. Egg produced from 75-weeks-old breeders resulted in significant improvement in the chick quality grades compared to those produced from 80 and 85-weeks-old breeders. First-grade chick's percentage was significantly decreased by the increased storage period, whereas second-grade chick's percentage was significantly increased. The deleterious effects of long-term egg storage on chick quality could be due to the reduction of embryo weight. This is an indication of decreased embryo quality that could affect hatch quality (Hamidu *et al*., 2011). Previously, embryos from broiler eggs, stored for 14 days showed a reduction in growth rate, hatchability and poor chick quality compared with eggs stored for 4 days (Fasenko *et al*., 2001a). In other studies, embryos from eggs stored for long periods showed a reduction in the rate of metabolism than those from eggs stored for a shorter period (Fasenko *et al*., 2001b) and a decline in relative lung weight (Yalçin and Siege, 2003) which resulted in poor chick quality. Significantly higher percentage of first-grade chicks obtain from eggs for four days followed by those stored for nine days while the lower percentage obtained from eggs stored for 14 days, respectively. There were significant interactions between the storage period and pre-storage incubation duration for chicks' grade. The obtained data indicated that the chicks produced from WEBS for six hours and stored for 4to 14 days at 75, 80 and 85 weeks of age breeder's eggs, respectively had higher percentages of grade A chicks. The significant improvement in grade A chicks' percentage in the six hours WEBS group, as compared to three hours WEBS group was observed, when eggs were stored for four, nine or fourteen days at75, 80- and 85-weeks old breeder's eggs, respectively. These results are in accordance with Reijrink *et al*. (2009) who suggested that pre-storage warming can affect the chick quality positively or negatively depending on the duration of pre-storage incubation. Marandure *et al*. (2012) found that pre-incubation of broiler breeder hatching eggs

significantly improved hatchability and post-hatch chick uniformity. Atif *et al*. (2015) showed that warming hatching eggs of White Hisex breeders at 37.5°C for four hours before storage improved hatchability reduced embryonic mortality and increased the percentage of first-grade chicks.

Early death, mid death, late death and total embryonic mortality percentages were significantly influenced by the experimental treatments. Warming eggs during storage (WEDS) treatment for (1 hour) resulted in significantly the lowest percentages of early, late and total embryonic mortality when compared with the other WEDS (2 hours) or (zero hour) group, while mid death was not influenced by the (WEDS) treatment. Early death, late death and total embryonic mortality percentages were significantly increased by breeder's age, while no significant effect in mid death. Four days of storage period resulted in significantly reduction in early death and total embryonic mortality followed by nine. Mid death results showed a significant reduction for eggs stored for four days compared to nine and fourteen days of storage period. Late death was not influenced by the storage period. The reduction in embryonic viability during egg storage is due to the apoptosis (cell death) in the egg (Bakst, 2016).Until seven days of proper egg storage, the number of embryonic cells remains stable, then after seven days, the number of dead and abnormal cells started to increase. Maintaining lower temperature and higher humidity during egg storage can dramatically improve cell viability for eggs to be stored long term (Fasenko, 2007). Similar findings w reported by Gharib (2013) who found that significantly higher rate of late embryonic mortality for egg stored for10 and 14 d compared to 4 and 7 d of storage. Hamidu *et al.,* (2011) explained the deleterious effect of prolonged storage on broiler and layer blast dermal cell viability, cell death and embryo survival. Significant interactions were also detected between the WEDS treatment duration and storage period on all embryonic mortality rates. The results indicated that WEDS treatment for 1 hour significantly decreased embryonic mortality within all storage periods as

compared to those not warmed or warmed for 2 hours at 75, 80 and 85 week of age breeder's eggs respectively except for mid death which showed no significant differences. When eggs were stored for more than four days, total embryonic mortality rates were significantly lower when eggs were exposed to WEDS treatment for 1 hour, as compared to those not warmed or warmed for 2 hours at 75, 80 and 85 weeks old breeder's eggs respectively. This results are in agreement with the previous reports on broilers, turkey and Japanese quail chicks (Anonymous, 2000; Fasenko *et al*., 2001a, b) warming eggs before or during storage was reported to increase hatchability and reduce embryonic mortality. Tag EL-Din, *et al*., (2017) recommended that when storage of eggs to more than seven days, one should warm eggs for 2.5 h every five days to minimize the harmful impact of storage. These results are in agreement with the present study. Reijrink *et al*., (2010) reported that significantly higher late embryonic mortality rate observed for egg stored for 10 and 14 d compared to 4 and 7 d storage.

In the current study, there were no significant effects of WEDS treatment for (0, 1 and 2 hours), storage period (4, 9 and 14days) on the true fertility percentage. Storage warming eggs did not affect apparent fertility. Fertility should not have been affected by the two main treatments because fertilization would or would not have occurred before the eggs were exposed to the treatments. Similar suggestions were reported by Fasenko *et al.,* (2001a) in chicken eggs, and Petek and Dikmen (2004) in quail eggs. They found that the differences for the apparent fertility among the main groups of pre-storage heating and storage duration were not significant. On the other hand, true fertility percentage was significantly affected due to breeder's age. The highest values obtained from eggs produced by 75 weeks old breeders followed by eggs produced from 80 weeks old breeders and the lowest values obtained from eggs produced from 85 week old breeders. True fertility percentage was not affected by the all interactions between factors. These results are in agreement

with those reported by Fasenko *et al.*, (2001) who showed that fertility of broiler breeder eggs was not affected by the interaction as fertilization would or would have not occurred before the eggs were exposed to the pre-storage incubation (0, 12 or 18 hrs) or by storage periods (4 or 14 days). Similarly, Elibol *et al.*, (2002) did not find any significant effects on the apparent fertility when they stored eggs for four, seven, ten and fourteen days at 18°C and 75% RH. Pre-warming treatment did not show any significant effect on the number of fertile eggs and fertility%.

