IMPACT OF EGG STORAGE DURATION AND STORAGE TEMPERATURE ON EGG QUALITY, FERTILITY, HATCHABILITY AND CHICK QUALITY, OF NAKED NECK CHICKENS' EGG

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DECLARATION

I hereby declare that this thesis submitted to the Department of Animal Science, is as the result of my work towards the award of MPhil degree in Animal Breeding and Genetics and has neither in whole nor in portion been presented for any other degree elsewhere. However, works of other researchers and authors which served as sources of information were appropriately acknowledged by references of the authors.

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ABSTRACT

It is a well known fact that when hatching eggs are stored for a number of days the quality of the eggs, fertility and hatchability are affected. A study was therefore conducted to evaluate the impact of different egg storage duration (1day, 3days, 7days, 10days and 14days) under two temperature conditions (coldroom;18^{o}C and ambient; 25-30^{o}C) on egg quality, fertility, hatchability of eggs from naked neck chickens and subsequent chick quality. Four hundred and fifty (450) eggs were collected from 45-week old heterozygote naked neck chickens. Two experiments were conducted in this research. Experiment one looked at the impact of different storage duration and different storage temperature on egg quality. Experiment two also looked at the effect of storage duration and storage temperature on egg quality, fertility, hatchability and chick quality parameters. Initially and subsequently for every level of duration of storage, eggs (n=90) were collected, divided into 2 groups weighed and stored at the 2 different temperature conditions to set up different levels of pre-incubation duration treatments prior to incubation. After the period of storage eggs were weighed, 15 eggs from each group were selected for egg break out to measure egg quality and blastoderm quality. The rest of the eggs were incubated for 21 days at 37.5^{o}C and 30-40% relative humidity. After 18 days of incubation, eggs were candled for live embryos. Data was analysed using the Proc. Mixed Model procedure of SAS at P < 0.05. However, fertility and hatchability data was expressed as a percentage and compared. Among the external egg quality characteristics measured, initial egg weight before storage, egg weight after storage and shell thickness were not significantly (P>0.05) affected by storage duration and storage temperature. Interaction between storage duration and storage temperature on egg weight before storage, egg weight after storage and shell thickness was also not significantly (P>0.05) different. However, egg weight loss, wet shell weight and dry shell weight were

significantly (P< 0.05) affected by different storage durations but were not significantly affected by storage temperature, except wet shell weight. All the Internal egg qualities (blastoderm diameter, albumin weight, and dry yolk weight) were significantly(P<0.05) affected by length of storage except wet yolk weight. Blastoderm diameter and dry yolk weight increased as storage duration increased whereas albumen weight decreased as storage duration increase. Storage temperature did not affect(P>0.05) internal egg quality characteristics measured except blastoderm diameter. Blastoderm diameter for the eggs stored under ambient temperature was higher (P<005) (6.96cm) compared to eggs stored under coldroom temperature (5.41cm). The interaction between storage duration and storage temperature on internal egg quality characteristics was significant(P<0.05) on blastoderm diameter and albumen weight but was not significant(P>0.05) on yolk weight. Duration of storage did not influence(P> 0.05) chick weight and chick length but influenced shank length significantly. Chick weight and chick shank length was higher in eggs stored under coldroom temperature than eggs stored under ambient temperature. Interaction between temperature and storage duration was significantly(P<0.05) different on chick weight and shank length but was not significantly(P>0.05) different on chick length. Percent fertility and hatchability decline after 7days of storage when eggs are stored under ambient temperature.

DEDICATION

This work is dedicated to my lovely wife, Ruth Gyamfuaa and my children Lois Gyamfuaa Antwi, Leslie Antwi Boasiako and Lester Kyerematen Antwi. I also dedicate this piece of work to my Sister and her Husband, Mr and Mrs Kyerematen for being an integral part of my upbringing and also supporting me in my education.

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TABLE OF CONTENT

LIST OF TABLES

LIST OF PLATES

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Poultry production is one area of animal production with significant contribution to food security. It provides products such as eggs and meat protein of high biological value (Jay and Michael, 2004). Eggs and meat are amongst the most nutritious foods. Eggs are rated with milk as the best protein foods rich in iron (Fe) and vitamins (Oluyemi and Roberts, 2000).

There are different breeds of chicken for egg production over the years. These comprised of more primitive and household types of chickens and in many parts of the world extensive practices of backyard farming still uses these birds. The naked neck bird is not an exception. Naked neck is mutant bird known for its high productive adaptability under high environmental temperatures, high postembryonic vitality and high carcass yield (Deeb and Cahaner, 2001, Yalcin *et al*.; 1997). It is common to find many commercial farms using exotic layer breeds for production. However, these foreign breeds normally face high tropical temperatures, which results in large economic losses due to reduction in general performances and higher mortality (Abera and Tegene, 2011). This situation is a major concern for farmers and breeders in Ghana. According to Adomako (2009) when indigenous naked neck male chicken was crossed with Lohmann commercial female chicken, the offspring produced had significant better body weight, body weight gain, number of eggs per clutch, hen-housed and hen-day rate egg production, egg size, Haugh unit, shell thickness, survivability and carcass yield. It appears that these birds have the potential to be used as commercial breeds in Ghana and therefore must be examined to determine potential limitations to their productivity.

Often naked neck birds are tagged with low hatchability (Tadelle *et al*., 2000; Dunga *et al*., 2013). This has been one of the setbacks for commercial production of naked neck chickens.

Peters (2000) discovered the highest number of dead-in-shell in naked neck chickens. Dunga (2013) also recorded a lower number of chicks hatched in naked neck compared to the frizzle (*nanaFf*). The embryonic mortality observed in this study mostly occurred during the last stages before hatching with majority pipping but not hatching (pipping mortality) (Merat, 1990). Our observation shows chicks that appear very exhausted after hatch (Dunga *et al*, 2013). Peters *et al*. (2008) recorded a reduction of 6.1 % for naked neck in embryonic survival when compared with normal feathered birds and explained that this embryonic mortality occurs normally during the last stage of incubation in (18-21 days). However, the embryonic mortality associated with the naked neck so far still remains unclear. The reason could be attributed to the position attained by the embryo before breaking out of the shell (Fathi *et al.*, 2013) Even though many factors including genetic strain or background of the parent stock may contribute to the increased late embryonic malposition and mortality, handling of eggs prior to incubation could also play a big role (ISA 2009). It has taken years for breeding practices to use these birds for commercial purpose in Ghana due to lower experimental numbers. Subsequently, small numbers of eggs are produced and collected at each time. Hence these eggs are stored till the numbers are enough to fill an incubator. It is hypothesized that frequently leaving eggs in the open due to the low value given them may have storage impact on the development of the blastoderm (or day zero embryo) (Hamidu *et al*., 2010; 2011). Meijerhof (1992) reported that during storage, hatching is influenced by the storage duration, storage temperature, position of the eggs, humidity and other environmental factors.

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).

1.2 General Objectives

To determine the effects of different storage temperature conditions and period of storage of egg quality, embryonic quality, fertility and hatchability of naked neck eggs.

1.3 Specific Objectives

- To determine the effect of different storage duration and storage temperature on egg and blastoderm quality.
- To determine the effect of different storage duration and storage temperature on chick quality, fertility and hatchability

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. The Naked Neck Chicken

2.1.1. Origin

The history about the origin of naked neck is not very well known. Due to differences in reports about the exact origin of the bird. Naked neck chickens are thought to originate from Malaysia (ARC, 2006). It may have been brought to Malaysia by early traders. (van MarleKoster and Nel, 2000). According to Fourie and Grobbelaar (2003), naked neck chickens were introduced by Dutch East Indian Company in the seventeenth century to Cape of Good Hope. The naked neck chicken is also thought to have originated from Transylvania, Romania and was spread all over the world by a Dutch East Indian Company in the course of trading around the 17th century (Ramsey *et al*., 2000)

According to Chunyan *et al.* (2011), the birds were first bred in Eastern Hungary. It is thought that naked neck birds are cross between chicken and domesticated turkey, but this fact isnot scientifically supported. However, a study had revealed that naked neck is due to random genetic mutation caused by a molecule called BMP12. The mutants were first spotted in northern Romania. The mutant gene(*Na*) makes the birds appear as chicken bearing the head of a turkey (chicken with long, deep-red bear neck). The *Na* gene is associated with significantly less plumage cover than chickens not carrying the gene (Nthimo, 2004). Naked necks are very colourful yet white, red, brown and black feather combinations can be found. The *Na* gene is an autosomal incomplete dominant gene. The heterozygote has feathered area on the body of 20% to 30% and the homozygote (*NaNa*) has 40% feathered area, (Islam and Nishibori, 2009).

2.1.2 Phenotypic and Genotypic Characteristics of Naked Neck

Naked neck is controlled by a single dominant autosomal gene, expressed phenotypically as chicken without feathers or reduced feather at the neck region. It is an incomplete dominant gene. The heterozygotes (*Nana*) have tuft of feathers at the ventral side of the neck (Scott and Crawford, 1977) whereas the homozygotes have no feathers on the neck. (Somes 1988). The *Na* gene is a heat tolerance gene and also possess productive adaptability (Horst, 1988). Naked neck birds can tolerate heat stress as compared to the normal feathered birds. This is due to their reduced feather mass which helps them to dissipate heat (Patra *et* al., 2002). According to Yahav *et al*. (1998), the reduced feather mass provides large surface area for effective heat dissipation and also increases the sensible heat loss from the neck region. The heterozygote female has 4.8% greater naked neck area covered as compare to the heterozygote male (Howlider *et al*.,1995). Bordas *et al*. (1978) also reported that the heterozygote (*Nana*) birds have more feather coverage (41 to 27%) as compared to the homozygote (*nana*) birds (33 to 22%) for males and females respectively.

According to Islam and Nishibori (2009) they are also well adapted to harsh tropical climate and poor nutrition. The birds are highly resistant to disease have good growth rate, high egg production and good egg quality, and meat yield trait as compare to the indigenous fullfeathered and exotic birds. When the birds are given good nutrition and better management conditions, they can double the egg production (Islam et al. 2009). An offspring from a crossbred between indigenous naked neck and exotic chicken perform better in terms of egg production performance and production traits (Islam and Nishibori, 2009).

