CHAPTER ONE

1.0 INTRODUCTION

Although world's poultry industry depends mainly on chicken production for both meat and egg, now there is an interest in other poultry species for food production or genetic conservation resources purpose (Hassan, 2011). Japanese quail, the smallest farmed avian species is getting more importance for commercial egg and meat production. It has marked advantages such as fast growth, early sexual maturity, high rate of egg production, short generation interval and short incubation period (Sarabmeet, *et al*., 2008). More recently, quail have become an important source of meat and eggs for human consumption (Kayang, *et al*., 2004).

It is well known that hatching eggs are often stored in farms or hatcheries to minimize transportation costs or to provide enough egg to fill large incubators (Petek and Dikmen, 2006). A common practice in commercial breeder farm and hatcheries is to store hatching eggs from 1-4 days to obtain the amount of eggs required for the normal flow of incubation process, however, the implementation of good hatchery and rearing practices begin with the management of eggs from laying to hatching (Boerjan,2010). Warming hatching egg prior to storage may increase the development stage of fertile eggs from older broiler

breeders to an inactive stage helping them to withstand storage (Fasenko,2007). Heating eggs before storage is an effective method of reducing loss of hatchability (Abdel-azeem, 2009). In recent study Cameron (2008) indicated that eggs of chickens can be warmed at incubation temperature (37.5 ^{0} c) for 15th hrs. without negatively affecting hatchability also Idress (2015) reported that warming hatching eggs of Hi-sex layer breeder at(37.5^{0} c) for 4 hours before storage significantly reduced early dead embryos and total un hatched eggs and the first grade chicks were increased. However, in Sudan, very little information is known concerning the effect of heating quail eggs. Therefore, the objective of this experiment was to Study the effect of pre-heating time of Japanese quail eggs on hatchability and post hatch performance.

CHAPTER TWO

2:0 LITERATURE REVIEW

2.1Incubation and Hatching:

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). The process from the time of egg formation to hatching is very complex. 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 heating eggs collected 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 laying, 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 (Chattock,1925, 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 settled 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 settled 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).

2.2Physical conditions required for successful incubation:

A fertile egg is a self-contained life support system for the developing embryo. However, the hatching egg depends on their environment for heat, gas exchange and movement, to ensure that chick development continues (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 (Garip and Dere, 2011).

2.2.1Temperature:

The internal temperature of the egg is the most important physical factor that affects the development of the embryo. Temperature had been indicated to be the most important factor controlling embryo growth and development (Meijerhof, 2000). The developing embryo hatches earlier if it is incubated at high temperatures up to a maximum of 39^0 c, however, a continuous $37.^0$c gives the best rate of embryo survival. Incubation temperatures can be reduced by up to $2\ ^0$ C shortly before hatching because of the increased activity of the embryo (Rose,1997). Hill (2001) and Lourens et al (2005) showed that environmental temperature is the most important factor in incubation efficiency. The optimum incubation temperature is 37.8°C and should not vary more than 0.3^0 c (Wilson,1991 Lourens, 2001; Lourens, et al., 2007). Egg initially need a very controlled heat input to maintain the optimum temperature of 37.5^0 C because the embryo is microscopic in size as embryo grows in size it produces more heat than it requires and may even need cooling (Kingori , 2011) High incubation temperature 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). 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. 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. 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).

2.2.2Humidity:

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) reported 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. Relative humidity is important to stop excess

moisture loss from the egg contents through the porous of 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). Fan speed that give changes of fresh air per hour generally give an adequate ventilation rate. Rate and direction of air flow over the eggs during incubation influence gaseous exchange through the shell and shell membranes (Wilgus and Sadler, 1954).Tullet and Deeming, (1982) demonstrated that embryonic oxygen consumption is proportionally related to the egg shell porosity. Embryonic development was stimulated during the first 48 hours by increment of carbon dioxide in the incubator up to 4 percent (Sadler, et al., 1954).

2.2.4 Egg turning:

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 45^0 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, or 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)

2.3 Factors affecting hatchability and embryonic mortality:

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). Hatchability was significantly related to the flock age, egg storage length, strain, feed, season as well as hatchery (Yassin et al., 2008). 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 (Van Schalkwyk et al., 2000; Hassan et al., 2004; Malecki et al., 2005).

2.3.1 Breeds strain:

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 (1966) 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 (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, 2005).

2.3.2 Breeder nutrition:

The diet of 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). A study with Kenyan indigenous chicken indicated that the dietary crude protein requirement for laying hens is 12% (Kingori et al.,2010). 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). Poultry diets must be formulated to provide all of the bird's nutrient requirement if optimum growth and production is to be achieved. there are six classes of nutrients. Carbohydrate, the major source of energy for poultry. most of the carbohydrate in poultry diets is provided by cereal grains (Waldroup et al.,1976). Fats, provide energy and essential fatty acids which are required for some body process (Bornstein et al., 1979). Proteins, is required for synthesis of body

tissues (particularly muscles), physiological molecules such as enzyme and hormones) and for proteins which also provide a small a mount of energy (Combs,1962) . Vitamins, organic chemicals (chemicals containing carbon) which help control body processes and are required in small amounts for normal health and growth (NRC, 1994). Minerals, Are required for the formation of the skeleton, as components of various compounds with particular function within the body, as activators of enzymes, and for maintenance of necessary osmotic relationships within the body of the birds (Davis et al.,1968). Water, must be regarded as an essential nutrient, although it is not possible to state precise requirements.

2.3.3 Breeder age:

The age of the breeders affects hatchability, because it is related to the quality of the Hatching egg , 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 breeder age increases the weight of the egg and percentage of yolk increase, while the percentage of shell declines (Tona 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 developmentaly 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).