Hatchability of fertile and total eggs was significantly affected by the experimental factors. The results showed that higher percentages of both hatchability of fertile or total eggs set were observed for groups exposed to WEDS treatment for 1 hour followed by those WEDS for 2 hours and the poorest values observed for control group (0 hour). The highest values for the two parameters obtained from eggs produced by 75 weeks old breeders followed by those produced by 80 weeks old breeders and the lowest values obtained from eggs produced from 85 week old breeders. Longer period of egg storage resulted in a linear significant decrease in the hatchability of fertile and total eggs. A significant improvement in hatchability from total eggs and hatchability from fertile eggs was observed for eggs stored for 4 days followed by those eggs stored for 9 days and the lowest values stand for eggs stored for 14 days. The interaction between WEDS treatment (hours) and storage period days showed a highly significant effect on both hatchability on fertile and total eggs. WEDS treatment for 1 hour significantly increased hatchability in eggs stored for more than 4 days as compared to those not warmed or warmed for 2 hours at 75, 80 and 85 week of old breeder's eggs respectively. Lower rates of fertilization, hatchability, and higher embryonic mortality at various incubation periods of older hens' eggs are caused by a number of biological factors such as decreased sperm retention in the uterovaginal sperm host glands (Fasenko *et al.*, 1992) and deteriorating egg quality (Reijrink *et al.*, 2008). In this study,

WEDS treatment for 1 hour for 4 days of eggs storage allowed for an improvement of hatchability, mainly from eggs of older hens and may be considered as a good practice to improve incubation results of eggs stored for short, intermediate, and long periods. These results are consistent with previous reports by Reijrink *et al.,* (2010), who improved hatching from eggs from older hens stored for 11 d and treated with PI.WEDS treatment for 1 hour may be considered as a good practice to improve incubation results of eggs stored for short, intermediate, and long periods. Tag EL-Din *et al.,* (2017) reported that warming egg at 2.5 and 5 hours showed the highest hatchability from total eggs and hatchability from fertile eggs. In further contrast to previous studies by Fasenko *et al.,* (2001b) and Reijrink *et al.,* (2009) found a positive effect on hatchability when advancing SPIDES embryos to early primitive streak formation over several short pre-incubation, advancement of embryos to hypoblast formation or primitive streak formation in 6- or 12-h pre-incubation, respectively, showed a detrimental effect. Dymond *et al.,* (2013) have shown that three-to-four 'short periods of incubation during egg storage' or 'SPIDES' of 21 days increased hatchability and reduced hatching time when compared with eggs stored for similar periods of 21 days (controls). These findings are in agreement with Damaziak *et al.,* (2018) who demonstrated that the 2 × 4 h pre-incubation during 12 d of eggs storage allowed for an improvement of hatchability, mainly from eggs of older hens.

The current results revealed that, WEDS treatment for 1 hour resulted in significant improvement in both chick quality grades followed by WEDS treatment for 2 hours, as compared to non-warmed eggs. Breeder's age significantly affected the chick quality. Chicks produced from 85 weeks of age breeders were significantly lower in quality (lower percentage of first grade chicks and higher percentage of second grade chicks) compared to those chicks hatched from 75 and 80 week of age of breeders. No significant differences between chicks hatched from 75 and 80 weeks of age breeders. Long storage

period 14 days resulted in significant lower quality hatched chicks compared to those hatched from eggs stored for 4 or 9 days. No significant differences in chick's quality between chicks hatched from eggs stored for 4 or 9 days. Longer periods of storage affected the vitality of the embryo, causing increased early and late embryonic mortality, a delay in hatch and reduced chick quality (Fasenko, 2007; Dymond, 2013)**.** There were significant interactions between the WEDS treatment duration for chicks' grade. The obtained data indicated that the chicks produced from WEDS treatment for 1 hour and stored for 4, 9 and14 days had significantly higher percentages of grade (A) chicks, as compared to non-warmed eggs at 75, 80 and 85 week old breeder's eggs respectively. Tag EL-Din, *et al.*, (2017) reported that warming egg at 2.5 and 5 hours showed highest significance for chick quality. Damaziak *et al., (*2018) showed that pre incubation had increased the hatchability of the set and apparently fertilized eggs, decreased the number of unhatched eggs, and improved chick's quality.

CHAPTER SIX

6. CONCLUSION AND RECOMMENDATIONS

6.1. The Study Concludes that:

- Warming hatching eggs of late layer breeder's eggs before for six hours or during storage for one hour at 37.5° C and 53% RH increased hatchability.
- WEBS for six hours or WEDS for one hour at 37.5° C and 53% RH is more efficient in increasing embryonic livability and decrease embryonic mortality percentage.
- WEBS for six hours or WEDS for one hour at 37.5° C and 53% RH and storage period 4-9 days increased the number of saleable first grade chicks which by far increases profits of eggs store for longer periods.
- WEBS for six hours or WEDS for one hour at 37.5° C and 53% RH and storage period 4-9 days could be used by the poultry industry as a method to improve hatchability and chick quality.

6.2. The Study Recommended that:

- WEBS or WEDS should be practiced if eggs are stored for seven days or more especially for late layer breeders to minimize the harmful impact of storage.
- Further research is needed to precisely determine the number of hours of WEBS required obtaining maximum hatchability and chick quality and the interaction with the storage period. Meanwhile, it should be kept in mind that the economic cost of WEBS and WEDS must be evaluated in comparison with its beneficial effects.

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