The performance of naked neck birds is much affected by temperature. According to the findings of Horst and Rauen (1986) and Rauen *et al.* (1986); Cahanar *et al*. (1993) the bird‟s performance is inferior when temperature is at 20^{o}C or below but their performance is superior when temperature is at 30^{o}C when compared to the normal feathered birds kept under the same condition. When the number and structure of feathers on a bird is reduced heat loss increases thereby indirectly enhancing feed intake and productivity (Rauen *et al*., 1985). This can reduce mortality rate due to heat stress. The birds can also survive under harsh environmental conditions like poor nutrition, poor housing, poor management, sudden change of feed and variable temperature and humidity (Barua *et al*.,1998). This higher adaptability of the naked neck birds makes them suitable for tropical climate leading to better growth rate, better feed intake and and conversion, and better egg production as compared to normal furthered (Fraga *et al.*, 1994; Ajayi, 2010).

2.1.3 Effect of Naked neck gene on Liveability

The naked neck chicken is noted for lower chick mortality due to high environmental temperature (Smith and E,le, 1997). High ambient temperature does not affect their fertility much (Ladjali *et al*., 1995). There is less body weight loss (Mazzi *et al*., 2002), superior levels of heat shock protein and high resistance to sudden death and ascites syndrome, (Gonzales *et al*.,
1999) clocal cysts, prolapse, Marek‟s disease, coccidiosis, osteodystrophy and Salmonellosis, when there is high ambient temperature (Fraga *et al*., 1999).

Islam *et al*. (2002) stated that the naked neck gene induces appetite in the birds because of their ability to tolerate heat. According to them when the birds find themselves in a cool climate, there is higher demand for energy and in hot climates, there is an increase in the upper limits of

the critical temperature; in both conditions feed intake increases. These result in improved body weight, egg size and liveability when the birds find themselves in the two temperature conditions. Introduction of the naked neck gene in chicken breeds will go a long way in improving the birds‟ ability to withstand heat stress (Islam and Nishibori, 2009) thereby improving their productivity. There is also a higher blood volume in birds with naked neck gene. This may be due to higher haemoglobin concentration in the packed cell volume in the cell of birds with this gene which is necessary for oxygen uptake and increase metabolism for multiple activities. (Luger *et al*.,1998).

2.1.4 Naked Neck gene and Carcass Characteristics

The reduction of plumage (20 - 40%) gives 1.5 - 3.0% more carcass yields to the birds with naked neck gene than normal feathered birds regardless of the temperature. Due to the higher pectoral muscles in naked neck chicken, there is 1.8-7.1 percent more meat in them than normal feathered birds when their carcasses are dressed (Merat, 1986). According to Fathi *et al*. (2008), birds with naked neck genes possess relatively higher dressed carcass weight, drumstick and breast muscles compared to full-feather birds. Also the proportion of abdominal fat was decreased in both naked neck birds compared with normally feathered ones. Intramuscular and subcutaneous fat is relatively low in birds carrying *Na* genes. This is because they are able to utilize large fraction of the fat for thermoregulation (Merat,1990). N‟Dri *et al*. (2005) observed that slow growing homozygous and heterozygous naked neck birds under fluctuating temperature, attain weight of 2kg in 3.3days sooner than normally feathered birds and that carcass yield of the birds are higher than that of full- feathered birds (81.6 % vs. 80.0 %).

At normal temperature, broiler chickens carrying *Na* genes had relatively higher growth rate and meat yield than the normal feathered birds, however, the effect of this gene was more obvious at higher temperature (Cahaner *et al*., 1993). Singh *et al*. (1996) added that heterozygote naked neck broilers achieved 3% more weight than full-feathered heterozygote bird when both birds were given commercial conditions during the spring and summer months. Comparatively, naked neck birds require less dietary protein due to their reduced mass feathers. This explains why there is less evidence of cannibalism and feather picking among the birds, (Merat, 1986; Bairagi *et al*., 1992; Barua *et al.*, 1998*;* Islam *et al*., 2002).

2.1.5 Growth and Reproductive Performance of Naked Neck

At 20°C, adult body weight of naked neck hen was lower as compared to their normal feathered counterpart. The reverse was observed when the temperature was above 30^{o}C. Reduced feather coverage makes them heat tolerant birds. This makes their performance superior than normal feathered birds under high ambient temperature (Cahaner *et al.,* 1993). Younis and Galal (2006) observed that the naked-neck birds also reached sexual maturity 5days earlier than the normally feathered birds. The naked neck birds were also heavier at 24, 40 and 72 weeks than normally feathered birds at 40 and 72 weeks of age. However, Younis and Gala, (2006) and Garces *et al. (*2001) stated that the *Na* gene is able to reduce feed intake by 12.40 and 13.60% in heterozygotes and homozygotes genotypes, respectively. They also added that naked neck birds had significantly better feed conversion efficiency than the normal feathered genotypes. Again, a study by Njenga (2005) on productivity and socio-cultural aspects of local poultry phenotypes in coastal Kenya revealed that naked neck birds had significant higher body weight as compared to

the normal feathered birds. They also had the highest egg weights compared to the rest of the local birds.

2.1.6 Egg Production of Naked Neck Birds

Egg numbers at moderate temperature were not affected by the *Na* gene (Mathur, 2003). The naked neck hen has a high laying rate at high temperature. The heterozygote layer has significant higher egg numbers, egg weight body weight and productivity index than the normal feathered ones under constant temperature stress (Somes, 1988; Hareen-Kiso, 1991; Mathur, 2003). According to Machebe and Ezekwe (2004), naked neck cock had higher ejaculate volume, sperm concentration, sperm motility, and total spermatozoa than that of frizzle and normal cocks.

The naked neck gene has been associated with high laying rate, egg size and egg weight during hot environmental temperature conditions (Garces *et al*., 2001; Younis and Galal, 2006). According to Hoque *et al*. (2003), naked neck chicken had the highest egg production rate than any other indigenous chicken assessed. Observation made by Yushimura *et al*. (1997) revealed that the homozygote naked neck was more superior in egg production, egg size and body weight than other indigenous chicken when they were managed under ambient temperature of about 30^{o}C.

According to Egahi *et al* (2013) plumage modifier genes of naked neck translate into superior performance in egg quality indices of birds possessing them. They stated that birds with *Na* modified genes have significant higher yolk length than that of frizzle and normal feathered birds. Njenga (2005), had also found out that among the birds of Kenya (naked neck, dwarf and normal feathered), the naked neck bird was superior in term of eggshell thickness. Eggshell thickness in many studies had shown that naked neck birds have higher shell thickness compared to frizzle and

normal feathered sibs (Njenga, 2005; Sharifi, 2006). However, according Garces *et al*., (2001) the *NaNa* has significant egg yolk reduction and shell percentages. Eggs from naked-neck birds had a lower breaking strength and egg shell thickness compared with the *na/na* genotypes.

Akhtar-Uz-Zaman (2002), was of view that the reduced feathers in the birds make them possible to receive much radiant energy which may contribute to vitamin D3 synthesis. The vitamin D3 in turn may contribute to better egg shell quality. Abdel-Rahman (2000) researched into the effect of the *Na* gene on egg production performance of Sharkasi birds in subtropical environmental conditions and reported that the naked neck birds showed significant increase in egg production and egg mass. Barua *et al*. (1998) showed that among the indigenous chickens of Bangladesh, the naked-neck fowl performed better in terms of egg and meat production, and were more resistant to diseases than their fully feathered counterparts.

According to Ikeobi *et al*. (1996) and Peters (2005), naked neck chicken has highest egg weight followed by frizzle feathered and lastly normal feathered chickens. Variation in egg weight, size, egg length and breadth is said to be influenced by the possession of major genes, dam genotype and environmental factors influencing on the chicken. The possession of major genes influences the utilization of available food reserve for egg production (Peters 2000; Ikeobi *et al.,* 1996; and Ibe 1993).

2.2 Hatchability in Naked Neck

Hatchability refers to the percentage of eggs hatched into chicks after eggs have been set in an incubator for a period of 21days (Yassin *et al*., 2008). Percentage hatchability can be determined on the basis of all the egg set into the incubator or the number of fertile eggs set into the incubator after candling; normally after 18days of incubation.

Hatchability in naked neck is low in relation to the hatchability in normal feathered birds. Peters (2000) reported that the highest number of dead- in- shell was observed in naked neck eggs. Merat (1990) observed several favourable characteristics associated with this gene but was unhappy with high embryonic mortality which most often occurs during the last stage of hatching. According to Horst (1998) naked neck has the highest egg production capacity than frizzle and the normal feathered birds but their fertility and hatchability is always lower among the rest of the birds. Peters *et al.,* (2008) indicated that a cross between pure breeding naked neck birds resulted in eggs with high percentage of dead-in-shell.

Hatchability is a complex age depended trait. It consists of several sub-traits which are affected by genetic and environmental factors (Wolc and Olori, 2009). According Wolc and Olori (2009), the genetic traits are egg production, fertility and egg quality traits, and environmental factors are storage time, temperature, relative humidity, ventilation, position of the egg, turning of the egg and candling. Similarly feed variation also affects hatchability (Mussadeq *et al*., 2002). Other environmental factors that affect hatchability of a breeding hen include egg size, age and shell quality traits (King,ori, 2011).

2.2.1 Genetic Factors Affecting Hatchability

2.2.1.1 Fertility

According to Wishart *et al.* (2001), fertile eggs are eggs that are capable of hatching. They are eggs that have been fertilized and have formed embryo at ovipositor. Beanmont (1992) reported the measure of fertility as the number of fertile eggs and hatched during a 21days period after Artificial Insemination. However, natural mating will result in similar results.

Hatchability to a large extent is a derivative of fertility of eggs (Peters *et al.*, 2005). This means that hatchability greatly depends on the fertility of the eggs under incubation. Peter *et al.* (2008) stated that the breed of the bird can affects the fertility and hatchability of their eggs. Egg fertility is affected by many factors which most of them originates from the hen. (Brillard, 2003). These factors mentioned are also affected by the age of the bird (Hocking and Bernard, 2000; Gumulka and Kapkowska, 2005).

2.2.1.2 Egg Quality Trait

Hatchability to a large extent also depends on the egg quality. The overall quality of an egg can be classified under two broad categories. These are external egg quality and internal egg quality characteristics (Monira *et al*., 2003). External egg quality characteristics are features of eggs such as egg weight, egg size, egg shape, shell thickness, shell colour and shell strength (Bain, 2005). The internal egg quality is involving the albumen quality, yolk quality and blastoderm size. Good quality traits are beneficial to poultry breeding industries. (Bain, 2005).