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. 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,2002 and Romanoff,1960). 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,2005). 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 (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. (Parkhurst and Mountney,1988).

2.3.5 Breeder flock management

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, eggs rotten, broken yolk, dead in- shell chicks, poor breeder nutrition, breeder age, contamination, incubator and hatchery malformations (Van Schalkwyk, et al., 2000,Chabassi, et al., 2004, Hassan, et al., 2004, Malecki, et al.,2005). Temperature and photoperiod are the main factors that influence fertility and hatchability. 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)

2.4 Periods of embryonic deaths

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

in layer breeder diet or embryonic abnormalities. The late dead embryonic mortality peak represents the embryos that die during the last period of incubation. In this case, death is often due to abnormal positioning, complication in physiological changes, and lethal genes (North and Bell, 1990). 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). Eggs shell from early production usually has thicker albumen, 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).Failure of eggs to hatch is

determined by two factors, infertility and embryonic mortality prior to and during incubation. Time of embryonic mortality is not distributed uniformly over the 17-days period of incubation of a quail egg. Time of increased mortality, instead, is distributed over two phases, the first phase occurring during the first and second week of incubation and the second phase occurring during the last days of incubation (14-17 days) (Moseley and Landauer, 1949). They added that increased embryonic mortality during incubation might be attributed to changes in physiological and developmental functioning of the embryo and it also has genetic and environmental causes.

2.5 Effect of heating hatching eggs on hatchability performance

Since older breeders have lower hatchability's this 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). Coleman and Siegel (1966) found that heating eggs from heavy body weight lines for four hours to obtain a comparable stage of embryo development as the eggs from low body

weight lines improved hatchability of these eggs. 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 (Kan et al., 1962; North and Bell, 1990), or for a few hours immediately before setting (Proudfoot, 1970). A number of methods have been investigated to improve the hatchability of eggs stored for more than seven days .one of these methods to heat eggs prior to storage (Fasenko, 1997 ; Deeming ,2000). The pre-heating of poultry eggs before storage was reported to result in more live chicks and lower level of embryonic mortality (Fasenko, et al ., 2001a, Petek and Dikmen ,2004) .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., (2001a) 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 significantly improved by exposure egg to a pre-storage incubation for 8 hours compared to the control. 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) found that warming quail eggs for short-term before storage increased total hatchability and decreased incubation length without any negative effect on chick quality. Idress (2015) revealed that warming hatching eggs of Hi-sex layer breeder at (37.5^0 c) for 4 hrs. before storage significantly ($P \leq 0.01$) reduced early dead embryos and total un hatched eggs , also the first grade chicks were significantly ($P \leq 0.01$) increased .Silva, et al.,(2008) stated that heating eggs for 6 hours before storage improve incubation results as it decreases incubation length and late embryonic mortality. Khan, et al., (1962) also found that daily warming improved hatchability for eggs stored up to three weeks but was detrimental to eggs stored between three and four weeks. They concluded that warming eggs the day after they are laid proved to be the most effective time for pre-incubation warming. Warming older breeder eggs during storage may increase the development stage to an active stage helping withstand storage (Fasenko, 2007). 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 (Fasenko and O'Dea, 2009). Gamble, et al., (2010) stared that a pre-storage warming protocol might increase hatchability in the commercial industry.

2.6 Water Loss and Influencing Factors

After oviposition, the egg starts to lose water to the environment due to the water pressure differences between the inside and the outside of the egg. The albumen contains the highest amount of water of all egg components. The albumen loses water to both the environment outside the egg and the yolk. Due to water movements, the osmolarity of the albumen and yolk changes. The loss of water to the environment outside the egg is affected by the environmental temperature, relative humidity, egg storage duration, and age of the breeder flock (Walsh, et al., 1995). Initially, water that evaporates through the pores of the avian egg comes from the shell membranes. This is replaced, to some extent, by recruitment of water from the albumen. The amount of water in the shell membranes depends on an equilibrium between the capillary tension of the membranes and the colloid osmotic tension of the albumen. Brake et al. (1993) stated that water loss from the albumen may have a negative

effect on the viscosity of the albumen, although later Benton and Brake (1996) were unable to find a direct relationship between water loss and albumen pH and height. Meijerhof, (1994) showed that water loss between egg collection and day 17 of incubation was not affected by the relative humidity of 55% or 75% during a storage duration of 7 days. Based on these results, they suggested that the effect of water loss during storage on hatching results is limited, under practical conditions. Although the loss of water during storage is minimal compared to the loss of water during the whole incubation period, it is often advised to minimize water loss during storage (Mayes and Takeballi, 1984; Walsh, et al., 1995).

2.7 **Chick quality and influencing factors**

Chick quality can be influence in many different ways as a reviewed by Tona, et al., (2005) and Decuypere and Bruggaman (2007). Pre incubation factors such as egg storage duration, breeder age, and breeder line influence embryonic development and also one- day- old chick characteristics (Christensen, et al., 2003, Peebles et al, 2001, Tona et al, 2003). According to Deeming (1995), day old chick quality can be related to several factors, such as incubator quality, incubation environment, and egg characteristics. Furthermore, the spread of hatch can be influence by the storage length of incubation eggs (Christensen, et al., 2003) resulting in an increase in the number of chicks that

experience delayed access to first feed (Decuypere, et al., 2001). Eggs incubated on the day of lay produced heavier chicks than eggs stored for a number of days (Reis, et al., 1997).

2.8 **Measurement of chick quality**

Chick quality has proven to be a difficult matter to define. It is very much a subjective matter depending on judgment of each individual farmer. Over the years, however, 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 chicks (Deeming, 2000, Decuypere et al., 2001). A second quantitative method for assessing chick quality is chick length (Hill, 2001; Wolanski *et al.,* 2003, Meijerhof, 2006, and Molenaar *et al*., 2008). In addition to quantitative method for assessing chick quality, qualitative measurements had been developed (Decuypere and Bruggaman, 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 (Tona, *et al.*, 2003). The Tona score can be described as a trapped scoring system with a total score between 0 and 100 based on a wide range of parameters each with a hedonic score. The score divides the chicks into groups of different qualities with those scoring 100 being free of any abnormalities and being of the best quality (Tona et al, 2003). Reijrink, *et al.,* (2009)

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 heating eggs significantly improved hatchability and post hatch chick uniformity.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site and duration:

This experiment was conducted at Animal Production Research Center, Department of Poultry Research (Hillat kuku). The experiment was undertaken during the period between April and May 2017 to study the effect of pre- incubation heating times (PREIHT) of quail eggs on hatchability and post hatch performance.

3.2 Experimental design:

Fresh laid quail eggs were collected from the Khairat farm in Al-Kadaro from stock at 15^{th} weeks old reared in open system in the Sudan and Male to Female ratio was (1:3). Eggs were collected three times a day.

A total of 1200 suitable hatching eggs were selected and used in this experiment. Eggs were randomly distributed in a completely randomized design (CRD) into four experimental groups of 300 eggs each; each group was subdivided into four replicates of 75 eggs each.

3.3 Management:

After collection, eggs were disinfected by simple fumigation with 7.7g par formaldehyde/m3 area for 15 minutes (Khan et al.,2012), Both eggs and hatchery were disinfected by simple fumigation according to methods described by Silva et al. (2008)

Eggs at different heating times (0.0, 3, 6 and 9 hours) were expose to heat at standard dry bulb temperature of 37^{0} c and 65% relative humidity

in Avimatic ® incubator. After heating, eggs were incubated in Avimatic ® incubator for 14.0 days at 37.5^0 C and 65.0 % RH. During incubation period eggs were turned once every 4 hours (6 times per day). At 14 days of incubation eggs were transferred to Avimatic ® hatcher at 36.6^0 C and 75% average relative humidity.

3.4 Hatchability performance

3.4.1 Fertility, Hatchability and Embryonic mortality

After hatch was completed hatched and piped chicks were removed and counted, un-hatched eggs were broken for identification of infertile or to determine the stage of embryonic mortality (1 to 7, 8 to14 and 15to 17days of embryo development, respectively).Early dead embryos were differentiated by the absence of an egg tooth, the intermediate dead were differentiated by the presence of an egg tooth, the beginning of feathers and the yolk sac outward the body cavity. The late dead was differentiated by the evidence of the yolk sac entering the body cavity and the beak positioned to pip to the air cell. Piped (piped and could not hatch) and rotten (contaminated eggs and nonspecific embryonic mortality) chicks were also determined. Total embryonic mortality was determined as the amount of the all dead embryos. At 17 days of incubation the investigated variables were determined using the following equations as describe by (Erensayin, 2000):

-True Fertility (%) = (Number of fertile eggs／total number of eggs set)* 100

-Total Hatchability(%) = (Number of chicks hatched／total number of eggs set)*100
-Hatchability of fertile (%) = (Number of chicks hatched／number of fertile eggs)*100
-Early phase mortality (%) = (Number of embryos died in early phase／number of unhatched eggs)*100
-Middle phase mortality (%) = (Number of embryos died in middle phase／number of unhatched eggs)*100
-Late phase mortality (%) = (Number of embryos died in late phase／number of unhatched eggs)*100
-Pipped unhatched eggs (%) = (Number of pipped eggs／total number of unhatched eggs)*100
-Total mortality (%) = (number of dead embryos / total number of fertile 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
-Chick yield = average chicks weight / average egg weight × 100
-Estimated hatchability = (100- reproductive failure)

3.4.2 Egg weight loss:

All eggs were weighed before incubation and at transfer to the hatcher (15 days) to determined egg weight loss during incubation using the following equation.

Egg weight loss = (egg weight at day 0 – egg weight at day 15) / (egg weight at day 0)*100

3.4.3 Chick quality evaluation

All chicks were weighed and examined to score them for assessment of different parameters to determine chick quality according to method described by (Decuypere and Bruggaman, 2007) (Appendix, 1 and 2).

3.2 Post hatch performance of Japanese quail chicks:

After quality scoring 120 chicks (30 / treatment) were weighed individually and randomly assigned into three replicates. (10 chicks / each treatment). Chicks were housed in deep litter system of housing. Feed and water were provided adlibitum, 24 hours day light was provided. Chicks were fed starter diet containing 24 CP and 2900 ME (Kg/Kcal). The rations were formulated according to Japanese quail requirements (NRC, 1994). The chicks were reared for 42 days. During this period mortality (%) was recorded when occurred. Also, feed conversion ratio (FCR), feed intake and body weight gain were recorded on weekly basis. At the end of the 6^{th} week the overall performance data were recorded.

3.3 Statistical analysis:

Collected data were subjected to analysis of variance (ANOVA) and LSD test was used to determine the differences among the treatment means according to Steel and Tori (1996). Statistical analysis was performed using SPSS® computer software version 10.0 (2008).

CHAPTER FOUTR
4.0 RESULTS

4.1 Effect of the pre-incubation heating time of Japanese quail eggs on fertility, hatchability and estimated Hatchability:

The results of apparent fertility, hatchability of total and fertile eggs and estimated hatchability are shown in table (1). There were a significant ($P \leq 0.05$) effects of pre-incubated heating times on fertility, hatchability and estimated hatchability. In general eggs heated for hours, had significantly higher fertility, hatchability of fertile and total eggs and estimated hatchability compared to non-heated eggs or those heated for 3 or 9 hours.