Furthermore, embryonic liveability depends on traits such as egg weight, yolk and albumen weight, genetic factors and age of the hen (Onagbesan *et al*., 2007). The albumen height is important criteria for analysis of internal quality of egg (Silversides *et al*., 1993). Extended storage time and higher storage temperature decrease the albumen height, and thus degrade the internal quality of the egg (Scott and Silversides, 2000; Raji *et al*., 2009). A number of studies had been made to assess egg quality in chickens (Tona *et al*., 2002*;* de Ketelare *et al*. 2004; Bain, 2005). Other researched had also looked at changes that occur in micro environment of the egg during storage

and during early incubation, and how these changes affect hatchability (Narushin and Romanov, 2002; Tona *et al*., 2002; Reijrink *et al*., 2008).

2.2.1.2.1 Egg Shell Quality Trait

Egg shell quality trait includes shell thickness, shell porosity, shell weight, shell colour etc. Egg shell has two main functions during the development of embryo. Eggshell provides adequate protection to the embryo by absorbing shocks from the external environment of the embryo. The shell is also a structure where gaseous exchange between the internal environment of the egg and the external environment take place (Narushin and Romanov, 2002). According to Narushin and Romanov (2002) shell thickness can affect the ability for the egg to hatch; thickness or thinness of shell has great effect on hatchability. They advised that shell should be adequately thin and fragile so as not to impede hatching process. Though pores on egg shell are important for gaseous exchange, with high pore concentration on shell can cause pathogens to enter to cause harm to the developing embryo, (Narushin and Romanov, 2002). Egg shell quality is one of the most important parameters in poultry production which can influence economic profitability. The hen eggshell consists of 94% of $CaCO_3$, 1% of $MgCO_3$, 1% of $Ca_3(PO4)_2$ and 4% organic substances mostly of albuminous character (Nys *et al.*, 2000).

Factors that affect egg shell quality includes the breed of the hen, age, nutrition, general stress and heat stress, disease and production system. Nu1tritional factors that may affect egg shell quality include phosphorous, calcium, vitamins and water quality. Calcium and phosphorous are important component of egg shell. (Hamidu *et al.*, 2011). Normally as the hen ages the eggshell

quality decreases This is due to an increase in egg weight without an increase in the amount of calcium carbonate deposited in the shells (Gary, 2015)

2.2.1.2.2 Egg Shell Thickness

One factor associated with the egg shell quality is the egg shell thickness. Egg shell thickness is an important trait for hatchability. Khan *et al.* (2004), stated that shell thickness between 0.33 and 0.35mm is suitable for optimum hatchability. They added that eggs with shell thickness less than 0.27mm rarely hatch. Hrncar (2012) concluded that eggshell quality plays very important key role in hatchability. According to Tsarenko (1988) reported that thick shelled eggs had 30% higher hatchability percentage than thin shelled eggs. Sergeyeva (1956) also affirmed the report of Tsarenko (1998) that when shell thickness is increased by one micrometer within the range of 0.29-0.35mm, hatchability of the eggs is also increased by 2%. He concluded that when the egg thickness of turkey was increased from 0.04mm to 0.05mm, hatchability of the egg also increased from 67% to 80%.

2.3.1.2.3 Egg Shell Porosity

Shell porosity is the number of pores concentrated on the shell of the egg. The number and diameter of pores on a shell have effect on egg hatchability (Chistyakova, 1998). Shell porosity plays a vital role in gas exchange between the external environment of the egg and the embryo. Both low and high pore concentration can affect embryo development negatively (Narushin and Romanov, 2002). However, low pore concentration or pores with small diameters can cause difficulties in exchange of oxygen which may result in high embryonic mortality. On the other hand, eggs with high pore concentrations or high diameter can result in high embryonic

development. High number of pores causes dehydration of the developing embryo. They can also contribute to the entry of pathogens (Tullet and Burton 1982; Burton and Tullett, 1985; Peebles and Marks, 1991; Demming, 1995).

2.2.1.2.4 Egg Shell Colour

The effect of egg shell colour on hatchability is not clear. Literature on shell colour gives conflicting results concerning its relationship to egg hatchability. Poultry producers believe that dark brown shells hatch better than light brown shells. However, research carried out on flycatchers which are certain species of songbirds- revealed that birds that are fed well lay deep coloured eggs depending on nutrient quality (Moreno *et al*., 2005). And this explains why producers have been trained to remove light coloured eggs from set eggs for hatching. Yoho *et al*. (2008) also observed that dark coloured eggs have high hatchability than light coloured eggs. They added that because shell pigments are secreted on shells before they are laid light coloured eggs may be a sign of premature laid eggs which may be caused by environmental stress.

2.2.1.2.5 Egg Weight and Egg Size

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). Egg 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.

According to Wilson (1991), the hatchling weight is determined primarily by egg weight and secondarily by weight losses during incubation, gender of chick and time after hatching. Aydi and Bilgehan (2007) observed significant effect of egg weight on feed consumption. They observed that heavy chicks consume more feed than lighter ones and reported significant effect of feed conversion ratio on egg weight. Additionally, they also found significant effect on chick weight at hatch on mortality to be insignificant.

In naked neck chickens, Peters *et al*. (2007) observe higher egg weights in the naked neck birds than the fully feathered birds. They observed that the major genes of naked neck increased full feathered egg size by 8.13 and 5.85 per cent respectively. Egahi *et al.* (2013) also observed an increase in egg size of 8.62 and 29.62 per cent respectively in birds carrying *Na* genes over the fully feathered birds.

2.2.1.2.6 Age of Chicken

Hatchability is likely to reduce when birds grow older (Tomhave, 1956 and 1958). Suarez et al. (1997) reported that age of hen at lay have significant effect on hatchability. Insko *et al.* (1947) was of the view that fertility and hatchability will decrease as the birds grow older. Sunde and Bird (1959) reported that eggs that were laid by chicken which had just reach their sexual maturation did not hatch well. However, their hatchability increased as they grew older. Garwood and Lowe (1982) added that birds produce maximum hatchability after six weeks of sexual maturity. Several researchers have reported that hachabiltiy decline with age (Bourassa,2003; Seker *et al*, 2004; Tona *et al.*, 2001; Yildirin, 2005; Elibol and Brake, 2006; Zakaria *et al*., 2009; Abudabos, 2010; Almarshade, 2011). It has been proven that albumen height is affected by hens age. (Zaman, 2004). Noddegaard (1992). According to Akbas *et al.* (1996) and Lapao *et al*. (1999) they stated that as hen ages the albumen height of their egg laid decreases. Akbas *et al*., (1996) confirmed that yolk height decreases with age. ISA (2009) advised that farmers should not expect good hatching results from birds who are 24 weeks or less. They stated that eggs of younger breeds should not be collected for hatching until they are 25weeks old because they will give poor results. This is because such eggs have relatively small yolk size. The best age to collect hatching eggs is between 32 and 52 months.

2.2.2 *Environmental Factors Affecting Hatchability*

2.2.2.1 Temperature

Temperature is one of the essential factors for growth and development of embryo during incubation. Suitable temperature for storage of hatching eggs depends on the genetic strain of birds. Extreme temperature is detrimental to egg hatchability during storage or incubation. Extreme low temperature leads to cold stress. Cold stress can cause embryonic mortality when storage

temperature falls below 4ºC. According to Deeming (1989) cold stress can impede yolk consumption by the embryo thereby reducing embryonic development. It also prevents water loss or vapour exchange between the egg and its external environment. This can cause embryonic death which can lead to low.

On the other hand, extreme high temperature can also cause embryonic death (Salahi 2012). Hot environmental condition is among stressors in poultry production. Heat stress comes as a result of interaction between the bird or the embryo and air temperature. Humidity, air speed and radiant heat. According to Charles (2002) suitable temperature for high bird performance is 19 to 22ºC for laying birds and 18 to 22ºC for broilers. Heat stress in birds occur when temperature requirement is above normal. Their ability to cope with this condition depends on the strain of bird, feather pattern, nutrition and production system. According to Lin *et al*. (2006) normally when birds find themselves in a hot environmental condition, they make effort to maintain their body temperature to normal functioning of the internal organs. Stress response in birds are controlled mainly by activation of hypothalmo-pituitary- adrenal (HPA) axis and orthosympathic nervous system. Negative effect of heat stress includes high mortality rate, reduced feed consumption etc. All these directly affect hatchability (Yahav, 2000).

Birds with naked neck genes are known to have superior body weight, better feed conversion rate, high egg production rate and resistance to diseases compare to the full-feathered birds at moderate (25ºC) to high (32ºC) ambient temperature. They are able to do this because of the ability to dissipate heat. (Mahrous *et al*., 2008). This reduces heat stress on them and as a result suffer less heat stress (Yahav *et al*., 1998; Adedeji *et al*., 2006). Furthermore, naked neck birds channel the conserved energy that might have been used for fighting heat stress to productive functions like growth (Yalcin *et al*., 1997; Patra *et al*., 2002). According to Hagan *et al*. (2013),

birds with naked neck genes are superior in growth and carcass yield parameters when compared to the normal feathered birds even under normal temperature.

In broiler production, naked neck gene is plays a vital role when it comes to heat tolerance in birds. (Merat, 1986; Lin *et al*., 2006). Ability for broiler bird to control heat stress is an inhibiting factor which is militating against broiler production in hot tropical climate (Horst, 1987).

2.2.2.3 Storage duration

This is the period between egg laying and incubation. Meijerhof (1992) reported that during storage, hatching is influenced by the storage duration, storage temperature, humidity, general environment and position of the eggs. Storage temperature should be lower for prolonged storage of eggs. Sarda- Jova (1992) reported that storing eggs for 7days had no significant influence on hatchability. He also reported that there was a highly significant deterioration in egg quality with increasing length of storage. The quality of fresh eggs is higher than that of egg stored in a refrigerator or at an ambient temperature and the quality of egg refrigerated was higher than that of eggs stored at ambient temperature. Prolong storage of eggs can influence the albumen pH because of loss of carbon dioxide (Dawes, 1975). For maximum hatchability, it is very important to focus on all factors that will maintain embryonic viability (Kirk *et al*., 1980; Deeming, 2000; Heier and Jarp 2001). Research has proved that eggs that are set into the incubator the very day they are laid produces heavier chicks (Reis *et al*., 1997). Petek (2003) reviewed that when length of storage increases hatching weight, hatchability and growth performance decreases. Ruiz and Lanam (2002) stated that when storage duration exceeds 3days hatchability reduces. Long storage of egg also prolongs incubation time thereby reducing post hatch chick weight and also retarding

embryonic development (Sahan *et al*., 2003). King‟ori (2011), also recommended that hatching eggs should not be stored beyond 10days because

hatcbility declines drastically after that period of storage.