4.2 Effect of heating time of Japanese quail eggs on embryonic mortality, total egg weight loss, chick yield and chick quality

Early, mid and late embryonic mortality, piped eggs and total embryonic mortality percentage were significantly influenced by the experimental treatments (Table 2). Heating eggs for six hours resulted in significantly lower percentage of early, mid, total embryonic mortality and piped un-hatched chicks. The control and 9hours group showed significant higher rates of early and total embryonic mortality. Significant higher percentage of late embryonic mortality was observed in non-heated eggs compared to those heated for nine, six and three

hours respectively. On the other hand, heating eggs for 3hrs showed significant lower percentage of late dead embryos compared to the other treatments.

Total egg weight losses were significantly affected by heating times. H Heating quail eggs for 9 hours resulted in significantly higher total egg weight loss (see also figure 2).

4.3 Effect of heating time of Japanese quail eggs on post hatch performance of quail chicks (42 days)

The Tona score chick quality and post hatch performances of the hatched quail chicks from (1-42 days) are presented in Table (3). The obtained data indicated that the initial body weight, final body weight, weight gain, feed consumption and feed conversion ratio were not significantly affected by pre-incubation heating times (0.0, 3, 6 and 9 hrs.). Meanwhile, mortality rate was significantly ($P \leq 0.05$) reduced when the eggs exposed to heat for 6 hours Pre-incubation heating compared to those heated for 9 hours, non-heated and to eggs heated for 3 hours. Chick yield percentage was not significantly affected by pre-incubation heating time. Also, No significant differences were observed in Tona score between chicks hatched from non-heated or heated eggs.

Table (1):

Effect of pre-incubation heating time of Japanese quail eggs on fertility, Hatchability and Estimated Hatchability (%).

Pre incubation heating time	Parameters (%)			
	Apparent fertility	Hatchability of fertile eggs	Hatchability of total eggs set	Estimated hatchability
0 h	$85.71^{c}\pm0.07$	$83.33^{c}\pm0.01$	$71.43^{c}\pm0.02$	$85.57^{b}\pm0.01$
3 h	$88.57^{b}\pm0.02$	$85.80^{b}\pm0.01$	$76.01^{b}\pm0.01$	$87.46^{a}\pm0.01$
6 h	$91.20^{a}\pm1.6$	$88.60^{a}\pm0.01$	$80.80^{a}\pm0.05$	$88.31^{a}\pm0.01$
9 h	$86.02^{c}\pm0.44$	$83.78^{c}\pm0.03$	$71.70^{c}\pm0.19$	$83.44^{c}\pm0.01$
Level of Sig.	**	**	**	**

^{a,b,c} Means in rows followed by different superscript letters are significantly different at (P 0.01)

** Significant ($P \leq 0.01$).

N S : Not Significant

Table (2):

Effect of pre-incubation heating time of Japanese quail eggs on embryonic mortality, chicks grade, egg weight loss and chick yield

Pre-incubation heating time	Parameters							
	Total embryonic mortality %	Early embryonic mortality %	Mid embryonic mortality %	Late embryonic mortality %	Pipped unhatched eggs %	Egg weight loss (%)	Chick yield (%)	Tona score
0 h	14.25^{b} ±0.03	3.41^{a}±0.01	2.85^{c}±0.01	5.42^{a}±0.02	2.57^{c}±0.02	12.89^{c}±0.02	67.84±0.05	96±2.65
3 h	12.54^{c}±0.02	2.57^{b}±0.02	3.41^{b}±0.02	2.85^{c}±0.02	3.71^{a}±0.01	13.52^{b} ±0.01	67.84±0.05	95±1.0
6 h	11.69^{c}±0.01	1.71^{c}±0.01	2.85^{c}±0.02	4.28^{b}±0.01	2.85^{c}±0.04	13.66^{b} ±0.01	67.86±0.05	96±2.0
9h	16.56^{a}±0.05	4.57^{a}±0.05	4.28^{a}±0.06	4.57^{b}±0.06	3.14^{b}±0.01	14.28^{a} ±0.02	67.86±0.05	94±3.0
Level of sig.	**	**	**	**	**	**	NS	NS

a,b,c Means within columns with no common superscript differ significantly ($p \leq 0.05$)

** Significant ($p \leq 0.05$)

N S: Not Significant

Table (3):

Effect of pre-incubation heating time on growth performance of Japanese quail chicks from 1-42 days of age:

Pre Incubation Heating Time	Parameters					
	Initial body weight (g/bird)	Final body weight (g/bird)	Weight gain (g/bird)	Feed consumption (g/bird)	Feed conversion ratio (g feed/g gain)	Mortality (%)
0 h	9.26±0.05	261.27±0.05	252.01±0.00	598.0±6.35	2.37±0.02	2.67^{a}±0.00
3 h	9.30±0.03	261.25±0.02	251.95±0.03	595.0±2.65	2.36±0.01	2.51^{b}±0.03
6 h	9.29±0.03	261.26±0.02	251.97±0.01	596.0±0.58	2.37±0.01	2.43^{b}±0.03
9 h	9.23±0.03	261.24±0.05	252.01±0.00	597.0±2.08	2.37±0.01	2.63^{a}±0.01
Level of sig.	NS	NS	NS	NS	NS	**

a,b,c Means within columns with no common superscript differ significantly ($p \leq 0.05$)

** Significant ($p \leq 0.05$)

N S: Not Significant

Figure (1): Effect of pre-incubation heating times on hatchability of total egg set:

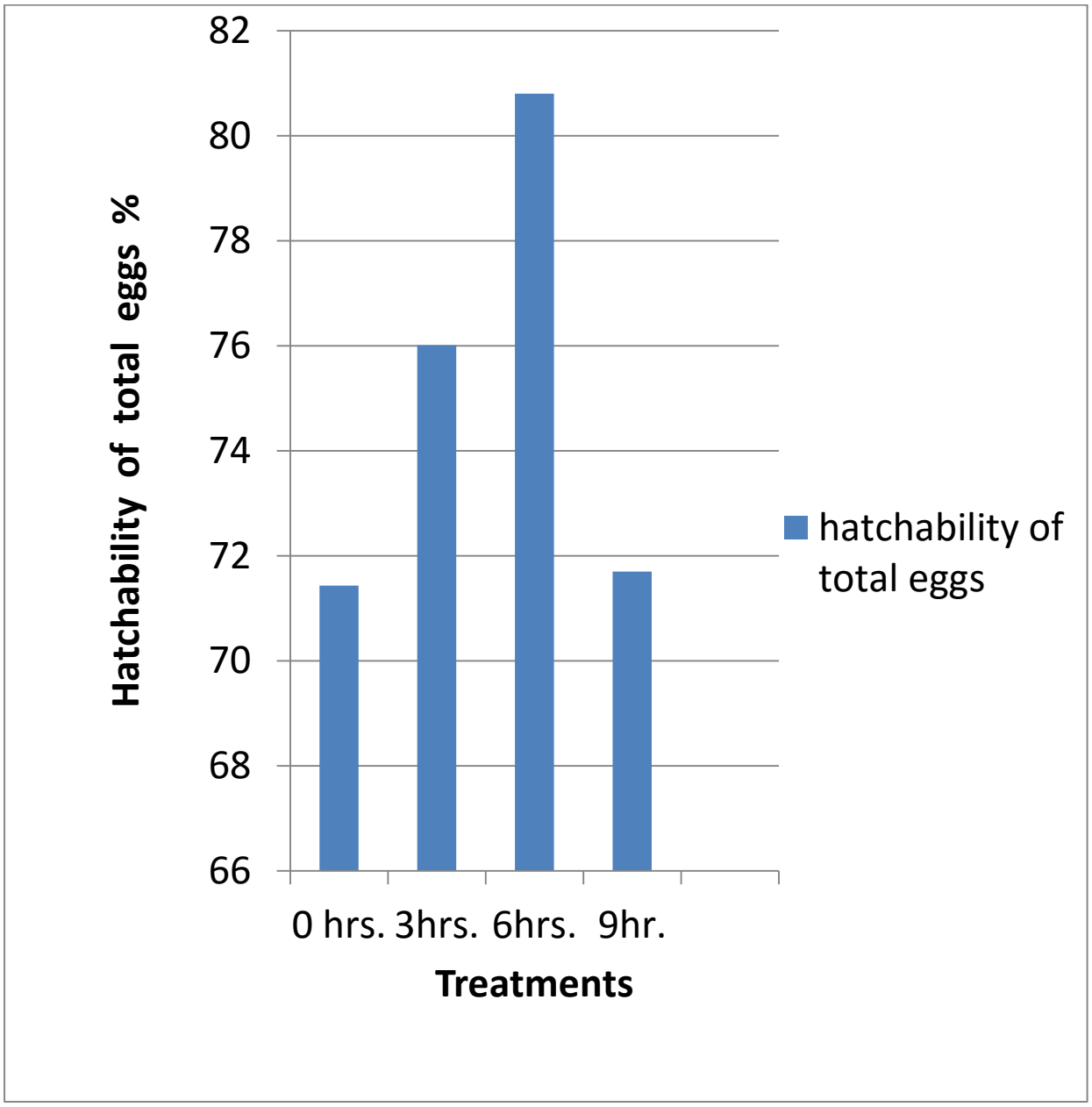
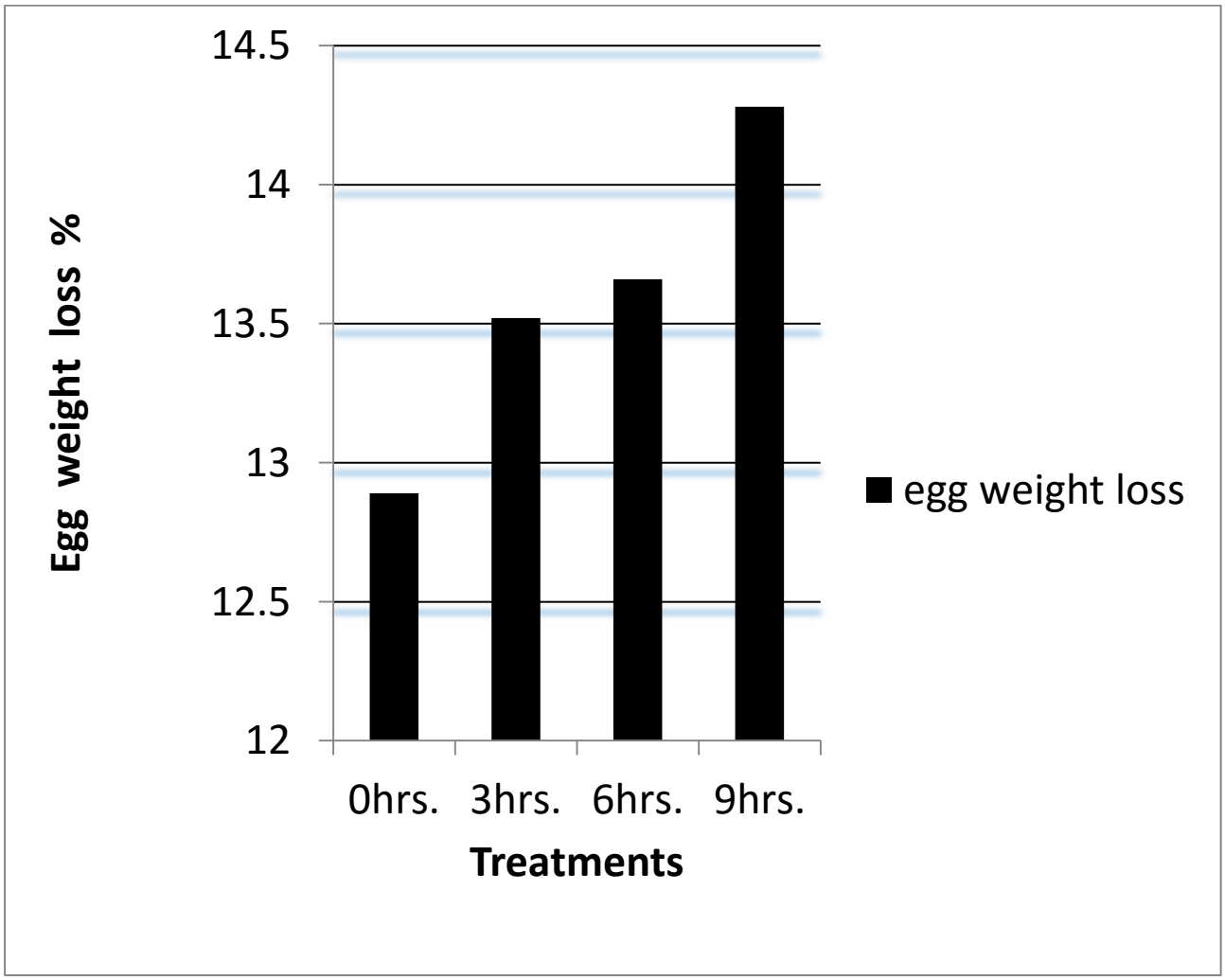


Figure (2): Effect of pre-incubation heating times on egg weight loss:



CHAPTER FIVE

DISCUSSION

5.1. Fertility and Hatchability performance:

The apparent fertility showed highly significant ($P \leq 0.05$) difference between treatments, the eggs heated for 6 hours showed very high fertility percent 91.20 % compared to those heated for (0, 3 and 9 hours) 85.71 , 88.57 and 86.02 % respectively. This result was in agreement with that obtained by (Petek and Dikmen (2006) who found a significant differences in apparent fertility of broiler breeder eggs using different pre- incubation heating time. However, Fasenko et al (2001 a) disagreed that, he found that fertility should not be affected by different PREIHT treatments because fertilization would or would not have occurred before the eggs were exposed to the different PREHTI treatments. Significantly lower percent fertility for the PREIHT 3h and PREIHT 9h group compared to PREIHT 6h treatment groups found in the current study might have been due to very early embryonic death because it is quite difficult to distinguish between infertile blasto discs and embryos that died before significant extra-embryonic membrane formation has occurred after 17d incubation period (Fasenko, 2001a). The current method of determining fertility at the end of the incubation period lumps truly infertile eggs at oviposition, eggs whose embryos died during storage and very early during incubation as infertile

eggs leading to an underestimation of true fertility and an overestimation of infertility. There were significant differences in hatchability of fertile eggs between the experimental groups. The Hatchability of fertile eggs in the 6h PREIHT treatment group was however significantly higher followed by the 3h PREIHT (85.80%) 9h PREIHT (83.78%) and the control group 0h PREIHT (83.33%). Fasenko et al., (2001a) also reported significantly higher hatchability of fertile eggs (81.9%) in the PRESI-6h group. Significantly higher hatchability of fertile eggs in the PREIHT 6h treatment group compared to the 3h,9h and the control in the present study clearly indicated that pre-heating of quail eggs for 6h at 37.5°C prior to incubation might be because reduces embryonic mortality. The reduction in embryonic mortality during incubation was clear by the PREIHT-6h group than the PREIHT-3h group. Prolonged heating of the eggs (PREIHT-9h) prior to incubation however presented no significant benefit to the hatchability of fertile eggs compared to the control group (PREIHT 0h) might be due to the high embryonic mortality during incubation in the two groups. Hatchability of set eggs was highest for the 6h PREIHT group (80.80%), followed by the 3h PREIHT group(76.01%), then control group 0h PREIHT group (71.43%) and lastly the 9h PREIHT(71,0%). Fasenko et al., (2001a) also reported significantly higher hatchability of set eggs of 79.0% in the PRESI-6h group in broiler breeder eggs, of Cobb breed. Lourens (2006) and Petek and Dikman (2004) also reported significantly better hatchability of broiler

breeder eggs and quail eggs in the PRESI-6h and PRESI-8h groups than the control group respectively. Relatively high hatchability of set eggs in the PREIHT -6h group compared to the control and other PREIHT treatment groups observed in the current study could be due to high embryonic mortality during incubation in the control and the other two PREIHT treatment groups compared to the PREIHT-6h group. The reason for lower hatchability observed in eggs exposed to 9h of pre incubation heating could have been due to prolonged duration of exposure to heat shock (Meijerhof, 1994). Lotfi et al. (2011) and Fasenko et al., (2001a,b) established that PRESI -6h treatment for broiler breeder eggs and PRESI-12h treatment for turkey eggs advanced embryonic development to the stage of development at which hypoblast formation is complete. At that developmental stage, embryos are at a relatively quiescent state and are better able to withstand developmental arrest during storage resulting in significantly higher hatchability of both fertile and set eggs. The PREIHT 6h quail eggs probably contain more cells than the control embryos and are in a more quiescent developmental stage than PRESI-9h embryos which make them more resistant (Rejrink et al., 2009). From the preceding discussion, it is clear that there is an optimal developmental stage or range of developmental stages and optimal PREIHT treatment or range of PREIHT treatments in different poultry species that maintain embryonic viability and consequently result in

improved hatchability of both fertile and set eggs. **(**Fasenko,1997;Fasenko et al,2001; Laurens,2002,Silva et al,2008 and Abdel-Azeem,2009*)*