Eggs store beyond 7days increases incubation time, affects hatchability negatively and reduces chick quality. (Mather and Laughlin, 1976; Tona *et al*., 2003; Becker, 1964; Fasenko *et al*., 2001; Tona *et al*., 2004; Yassin *et al*., 2008; Bynb and Nas, 1962; Merritt, 1964)

2.2.2.4 Relative Humidity

Humidity is one of the important parameters which needs to be considered in poultry production, especially during incubation. Evaporation of water vapour from egg through the shell pores is directly affected by relative humidity surrounding the egg. Eggs tends to loss about 12% to 14% of water vapour during normal incubation. This is evident on the fact that air cell continues to enlarge during incubation. Chick weight forms approximately 68% of the initial weight of the egg. Deductively an egg which weighs 60g should produce 41g of chick after hatching with 8g of the initial weight of egg loss as a result of vapour loss (humidity). The percentage of water loss from egg depends on many factors. The breed, age of egg, age of flock, weight of egg and type of incubator are some the factors that affect relative humidity. However, one thing farmers should be mindful of is that high level of humidity can lead to difficulty in pipping. Very low level of humidity result in egg dehydration which leads to smaller chick size. Again, dehydration of eggsa can cause shell membrane sticking to the embryo and causing difficulties in hatching (CEVA, 2007). In storage, relatively high humidity can improve hatchability (Meijerhof, 1992).

2.2.2.5 Ventilation

Egg is a living thing which respires. Respiration is an important live process in eggs. The eggs take about 7g of oxygen throughout the 21days of incubation and gives off 9g of carbon dioxide. The exchange of this gasses is made possible by ventilation. The fresh air which the incubator draws from the incubator room makes oxygen available to the eggs. By ventilation, carbon dioxide produced as a result of respiration by the eggs is taking out. The air circulation inside the incubator reduces excessive heat surrounding the eggs. Ventilation is a major factor which affects incubation time and hatchability. During laying, as the egg moves down the reproductive tract, the egg finds itself in acidic environment. However, when the egg is released, atmospheric oxygen takes over, which increases the internal pH of the egg. This slows down embryonic development in the egg. Incubators should be adjusted so that oxygen level is kept at 21% and
0.4% for carbon dioxide level (CEVA, 2007).

2.2.2.6 Nutrition

Development of egg embryo is an external process. Embryo development depends on the nutrient in side the eggs. The nutrient in the egg which is supplied by the egg yolk provides energy and building blocks for the development of the embryo through the period of incubation (Foye *et al*., 2006). The only nutrient which is not supplied by the egg and are taking from the environment in which the egg finds itself is oxygen. The ability of the embryo to consume the nutrients packed in the egg yolk depends greatly on oxygen availability for respiration (Moran, 2007). In a nut shell hatchability depends on the nutrient composition of the hatching egg and how the nutrients are utilised in the presence of oxygen. Moran (2007) determined that glucose is the main source of energy in the first week of development. Fatty acids are metabolized during the second half of

incubation. According to Sato *et al*. (2006), greater amount of yolk nutrient is utilised in the development of embryo during peri- hatch period and the hatching period (Richards, 1991; Vieira and Moran, 1999). At the later stage of incubation, fat metabolism decrease whiles glycogen metabolism increases (Moran, 2007). The main energy source during hatching is the glycogen stored in the muscles and the liver of the embryo (Donaldson, 1995; Moran 2007).

Deficiency of energy during hatching is detrimental to hatching process. The lack of energy can lead to weak hatched chicks and in more severe cases embryonic death may result. Energy is therefore the most important factor when it comes to chicks hatching out of the egg.

In embryo development, temperature is one of the factors which causes the embryos to switch form aerobic to anaerobic respiration. This affects the reserved level of glycogen for the embryo during the last stage of hatching. When temperature inside the incubator is increased at the last stage of incubation, chicks with poor qualities develop (Wineland *et al*., 2000a; Wineland and Christensen, 2001; Lourens *et al*., 2005; Leksrisompong *et al*, 2007; Molenaar *et al*., 2009 Piestun *et a*l., 2009). Temperature also affect the uptake of protein and lipids by the embryo secreted by the yolk. High temperature leads to decrease level of oxygen resulting in an increase embryonic mortality (Speake and Powell, 2003; Powell *et al*., 2004).

2.2.2.7 Egg turning frequency

Egg turning is very important during incubation. According to Tona *et al*. (2003), when eggs are turned in the first week of incubation, it enhances the formation of extra-embryonic membrane which reduces the incidence of malposition. Elibol and Brake (2004) also added that

lack of turning result in malposition in which the head of the embryo is found at the small end of the egg. Turning of egg during incubation also enable yolk nutrients to be transfer to the embryo through sub- embryonic fluid (Deeming, 1989). New, (1957), and Robertson (1961) reported that turning of egg results in the development of the embryonic membrane which leads to correct positioning of the embryo.

Several research had proven that when egg is turned 24times a day, high hatchability is achieved as opposed to less frequent turning (Kuiper and Ubbels, 1951; Kaltofen and Ubbels, 1954, Kaltofen, 1995. Elibol and Brake (2003) suggested that when turning frequency of broiler hatching eggs is adjusted to 96times a day after 3days of incubation till 11th day of incubation, it yields better results than 24 times and 48 times turning frequency in a day. However, when turning frequency is too high, hatchability is negatively affected. For example, turning frequency 0f 480times per day decreases hatchability (Robertson, 1961). Wilson *et al*., (1997) suggested that turning of hatching eggs could be halted at 16days of incubation without negatively affecting hatchability. According to Elibol and Brake (2006, 2008), an increase in turning frequency enhanced not only hatchability but also fertility of egg of older hens but not younger hens.

In chicken, the most important period which needs egg turning is 3days of incubation to 7days of incubation. (Deeming, 1990; 1991). Failure to turn eggs may have negative effect on gas exchange through the chorioallantois; the unabsorbed albumen is interposed between the chorioallantois and inner shell membrane, hence reducing the gas exchange, decreasing the arterial oxygen pressure (paO_2) of late embryos and increasing haematocrit values (Deeming, 1989; Wilson, 1991). Also, the partial pressure of oxygen of unturned eggs is lower and slows down embryo development (Tazawa, 1980). All this may result in an increase in the length of the incubation time and decreased hatchability

The position of the eggs during storage has an effect on hatchability. Roovert-Reijrink (2011) stated that the normal way of storing egg is broad end up and the small end down. This position makes the embryo to be beneath the air cell. He addad that embryos tends to dehydrate or stick to the internal membrane when the egg is positioned with the broad end down.

On the contrary, Proudfoot (1967, 1969) reported high hatchability when eggs were stored with small end up. He added that small end up storage of eggs resulted in high hatchability in all storage durations, even up to 28 storage duration. Mujeer *et al*. (1986) obtained similar results when they stored eggs with small end up. However, Oluyemi and George (1972); Mouldgsl *et al* (1976) did not see any significant differences in fertility and hatchability of eggs stored either with small end up or large end up, when they were store for 10days.

2.2.2.8 Disinfectants

Disinfecting hatching eggs is a critical control point (CCP) in the poultry incubation chain, aimed at reducing the introduction of pathogens into the hatchery for the production of healthy day-old-chicks. Some common examples of disinfectants are Formaldehyde, Iodophors, ultra violet light, ammonium, etc. How eggs are handled after it had been laid till incubation is very important. When it is not well handled egg shell quality is greatly affected which can also affect hatchability (Moyle *et al*., 2008). Davies and Breslin (2003) are of the view that when the surface of egg is sanitized it reduces bacterial population on the shell and consequently increasing hatchability and improving egg quality. Davies and Breslin (2003) noted that incidence of salmonellae decreased when sanitation in farms and egg packing plants was improved. However, salmonellae were not eliminated from the surface of the shell. There have been several ways farmers have tried to

sanitized hatching eggs and table eggs. Some of these methods includes UV light, egg washing and fumigation. (Berrang *et al*., 2000; Wilson, 2002; Coufal *et al*., 2003). In recent times ultrasonic vibration has been employed to control pathogens on the surface of the egg (Sert *et al*. 2011; Aygun and Sert, 2012). However, according to Shafey *et al.* (2013), ultrasonic method create pressure on the surface of the shell and causes a number of physical and mechanical damages on the shell. That is the surface of the shell may be eroded or the shell thickness will decrease. This method can cause invisible cracks in the shell and may affect shell characteristics and can cause embryonic death as well as low hatchability of hatching eggs (Dawson *et al*.1962; Wladyka *et al*. 1963).

Dawson *et al*. (1962) and Wladyka *et al*. (1963) indicated that ultraviolet vibration enhances the penetration of the cleaning solution. Research conducted by Sert *et al.* (2011) review no significant differences in shell strength between shell treated with ultrasonic vibration at first day and the control. Again, they also found that eggs that were stored for 10days had higher shell strength as compare to other eggs stored for the same day without treatment with ULT.

2.2.2.9 Egg Contamination

Egg contamination can be lethal to the embryo even at low doses. The degree of yolk contamination is influenced by the degree of egg contamination before egg setting (Musara and Dziva, 1999; Cabassi *et al*., 2004). Mushi *et al*. (2008) observed a 7.3% hatchability depression associated with microbial contamination of eggs. Microbial contamination of eggs can result from the dipping or washing of eggs in liquid disinfectants before setting them into incubators that possibly leads to the disruption of the protective cuticle of the egg shell (Richards 2002). Because of egg contamination and infection of day old chicks, fumigation should be routinely carried out

before setting eggs into the incubator. (Huchzermeyer, 1996; Mushi *et al*., 2008). Various microbes have been associated with egg contaminations, including bacteria and fungi (Penicillium sp. and Fusarium sp.) (Musara and Dziva, 1999; Cabassi *et al*., 2004; Mushi *et al*., 2008).