5.2. Embryonic mortality, egg weight loss and chick yield:

It was found that eggs subjected to pre-incubation heating for 3 and 6 hours had significantly reduced total embryonic mortality compared to the other groups. Most probably embryos in PREHTI are being pushed to the optimal stage of development to safely store eggs (Gupta et al.,1993 and Fasenko, 2001:). Heating eggs for 6 hours significantly ($P \leq 0.05$) reduced early embryonic mortality, mid embryonic mortality, late embryonic mortality and piped unhatched chicks compared to other groups. The late embryonic mortality was high in non- heated eggs (5.42 %) compared to heated ones, this could explain the beneficial effect of heating in decreased late embryonic mortality. In the recent decades, several studies reported that heating of poultry eggs before storage resulted in more live chicks and lower level of embryonic mortality compared to the non heated eggs. The total embryonic mortality was significantly lower when quail eggs exposed to 6h PREIHT (11.69%) and 3h PREIHT (12.54%).These results are in consistent with that obtained by Laurens (2002) and Petek and Dikmen (2005) who stated that pre-heating of poultry eggs before storage resulted in more live chicks and in a lower level of embryonic mortality. Also Abdel-Azeem (2009) showed that mortality rate was reduced when eggs exposed to 7 hrs. PRESI as compared with control group or group of eggs exposed to

10 hrs. However, the highest total embryonic mortality was detected in groups exposed to 0 or 9 hrs. (14.25% and 16.56 %, respectively). These results are in accordance with Petek and Dikmen (2006) who indicated that total embryonic mortality rate during incubation were significantly affected by pre-storage incubation warming time. However, researchers have reported that preheating can be harmful to some freshly laid eggs due to progressing eggs that were laid in an "optimal" stage to a "less-optimal" stage during and after the preheating treatment (Kosin and Pierre, 1956). The piped unhatched embryos were significantly ($P \leq 0.05$) affected by pre-incubation heating time in that eggs heated for 6h recorded significantly low percent of un hatched (2.85 %) compared to the other treatments, these findings are in agreement with that of Silva, et al. (2008) who reported that warming eggs for 6 hours resulted in lowest piped un hatched eggs stored for nine and 14 days. Tona scores were not significantly affected by pre-incubation heating time as compared to non-heated eggs. Also, PREIHT had no significant effect on chick yield. These results are in accordance with that obtained by Reijrink, et al. (2009) who suggested that pre-storage warming can be positive or negative for chick quality in dependence of pre-incubation time. The highest egg weight loss percentage (14.28%) was observed in eggs exposed to 9hr of pre-incubation heating. This result in consistent with that reported by Fasenko and O'Dea (2009) who observed that pre-heating eggs for long periods of time increases weight loss. They

attributed this weight loss to loss of moisture from eggs; the moisture loss is progressively enhanced by continued exposure of eggs to high temperatures.

5.3. Post hatch performance of Japanese quail chicken:

The obtained data indicated that the post hatch performance parameters where not affected by pre-heating. These results are in agreement with Petek and Dikmen(2004) and Abdel-Azeem (2009) who reported that pre-storage heating of quail eggs had no significant effect on subsequent growth performance of quail progeny. On the other hand (Marandure, et al., 2012) reported that pre-incubation of broiler breeder heating eggs significantly improve hatchability and post hatch chick uniformity.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Based on the result of this study the following conclusions can be withdrawn **:-**

- Heating quail eggs for 6 hours improve hatchability of both fertile and set eggs.
- Heating quail eggs for 6 hours reduce total embryonic mortality.
- Heating quail eggs had no effect on post hatch performance.

6.2 RECOMMENDATION

Further research is needed to determine the optimum PREIHT time that gives the highest hatchability as the optimum could be anywhere between 3 and 9 hours of pre incubation heating time.

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Appendix (1):

Assessment of different parameters to determine chick quality:

Parameters	Assessment
Vitality **(Reflex)**	The chick is vital if it turns immediately within seconds from lying on its back 10 standing on its feet (score= 0). If this action takes more than three seconds the chick score one point for reflex (score:1)
Navel	The navel of the day-old chick is normal if it is fully closed, which means that the yolk is fully retreated (score: 0). If the navel is open or a block knob is visible, the navel score 1.
Legs	The normal legs of a day-old chick are not swollen and show a normal color (score: 0). Legs are score 1when they are swollen and/or red.
Beak	The normal (beak) including nostrils of a day-old chick is clean (score: 0). The beak score 1 if it is dirt and/or has a red dot (score:1)
Belly	The thickness of the belly = volume of the yolk sac, depends on the volume of the yolk sac before the yolk is withdrawn into the abdomen. Animal belly feels smooth and these chicks (score: 0) for belly. If the belly feels hard and the skin in fines, the belly score 1.

Source:Tona score chick quality(Decuypere and Bruggaman,2007)

Appendix (2):

Table (7) Assessment of chick quality based on physical apparent:

Treatment	Chick number	Reflex	navel	Legs	Beak	belly	Score
0 hours	1	0	0	0	0	0	10
0 hours	2	1	0	0	0	0	9
0 hours	3	0	0	0	0	0	10
0 hours	4	0	0	0	0	0	10
0 hours	5	0	0	0	0	0	10
0 hours	6	0	0	0	0	0	10
0 hours	7	1	0	0	0	0	9
0 hours	8	0	0	0	0	0	10
0 hours	9	1	0	0	1	0	8
0 hours	10	0	0	0	0	0	10
Total		96					
0 hours	11	0	0	0	0	0	10
0 hours	12	0	0	0	0	0	10
0 hours	13	1	0	0	0	0	9
0 hours	14	0	0	0	0	0	10
0 hours	15	1	0	0	0	0	9
0 hours	16	0	0	0	0	0	10
0 hours	17	1	0	0	0	0	9
0 hours	18	0	0	0	0	0	10

0 hours	19	0	0	0	0	0	10
0 hours	20	1	0	0	0	0	9
Total		96					
0 hours	21	0	0	0	0	0	10
0 hours	22	0	0	0	0	0	10
0 hours	23	1	0	0	0	0	9
0 hours	24	0	0	0	0	0	10
0 hours	25	1	0	0	0	0	9
0 hours	26	0	0	0	0	0	10
0 hours	27	0	0	0	0	0	10
0 hours	28	1	0	0	0	0	9
0 hours	29	0	0	0	0	0	10
0 hours	30	0	0	1	0	0	9
Total		96					
Average (0hours)		96					
Treatment	Chick number	Reflex	navel	Legs	Beak	belly	Score
3 hours	31	0	0	0	0	0	10
3 hours	32	1	0	0	0	0	9
3 hours	33	0	1	0	0	0	9
3 hours	34	0	0	0	0	0	10
3 hours	35	1	0	0	0	0	9
3 hours	36	0	0	0	0	0	10
3 hours	37	1	0	0	0	0	9
3 hours	38	0	0	0	0	0	10

3 hours	39	0	0	0	1	0	9
3 hours	40	0	0	0	0	0	10
Total		95					
3 hours	41	0	0	0	0	0	10
3 hours	42	0	0	0	0	0	10
3 hours	43	1	0	0	0	0	9
3 hours	44	0	0	0	0	0	10
3 hours	45	0	0	1	0	0	9
3 hours	46	0	0	0	0	0	10
3 hours	47	1	0	0	0	0	9
3 hours	48	0	0	0	0	0	10
3 hours	49	0	0	0	0	0	10
3 hours	50	1	0	0	1	0	8
Total		95					
3 hours	51	1	0	0	0	0	9
3 hours	52	0	0	0	0	0	10
3 hours	53	1	0	0	0	0	9
3 hours	54	0	0	0	0	0	10
3 hours	55	1	0	0	0	0	9
3 hours	56	0	0	0	0	0	10
3 hours	57	0	0	0	0	0	10
3 hours	58	1	0	0	0	0	9
3 hours	59	0	0	0	0	0	10
3 hours	60	0	0	1	0	0	9
Total		95					
Average (3hours)		96					

Treatment	Chick number	Reflex	navel	Legs	Beak	belly	Score
6 hours	61	0	0	0	0	0	10
6 hours	62	0	0	0	0	0	10
6 hours	63	1	0	0	0	0	9
6 hours	64	0	0	0	0	0	10
6 hours	65	1	0	0	0	0	9
6 hours	66	0	0	0	0	0	10
6 hours	67	1	0	0	0	0	9
6 hours	68	0	0	0	0	0	10
6 hours	69	0	0	0	1	0	9
6 hours	70	1	0	0	0	0	9
Total		95					
6 hours	71	0	0	0	0	0	10
6 hours	72	1	0	0	0	0	9
6 hours	73	0	0	0	0	0	10
6 hours	74	0	0	0	0	0	10
6 hours	75	0	0	0	0	0	10
6 hours	76	0	0	0	0	0	10
6 hours	77	1	0	0	0	0	9
6 hours	78	1	0	0	1	0	8
6 hours	79	0	0	0	0	0	10
6 hours	80	0	0	0	0	0	10
Total		96					

6 hours	81	1	0	0	0	0	9
6 hours	82	0	0	0	0	0	10
6 hours	83	1	0	0	0	0	9
6 hours	84	0	0	0	0	0	10
6 hours	85	0	0	0	0	0	10
6 hours	86	0	0	0	0	0	10
6 hours	87	0	0	0	0	0	10
6 hours	88	1	0	0	0	0	9
6 hours	89	0	0	0	0	0	10
6 hours	90	0	0	0	0	0	10
Total		97					
Average (6 hours)		96					
Treatment	Chick number	Reflex	navel	Legs	Beak	belly	Score
9 hours	91	0	0	0	0	0	10
9 hours	92	1	0	0	0	0	9
9 hours	93	1	1	0	0	0	8
9 hours	94	0	0	0	0	0	10
9 hours	95	1	0	0	0	0	9
9 hours	96	0	0	0	0	0	10
9 hours	97	1	0	0	0	0	9
9 hours	98	0	0	0	0	0	10
9 hours	99	0	0	0	1	0	9
9 hours	100	0	0	0	0	0	10
Total		94					

9 hours	101	0	0	0	0	0	10
9 hours	102	0	0	0	0	0	10
9 hours	103	1	0	0	0	0	9
9 hours	104	0	0	0	1	0	9
9 hours	105	1	0	0	0	0	9
9 hours	106	0	0	0	0	0	10
9 hours	107	1	0	0	0	0	9
9 hours	108	0	0	0	0	0	10
9 hours	109	0	0	0	0	0	10
9 hours	110	1	0	0	1	0	8
Total		94					
9 hours	111	1	0	0	0	0	9
9 hours	112	0	0	0	0	0	10
9 hours	113	1	0	0	0	0	9
9 hours	114	0	0	0	0	0	10
9 hours	115	1	0	0	0	0	9
9 hours	115	1	0	0	0	0	9
9 hours	117	1	0	0	0	0	9
9 hours	118	1	0	0	0	0	9
9 hours	119	0	0	0	0	0	10
9 hours	120	0	0	0	0	0	10
Total		94					
Average (9 hours)		**94**					