2.3 The Egg

This is a structure that is usually laid by some female of certain species of animals as a means of reproduction. Egg consists of four main parts namely; shell, membranes, albumen and yolk. The proportion of each component in terms of weight of freshly laid chicken egg is 32% forming yolk weight, 58% representing albumen weight and 10% being shell weight (Leeson, 2006). The shell serves as a protector and a container for the embryo throughout its developing stage till it hatches. The chicken egg has about 7000 pores on its shell (The Franklin Institute Science Museum, 2008). This serves as the medium for the exchange of gases. Carbon dioxide and moisture are given off and oxygen is taken in for embryonic development. The air cell is located at the broad end of the egg which also helps in exchange of gases during the embryo‟s development. The shell membranes give protection to the internal content of the egg against bacterial infection.

The albumen is the fluid matrix where embryo develops. It provides the embryo with protein which helps in the growth of the embryo. The egg albumen is made up of four structures. They are:

- **Chalazae:** This immediately surrounds the yolk and forms about 3% of the egg white. This is a double helix structure of the albumen which can be seen in a broken out egg as yolk

extension. The twisted nature of the chalaza comes about because of the turning of the yolk as it moves down the oviduct during egg formation.

- **Inner thin layer**: This surrounds the chalaza and makes up of about 17% of the egg albumen.
- **Thick layer**: This is an envelop that surrounds the inner thin white layer and the yolk. It is attached to the shell membrane at each end of the egg and constitute about 57% of the egg albumen.
- **The outer thin layer:** This is found just inside the shell membrane. It accounts for about 23% of the albumen (USDA, 2000; HHS, 2010).
- **Yolk**: It is the main source of food for the embryo. The yolk is also surrounded by a yolk sac membrane which is composed of two germ layers, the endoderm and vascular mesoderm. The endoderm has been described as a thick layer with villus-like projections and corrugations running in a generally meridional direction, and is responsible for the absorption of nutrients from the yolk (Lin, 2012).

2.3.1 Egg Quality

Egg equality is a term that explains the standards which defines both the internal and external qualities of egg. The external quality of egg encompasses thickness, texture, shape, colour and even how clean the shell is. The internal quality also focuses on size of air cell, yolk shape, yolk height, albumen viscosity, strength and blastoderm diameter (De Ketelaere et al., 2004).

2.3.1.1 Internal Egg Quality

Internal egg quality characteristics begins to deteriorate as soon as the egg is laid. As the storage time increases internal egg qualities decreases. However, the chemical composition of the yolk and the albumen do not change much. When an egg is laid the albumen pH is between 7.6 and 8.5 but the pH increases depending on the storage temperature to a maximum of 9.7 (Heath, 1977). Sharp and Powell (1931) observed an albumen pH of 9.18 even when the eggs were stored at temperature of 3^{o}C for 3days. Regardless of storage temperature the pH of egg will increase when the storage duration increases (Li-Chan *et al*., 1995). The Albumen pH eggs increase because of loss of carbon dioxide through the shell pores. Albumen pH is also affected by dissolved carbon dioxide, carbonate ions, bicarbonate ions and protein equilibrium (Heath 1977).

In a freshly laid egg, the yolk is round and firm. When the storage duration increases the yolk imbibes water from the albumen and increases in size. This put pressure on the vitelline membrane and weakens it. The yolk there looks flat and mottled. Albumen therefore after losing moisture to the yolk loses weight as well (Heath 1977; Li-Chan et al. 1995). The temperature and storage time also negatively affects the internal egg quality (Akyurek and Okur, 2009). Research has shown that an albumen index of 10.31 in fresh eggs decreased to 6.63 after 10 days of storage. Also Haugh unit of 82.1 in the fresh eggs decreased to 66.6 in eggs stored for 11 days (Onbasilar *et al.*, 2007). It can be state categorically that the decrease in internal egg quality is as a result of water and carbon dioxide loss from the egg resulting in albumen becoming watery.

2.3.1.2 External Egg Quality

External qualities of an egg is basically the characteristics of the eggshell. A good quality shell must be without cracks, clean, smooth with uniform colour, shape and size. Kouszek *et al*. (2009) reported that, eggs with dark colour eggshells stored for a period of 0 to 10 days, in comparison with their light colour counterparts were characterized by higher numbers of blastodermal cells already on the day of laying. One factor associated with the egg shell quality is the thickness of the egg shell. It is an important trait which affects hatchability. According to Khan *et al*. (2004) for high hatchability, egg shell should be moderately thin and fragile which should not impede hatching process. This is because egg shell thickness and thinness has effect on hatchability.

The number of pores concentrated on a shell also affects gaseous exchange of the embryo. Too much or less pores on the shell can be detrimental to the development of embryo (Narushin and Romanov, 2002). The size of the pore can affect embryonic liveability and hatchability. Very small pore size on a shell can cause difficulty in exchange of oxygen between the embryo and its external environment. On the other hand, when the pores are too large they enhance dehydration which can also affect embryo development (Demming, 1995).

2.3.2 Egg Quality Evaluation

Quality of egg is very important for marketing and also for hatchability that is why farmers try to sort eggs according to their quality. Eggs are normally sorted by packing plants based on their quality. Eggs with dirt, cracks, meat spots and blood spots are not classified as quality eggs. However, classification or sorting of eggs is subjective to human error. There are two main methods of determine egg quality. These are:

2.3.2.1 Destructive Method

Haugh Unit (Haugh, 1937) is the most widely used method and the most accepted method of determine albumen quality. It is based on the relationship between the weight of the whole egg and its albumen height when the egg is broken out. Fresh egg has its yolk centrally placed, surrounded by thick albumen when the egg is broken out on to a flat surface. However, eggs that have been stored for long have the yolk displaced at one side with a thin albumen surrounding the yolk. This results in a decrease in albumen height which causes the haugh unit to decrease (Kemps *et al*.,2006). Contrary to the work of Haugh (1937), Siversides and Villeneuve (1994) argued that albumen height and egg weight is weakly related. They stated that Haugh unit is only dependent on the albumen height but independent of the albumen weight. According to Li-Chan *et al*. (1995) pH can be used to measure albumen freshness because pH changes with time. The change of pH is as a result of carbon dioxide lost from the egg through eggshell pores. The pH is directly dependent on the balance between dissolved carbon dioxide, carbonate ions and proteins. Donovan and Mapes (1976) stated that refractive index of an albumen is a good measure of freshness of the egg. The conversion of ovalbumin into S-ovalbumen is also a good indicator of measuring aging egg. According to Hildago *et al*. (2006) Maillard reaction; reaction as a result of acid hydrolysis of Amadori compounds (Furosine) can also be a reliable index for measuring albumen quality

2.3.2.3 Non-destructive methods

The most common non destructive method used to determine egg quality is the candling. This is simple and fast way for assessing equality. Candling devices ranges from simple unit to more

sophisticated unit. However, whether simple or sophisticated, the underlining technique is passing a white light through the egg. This is more accurate when the candling is done in the darkroom. During candling the yolk of a freshly laid egg is seen as a faint shadow. As the storage period increases and albumen quality decreases causing the yolk to be closer to the shell, the shadow of the yolk appears darker. Darker shadow can also result when the yolk enlarges or when the vitelline membrane becomes weak (Jacob *et al*., 2000).

2.4 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 have 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 cleaned, 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 comes 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; Tonal *et al*., 2003; Decuypere and Bruggeman, 2007)

2.4.1 Factors Affecting Chick Quality

2.4.1.1 Incubation Temperature

Very high or low temperature during the incubation has a negative effect on the day old chick quality. The temperature of an embryo is determined by number of factors. These factors include air temperature in the incubator, air velocity, humidity and embryonic metabolic heat. Deeming (2000) stated that extremely low or high temperatures in the incubator may result in high embryonic death. This can lead to prolong hatching process resulting in number pipped embryo without hatching, small size sticky chicks with unhealed navel and deformed toes. Extreme temperature in setters or hatchers negatively affect chick quality. According to Decuypere *et al*., (2001) environmental temperature surrounding the embryo should be within 37 to 38^{o}C. This range of temperature can produce chicks with good qualities as well higher number of eggs hatched

Joseph *et al*. (2006) found out that eggs that were set in an incubator of low temperature (36.6^{o}C) for 10days recorded lower hatchability. They also found that lower incubation temperature resulted in high hatch chick weight and shorter chick length. Similarly, high incubation temperature results in lower hatching weight.

According to Deeming (2000), embryonic development and rate of oxygen demand is enhanced by high incubation temperature. High incubation temperature results in high metabolism waste production in the embryo. High rate of metabolism leads to heat production which in turn increases egg shell temperature (Lourens, 2003). The consequence of high temperature leads to poor embryonic growth, poor utilization of albumen protein, embryonic stress, poor hatchability and poor chick quality which accounts for high rate of mortality (Deeming, 2000). According to Lourens (2003), embryonic temperature is the most important

factor that affects chick quality.

2.4.1.2 Incubation Relative Humidity

Level of humidity in the incubator has significant effect on the temperature in the incubator which invariably influences chick quality. According to Deeming (2000), low humidity result in dehydration, sticky and small. Low humidity hinders yolk sac cosumption, influences yolk sac infection and increases mortality of chicks in the first week after hatch. Temperature in the incubator is one factor that affects hatchability greatly. High humidity during incubation is also associated with unhealed navels in day old chicks. On the other side when humidity is too high chicks become large, weak and sticky. It is also cause unhealed navel in the day old chicks The amount of moisture surrounding the egg is greatly affected by the temperature in the incubator.

Its well known fact that chick hatching weight is dependent on initial egg setting weight. Chick weight is also influenced by moisture loss during incubation. Bruzual *et al*. (2000) looked at the effect three of different level of relative humidity on hatchability and chick quality. They determined that eggs incubated at relative humidity of 53% had the highest hatchability of 89.1%. Eggs incubated at a relative humidity of 63% had the lowest hatchability as result of late embryonic mortality as compared to the rest of group (43% and 53%). They explained that at later period of incubation, oxygen demand is high because the embryo development is at its maximum, this increases water vapour content and also decreasing oxygen partial pressure in the incubator. Therefore, high incubation relative humidity may result in low hatchability especially during the later period of incubation.

2.4.1.3 Effect of Ventilation on Chick Quality

Incubators are manufactured to provide conditions similar to that of breeding hen. They are made to control the exchange of gases and heat produced as a result of embryonic development. Ventilation is responsible for the circulation of air in the incubator. This supply oxygen in the incubator and take away carbon dioxide and water vapour. Temperature in around the egg is controlled by ventilation. According to Deeming (2000), when ventilation in the incubator or the room in which the incubator is installed is not enough, it results in poor embryonic development. The embryos will be surrounded by fluids. This is an evidence of low oxygen concentration and high carbon dioxide concentration. Higher carbon dioxide concentration in the incubators forces chicks to hatch early, However, chicks normally have problem with the maturation of the heart and lung (Coleman and Coleman, 1991).

2.4.1.4 Effect of Turning on Chick Quality

Turning is very important factor in incubation. It prevents the embryo from attaching itself to the eggshell membrane at early stages of embryonic development. Aside preventing embryo from sticking to the egg shell membrane, it also reduces an increase in temperature in the incubator. Turning enhances the development of chorio-allantois sac which play a vital role in respiration and nutrition of the embryo. Again, turning helps in the formation of extra- embryonic membrane in the first 18days of incubation (Deeming, 1999). Enough turning leads to late hatch, low hatchability and also hatched birds do not dry off normally (Deeming, 2000). Wilson (1991) stated that lack of turning leads to embryo attaching itself to the inner shell membrane, malposition of the embryo, reduced consumption of yolk and albumen and decreased oxygen etc.

2.5.2 Methods of Evaluation Chick Quality

Chick quality can be measured quantitatively or qualitatively by a standard scoring criteria (Decuypere and Bruggeman, 2007). Chick quality parameters that are measure include chick weight, chick length, chick shank length, leg and toe length, chick appearance, alertness and navel condition. (Tona *et al*., 2004; Willemsen *et al*., 2008).

2.5.2.1 Qualitative Method

This is a subjective method of evaluating chick quality. It is usually based on the farmer‟s experience (Decuypere 2005) and sometimes his own standards. According to Meijerhof (2005) qualitative method of measuring chick quality is usually based on visual scores as defined by Deeming (2000), his definition considers a quality day old chick as a chick which is cleaned with dried feathers, bright and clear eye, sealed navel and without deformities (Decuypere *et al*., 2001). Preez (2007) also added that quality chick should not have yolk sac or dried membrane and should have the yolk sac bulging out from the navel. There should be no sign of respiratory diseases and should have normal leg and body conformation. The skin and the hock joint should not have any swellings. The beak and toes should have a normal conformation (Decuypere and Bruggemen, 2007).

2.5.2.2 Quantitative Method

Quantitative method of evaluating chick quality is an objective way of assessing a day- old chick. Quantitative method was designed to make chick quality assessment objective for measurement (Raghavan, 1999; Deeming, 2000; Boerjan, 2002; Tona *et al*., 2003). With this

method a day old chick quality can be assessed and prediction on its growth potential can also be determined. There are three main ways for scoring chick quality quantitatively. These methods are a day old chick weight, measuring yolk free chick weight and measuring chick length (Meijerhof 2009.

2.5.2.2.1 Tona or Pasgar Score

This method uses a homogenous scoring system which considers some lay down parameters like yolk sac uptake, closed navel, viability, and ability of the chick to recover after it has been placed on its back (Preez 2007). The method put visual scores of hatchery managers into a standard scale with reproducible figures. This standard scale converts qualitative scores to quantitative scores (Boerjan, 2002; Tona *et al.*,2003). The assessing system of Tona method ranges from zero to hundred (Willemsen *et al*., 2008). Based on the system, chicks with hundred score means high quality day old chick. This is done by well trained personnel who are able to observe and score accurately to give reproducible figures. Unlike Tona method, Pasgar score is determined by conditions in the hatcher. However, Tona *et al*. (2008) is of the view that the scoring system should be revised with time.

2.5.2.2.2 A Day Old Chick Weight

This is one of the most objective method of determining chick quality (Deeming, 2000; Decuypere *et al*., 2002). However, there has been questions on whether the chick weight is the most perfect measure of the chick development. This is because several research has shown that

setting egg weight is positively correlated to chick hatching weight but did not include chick development. (Wiley,1950). Additionally, Sklan *et al.,* (2003) found positive correlation between initial egg hatching weight and slaughter weight. But Wolanski *et al* (2003), Tona *et al.,* (2004) did not observe positive correlation between initial egg hatching weight and slaughter weight.

They explained that a day old chick weight consists of the actual chick weight and the yolk sac weight. At hatch chicks with higher body weight tend to have smaller yolk sacs but birds with smaller body have larger yolk sacs which enhances their survival before they are introduced to external feed (Skewes et al., 1988). Large amount of yolk sac weight means that the chick utilised less energy source in the yolk during and chick is less developed.

2.5.2.2.3 Yolk Free Body Weight

It is used to correct egg setting weight. This method involves the chick weight at hatch and the weight of the remaining yolk sac. This reveals the content of egg used for embryonic development. This is a reliable method of determine chick quality. This is because when egg spend about 19days in the incubator, the yolk sac absorption into the embryo begins. Therefore, at hatch yolk sac should have been fully absorbed into the skin of the navel (Meijerhof, 2009). The yolk sac content provides internal feed for the survival of the chick in the first few days of its life, until external feed is introduced (Sklan, 2000). The yolk that remains in the body of the chick contains fats, proteins and maternal antibodies which protect chicks from infections from the causative agents the dams had been exposed to during laying (Vierna and Moran, 1999). In some large scale hatcheries, yolk sac may not be completely absorbed and this results in a closed navel with scab. This is known as navel button. The navel button is characterised by leakage of liquid from the

navel. This can lead to lower body weight at slaughter age (Fasenko and O"Dea, 2008). Again this problem can cause yolk sac infection known as omphalitis and can also result in high post- hatch mortality, especially in the first week of hatch. (Meijerhof, 2009). In birds, the yolk sac performs functions as colostrum in mammals, providing antibodies to fight against diseases. It is therefore an important tool in the assessment of quality chick (Alexander, 1988).

However, this method involves killing the bird and its also labour intensive (Lourens *et al*., 2006).

2.5.2.2.4 Chick Length

This is normally taken after the chicks have been pulled out of the incubatorr. The chick length is determined by placing the tip of the beak at zero mark of the ruler and stretching the chick along the ruler to the end of its middle toe. It has been found that chick length and body weight of chicks at 42days old are positively correlated (Meijerhof, 2006; Molenaar *et al*., 2008). Wolanski *et al.* (2006) also observed a positive correlation between chick length and chick weight at hatch. According to Meijerhof (2005), Deeming (2005) and Wolanski *et al*. (2006) chick length is considered a better indicator for chick quality than chick weight. Molenaar *et al*. (2008) reported that an increase in length in male broiler resulted in increase of chick weight from same size eggs.

CHAPTER THREE

3.0 MATERIALS AND METHODS

Two experiments were conducted in this research. The first was to evaluate the effect of different storage duration and different storage temperature on the quality of eggs and blastoderm of naked neck breeders. The second experiment look at the effect of different storage duration and different storage temperature on fertility, hatchability and chick quality of naked neck egg.

3.1 Study Area and Study Period

An on farm research was carried out at Akate Farms and Trading Company Limited, (poultry production section and Hatchery section, all in Kumasi Bosore) and The Department Animal Science Department Laboratory, Kwame Nkrumah University of Science and Technology Kumasi- Ghana from January 2014 to June 2014. Kumasi lies between latitude 06º 41‟N and longitude 01º 33‟W with an altitude of 261.4MSL, above sea level (Meteorological Services Department, Kumasi 2015; Adomako, 2009). The on farm sites have prevailing tropical climate with the mean ambient daily temperature ranged from 23° to 31°C. Chick quality, fertility and hatchability characteristics were carried out at Akate farms. Egg quality characteristics analysis were also carried out at the Department of Animal Science, KNUST.

3.2 Experimental Birds

The birds for this research were obtained from Akate farms. Four hundred and fifty (450) eggs were collected from forty -five (45) weeks old heterozygote naked neck chickens. These are locally developed commercial naked neck birds following generation of crossing between naked neck male and lohmann hens.

3.3 Experiment One

3.3.1 Egg Collection

The eggs were collected for a period of two weeks at specific days to determine the effect of egg storage on egg quality, fertility, chick quality and hatchability. Eggs were collected in five (5) batches within the fourteen days. The first batch of the eggs were picked up on the first day of

the fourteen days, these were the eggs stored for 14 days. The second batch was collected four days later and was stored for 10days. The third batch of eggs was also collected three days after collecting the second batch. These were eggs stored for 7days. The eggs stored for 4days and 1day were collected on the 10th and 13th days respectively.

Eggs were divided into two groups in each batch and labelled as cold room temperature and ambient temperature with their storage duration written on them. The eggs in each group was labelled individually and weighed to determine the initial egg weight before storage using Pro Scout balance.

3.3.2 Egg Sample Size and Sampling Technique

Egg were randomly collected from 120 naked neck birds. 90 eggs were collected in each batch and were divided into two treatments of 45 eggs.

3.3.3 *Egg Storage and Egg Quality Characteristics*

3.3.3.1 Egg Storage

After collection and weighing, the eggs were stored under ambient temperature and under coldroom temperature. The eggs for ambient temperature were stored at Saaman farm with temperature 23^{o}C to 30^{o}C. And the eggs for coldroom temperature were sent to Akate farms hatchery at Bosore and stored in a cold room at 18^{o}C. After the period of storage, the eggs were

reweighed to determine the loss in weight during storage. In all, 150 eggs were selected for egg quality characteristics.

3.3.3.2 Egg Quality Characteristics

Out of the 150 eggs selected, 15 eggs were selected from each group. Ten out of the 15 eggs were weighed and broken out. The yolk and the albumen were separated using egg separator. When an egg was broken out, it was poured unto egg separator and liquid albumen pass through the separator into a beaker leaving the yolk on the egg separator as described previously (Hamidu *et al*., 2011). The eggshell was washed thoroughly making sure the eggshell membrane was not removed. The wet yolk weight and wet shell weight were determined using a digital scale (Scout Pro SPU402; S/N: 7129141296, Ohaus Corporation). The egg shell thickness for each egg was determined using a micrometer screw gauge. The wet yolk and wet shell were placed in Gallen camp oven at 70$_{o}$C for 4 days to obtain the dry weights. The wet and dry egg components were expressed as percentage of the initial egg weight after storage.

3.3.3.3 Blastoderm Diameter

The remaining 5 eggs, out of the 15 eggs per replicate were also broken open and the yolk, albumen and eggshell separated as described. The fertility of the eggs was determined using the clear observation technique of an intact blastoderm with clearly displayed area opaca and area pellucid (Hamidu *et al*., 2010, 2011). The bastoderm for fertile eggs could be seen as two white rings like „doughnut". The egg yolks were rotated carefully until the blastoderm was visible on

the upper surface (Plate 3.1). A Vernier caliper was used to measure the diameter of the blastoderm on the surface of the yolk.

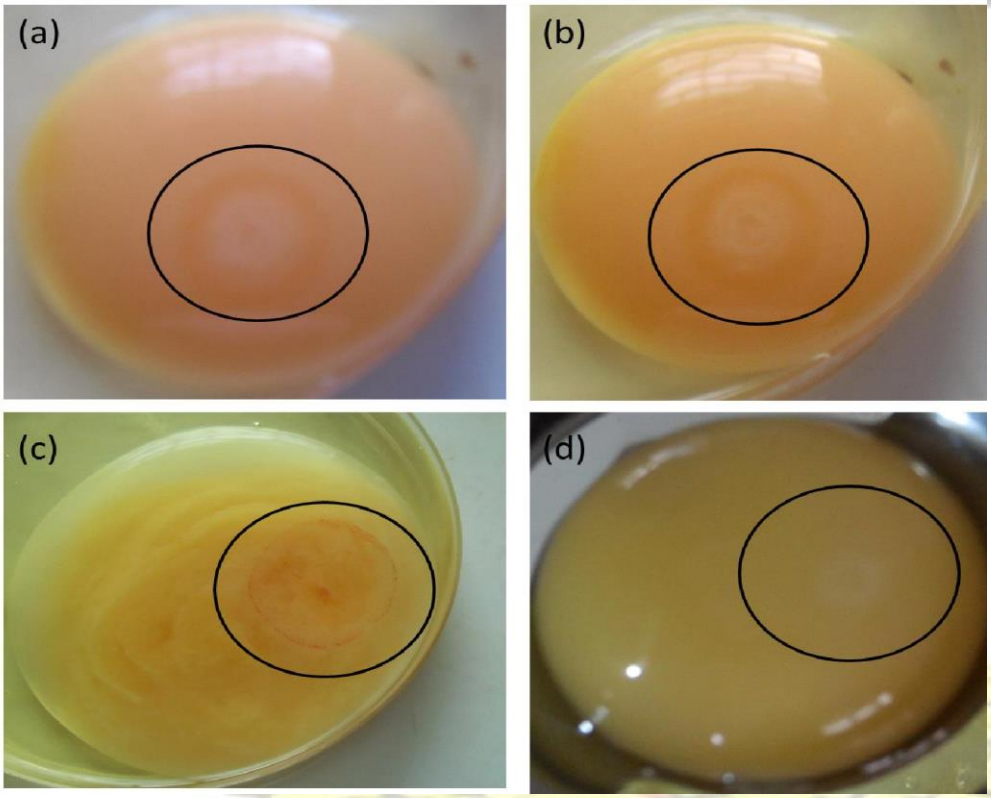

Plate 3.1. Effect of storage temperature and storage duration on blastoderm size and shapes for eggs stored under ambient temperature: a) Blastoderm of egg stored for 3days; b) Blastoderm of egg stored for 7days; c) Blastoderm of eggs stored for 10days and; d) Blastoderm of egg stored for one day.

3.3.4 Data Collection

Data on internal and external egg quality characteristics were collected. External egg quality characteristics collected were egg weight before storage, egg weight after storage, egg weight loss, shell thickness, wet shell and dry shell weight. The internal egg quality characteristics taken are blastoderm diameter, percentage albumen weight, wet yolk weight and dry yolk weight.

Egg number: This was the total number of hatching eggs collected and used through out the experimental period.

Egg weight before storage: This was taken immediately after the eggs have been collected using electronic balance (Pro Scout balance)

Egg weight after storage: This was taken before the eggs were set into the incubator (at the end of the storage duration).

Egg weight loss: This was taken as the difference in egg weight before storage and egg weight after storage. ***(Egg weight loss = egg weight before storage – egg weight after storage)***

Shell thickness: This was taken using micrometer screw gauge

Wet egg shell weight: This was taken after the shell had been air dried for a day and then weighed using electronic balance (pro scout balance)

Percentage wet egg shell weight: This was taken as the wet egg shell (air dried shell) expressed as a percentage of the egg weight before storage.

$$\%\ wet\ egg\ shell\ weight = \frac{Wet\ shell\ weight}{Egg\ weight\ before\ storage} x\ 100$$

Dry egg shell weight: The shell was oven dried at 70^{o}C for 4days and weighed using electronic balance

Percentage dry egg shell weight: This was the weight of an oven dried egg shell expressed as a percentage of egg weight before storage

Wet yolk weight: This was determined by carefully separating albumen from yolk after egg break out and weighed using electronic balance

Percentage wet yolk weight: This was the weight of wet yolk weight expressed as a percentage of egg weight before storage.

$$\% \text{ wet yolk weight} = \frac{\text{Wet yolk weight}}{\text{Egg weight before storage}} \times 100$$

Yolk dry weight: This obtained by oven drying the yolk for 4days at temperature of 70^{o}C and weighing it using electronic balance

Percentage dry yolk weight: This was obtained by expressing the dry yolk weight as a percentage of the egg weight before storage

$$\% \text{ dry yolk weight } \% \text{ dry yolk weight} = \frac{\text{dry yolk weight}}{\text{egg weight before storage}} \times 100$$

Percentage albumen weight: This was obtained by finding the difference of 100 and the sum of percentage dry yolk weight and percentage dry shell weight.

Percentage albumen = [100 – % dry yolk weight + % dry shell weight]

Blastoderm diameter –This was done after the yolk had been separated from the albumen using egg separator. The blastoderm was then located and caliper was used to determine its diameter.

3.3.5 Experimental Design

Experimental design used was 2x5 factorial design. It consisted of 2 levels of treatments of eggs storage temperatures and 5 levels of treatments egg storage durations.

3.3.6 Statistical analysis

Fertility and hatchability data was expressed in percentages and the chick quality data was analysed using the Proc. Mixed Model procedure of SAS at $P < 0.05$ (SAS statistical Institute 2012). The statistical model used was defined as below

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij} \quad [1]$$

Where, Y_{ij} = response recorded on a measured parameter, μ Overall mean, α_i = main effect of storage duration, β_j main effect of egg storage temperature, $(\alpha\beta)_{ij}$ =interaction effect of egg storage duration and storage temperature and ε_{ij} = random error term.

Where significance was observed, the means were separated using the PDIFF procedure of SAS. For hatchability and fertility data, they were expressed in percentages and compared.

3.4 Experiment Two

3.4.1 Incubation, Candling and Hatching

3.4.1.1 Incubation

Three hundred eggs were set into two setter trays. Each tray was labeled according to the condition of storage (ambient temperature and cold room temperature). The eggs were arranged according to the period of storage in each tray and storage period indicated on the eggs. Before incubation, eggs were cleaned, disinfected and fumigated. The eggs were placed in the setter trays

with the large end up and were set into an incubator of temperature 37.5ºC and humidity of 60% for eighteen days.

3.4.1.2 Candling

On the 18th day of incubation, candling of eggs was carried out to select fertile eggs and infertile eggs (early embryonic mortality). Each group of eggs were set on the candler in a dark room and a ray of light passed through the eggs. The fertile eggs were seen to be densely clouded and opaque with network of veins indicating development of embryo within the eggs while the infertile eggs were translucent under the light. Numbers of infertile eggs and early embryonic mortality in each group were recorded.

3.4.1.3 Hatching

After candling, the fertile eggs were transferred into pedigree hatching baskets which had been partitioned with perforated cardboard according to how they were set in the incubator (Plate 3.2). The hatching baskets were also transferred to a hatching unit of temperature of 37ºC and humidity of 85% for 3days. On the 21st day, the hatch was pulled and the chicks left to dry.

Plate 3.2. Improvised pedigree hatching basket as a result of partitioning with perforated cardboards

3.4.2 Data Collection

The data collected were egg fertility, chick quality and hatchability. Chick quality characteristics included chick weight, chick length and chick shank length.

3.4.2.1 Fertility Data Collection

Fertility: Number of eggs that were seen to be opaque after 18 days of incubation, when white light passed through them, was classified as fertile eggs (living embryo) for hatching.

Percentage fertility: This was taken as the total number of eggs whose embryo were alive (fertile eggs) after18days of incubation expressed as a percentage of total number of egg set.

$$\textit{Percentage Fertility} = \frac{\textit{Total number of fertile eggs}}{\textit{Total number of set eggs}} \times 100$$

3.4.3 Data Collection for Chick Quality

Individual weight of chicks was determined with a weighing scale after the chicks were pulled out of the incubator using Pro Scout electronic balance.

Chick lengths were also taken after the chicks pulled out of the incubator using 30cm ruler. The chick length was measured by placing the tip of the beak at zero mark of the ruler and stretching the chick along the ruler to the end of its middle toe

Chick shank length were measured right after hatching using 30cm ruler. The chick shank length was measured from the *hock* joint to the *metatarsal* pad.

3.4.5 Hatchability Data Collection

Hatchability: Total number of chicks hatched

Percentage Hatchability: This was taken as the total number of chicks hatched expressed as a percentage of total number of fertile eggs set.

$$\textit{Percentage Hatchability} = \frac{\textit{Total number of chicks hatched}}{\textit{Total number of fertil eggs set}} \textit{ x 100}$$

3.4.6 Experimental Design

Experimental design used was 2x5 factorial design. It consisted of 2 levels of treatments of eggs storage temperatures and 5 levels of treatments egg storage durations.

3.4.7 Statistical analysis

Fertility and hatchability data was expressed in percentages and the chick quality data was analysed using the Proc. Mixed Model procedure of SAS at $P < 0.05$ (SAS statistical Institute 2012). The statistical model used was defined as below

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij} \quad [1]$$

Where, Y_{ij} = response recorded on a measured parameter, μ Overall mean, α_i = main effect of storage duration, β_j main effect of egg storage temperature, $(\alpha\beta)_{ij}$ =interaction effect of egg storage duration and storage temperature and ε_{ij} = random error term.

Where significance was observed, the means were separated using the PDIFF procedure of SAS. For hatchability and fertility data, they were expressed in percentages and compared.

CHAPTER FOUR

4. 0 RESULTS AND DISCUSSION

4.1. EXPERIMENT ONE

4.1.1. The External Egg Quality Characteristics of Naked Neck Eggs

Egg storage weight was not significantly different between storage durations. (Table 4.1). In addition, egg weight after storage was not significantly different from the eggs stored under five different storage durations. Neither storage temperature also had little or no effect($P>0.05$) on egg weight before and after storage. Similar observations were found for the interaction between temperature and storage durations and interaction on storage duration (Table 4.1).

Length of storage significantly affected egg weight loss ($P< 0.0001$) with 10days storage duration, being affected much and 1day storage duration least. It could be observed that as storage duration increased, amount of water loss from eggs also increased (Table 4.1). This agrees with the work of Siyar *et al*. (2007). Where they reported significant difference in egg weight loss when eggs were stored for 7days and 14 days respectively. When length of storage increases, egg weight declines. The loss in weight could be attributed to loss of water, ammonia, carbon dioxide, nitrogen and hydrogen sulphide from the eggs (Dudusola, 2009; Alsobayel and Albadry, 2011; Jin *et al*., 2011). The results however show that the egg moisture loss was higher in eggs stored over a longer time compared to shorter durations (14days > 10days > 7days > 3days and 1day) ($P < 0.001$).

Storage temperature as well as interaction between storage duration and temperature had little or no influence (P>0.05) on egg moisture loss with eggs stored under ambient temperature losing a higher percentage of moisture. Eggs progressively lost weight irrespective of the temperature of storage (ambient or cold room treatment) and the length of storage. Eggs stored over longer period lost more moisture compared to eggs stored for short time duration. This is true because as storage duration increases evaporation of water from the eggs through the eggshell pores also increases (Fasenko *et al*., 2001; Hamidu *et al*., 2010, 2011). This research is in agreement with the results obtained by (Samli *et al*., 2005) and Hasan and Okur (2009) who observed a decrease in weight when eggs were stored for 10 days at temperature of 29ºC and18ºC. Yeasmin *et al.* (2014) also observed this trend of egg weight losses as storage duration increased. They added that water loss from eggs during storage is due to temperature of the environment and the storage duration.

Egg shell thickness was not significantly(P>0.05) influenced by storage duration, storage temperature and interaction between storage temperature and storage duration (Table 4.1). However, percentage wet shell weight of naked neck egg was significantly(P<0.01) affected but the egg percentage dry shell weight was not influenced (P>0.05) by storage duration, storage temperature or interaction. Wet shell weight seems to decrease significantly after 7days of storage. This is inline with the work of Samli *et al.* (2005) and Ihsan (2012). According to Samali *et al* (2005) shells are in direct contact with the external environment and an as results shell turns to lose more water when storage duration increased, hence a decrease in shell weight. Storage temperature and interaction between storage duration and storage temperature did not affect percentage wet shell weight and dry shell weight significantly (Table4.1).

Table 4.1. Effect of ambient and cold room storage temperature on external egg quality of naked neck eggs stored for five different storage durations

Storage duration	EWBS (g)	EWAS(g)	EWL (%)	ST (mm)	WSW (%)	DSW (%)
1day	56.78	56.52	0.42^{d}	0.039	8.89^{d}	8.53^{d}
3days	53.22	52.77	0.81^{d}	0.044	9.86^{b}	9.56^{b}
7days	54.26	53.52	1.35^{c}	0.052	10.38^{a}	10.10^{a}
10days	55.98	54.36	2.91^{a}	0.053	9.30^{c}	9.11^{c}
14days	56.52	54.91	2.83^{b}	0.049	8.99^{d}	8.78^{d}
SEM	1.176	1.1814	0.3446	0.0022	0.3234	0.3068
P-value	0.0642	0.0937	<.0001	0.0623	0.0044	0.0008
Storage temperature						
Ambient(23^{o}C – 31^{o}C)	55.39	54.36	1.82	0.046	9.32	9.04
Cold room (18^{o}C)	55.31	54.47	1.51	0.048	9.66	9.39
SEM	00.897	0.9116	0.1404	0.0013	0.2137	0.2259
P- value	0.9364	0.9063	0.0621	0.6150	0.2134	0.1409
Interaction						
Ambient x 1day	58.79	58.49	0.45	0.039	8.50	8.18
Ambient x 3days	52.32	51.86	0.83	0.039	9.57	9.19
Ambient x 7days	52.86	52.07	1.46	0.052	10.22	9.94
Ambient x 10days	56.62	54.82	3.19	0.055	9.28	9.09
Ambient x 14days	56.69	54.57	3.16	0.049	9.00	8.79
Coldroom x 1day	54.77	54.55	0.38	0.043	9.28	8.89
Coldroom x 3days	54.13	53.68	0.79	0.051	10.15	9.22
Coldroom x 7days	55.67	54.98	1.24	0.052	10.54	10.27
Coldroom x 10days	55.33	53.63	2.62	0.051	9.32	9.14
Coldroom x 14days	56.69	55.24	2.52	0.051	9.00	8.76
SEM	01.523	1.510	0.2698	0.0029	0.4344	0.4129
P- value	0.1391	0.1321	0.6536	0.1006	0.8662	0.7665

Superscripts $^{a-d}$ indicate significant differences among means in the same column ($p< 0.05$), SEM= Standard Error of Mean, EWBS=egg weight before storage, EWAS= egg weight after storage, EWL= egg weight loss, ST= shell thickness, WSW= wet shell weight, DSW= dry shell weight.

4.1.2 The Internal Egg Quality Characteristics of Naked Neck Egg

Blastoderm diameter increased significantly as storage duration increased($P<0.0001$) (Table 4.2). Blastoderm diameter increased linearly from 7days of storage to 14days. (7days<10<14). The 14days recorded highest blastoderm diameter and 1 - 3days recorded least diameter (Table 4.2). However, eggs stored for 1day, 3days and 7days were not significantly different from each other. The eggs stored for 10days and 14days were different. (Table 4.2). An increase in blastorderm diameter suggests embryonic development as storage duration increases. The embryonic development seems to be significant when storage duration exceeds a week. Van Schalkwyk *et al.* (1999) had also reported significant increase in blastoderm diameter after 7days of storage. Effect of storage temperature on blastoderm diameter was also significant($P<0.05$). Eggs stored under ambient temperature recorded higher blastoderm diameter(6.96mm) than eggs stored under coldroom temperature (5.41mm). It appears that blastoderm development during storage is also influenced by increasing temperature greatly.

Chickens embryos are robust and with the slightest of temperature above physiological zero they will initiate development (Edwards 1902).

Interaction on storage duration and storage temperature affected blastoderm diameter significantly ($P<0.0001$). Generally, it can be observed that blastoderm diameter increases irrespective of the storage temperature as long as storage duration increases (Table 4.2). However, eggs stored under ambient temperature had higher blastoderm diameter than eggs stored in the

coldroom temperature as observed ealier. Under the two temperature conditions, an increase in blastoderm diameter seems to be significant after 7days of storage. This could contribute to early embryonic mortality when eggs are incubated. This is because when eggs are stored in a temperature closer to the incubation temperature (ambient temperature), embryonic development occurs and sudden change in the temperature surrounding the developing embryo as eggs are exposed to incubation temperature could result in early embryonic mortality. The asymmetrical shape of the blastoderm changes and so is the yolk which become mottled (Hamidu *et al*., 2010, 2011; Bakst, 2003). This is because a developing embryo becomes used to its surrounding storage temperature which had aided its development and when such temperature condition is changed the embryo is affected negatively. Similar work carried out with ostrich eggs showed that eggs stored at 17°C which is within recommended storage temperatures resulted in lower embryonic mortality (26.7%) compared to 25°C (44.8%). The mortality in the late period of incubation were particularly higher in both cases (21.1% vs. 42.7%) (Van schalkwyk *et al*., 1999). There is also death o cells resulting in small embryo sizes (Hamidu *et al*., 2011). The results are consistent with the work of van Schalkwyk *et al.* (1999) and Malechi *et al.* (2005) who also observed a significant increase in blastoderm size when storage temperature and storage duration was increased.

Percentage albumen weight significantly (P=0.0302) decreased as storage duration increased. Albumen weight decreased from 1day storage duration to 10days storage duration. There was no significant increase in albumen weight for eggs stored for 1 – 3days and 10days - 14days respectively. However,10 - 14 days stored eggs were significantly higher than the rest of storage durations in albumen weight. A decrease in albumen weight may be due to the movement of albumen fluid into the yolk as storage duration advances and as a result, causing an increase in yolk weight. Silversides and Scott (2001), and Jin *et al*. (2011) also reported a significant decrease

in albumen and percentage albumen weight as storage duration increases. Storage temperature did not affect percentage albumen weight significantly(P>)0.05) but interaction on different storage duration and different storage temperature significantly(P<0.05) influenced percentage albumen weight. Percentage albumen weight significantly decreased after 3days of storage and tends to increase after 10days of storage under the two temperature conditions. Though eggs stored for 1day recorded highest albumen weight in the two temperature conditions, they were not significantly different from eggs stored for 14days. It appears that the albumen tends to regain the water from the yolk when storage duration exceeds 10days, whether the eggs were stored in a coldroom or under ambient temperature (Table 4.2). Yeasmin *et al.* (2014), found higher percentage albumen weight loss in eggs stored under room temperature and suggested that loss in albumen weight was as a result of water loss into the yolk (Tona *et al.*, 2004 and Akyurek, 2009). Raji *et al*., 2009; Tebesi *et al*. 2012; Gavril and Usturoi, 2012), further explained that when eggs were stored under a cold temperature, it reduces loss of carbon dioxide and retards the conversion carbonic acid to carbon. This maintains the mucin fiber in the albumen and yolk thereby maintaining the gel- like texture in the albumen and yolk.

From table 4.2, different storage duration did not significantly (P>0.05) affect percentage wet yolk weight but affected (P<0.01) percentage dry yolk weight significantly. Percentage dry yolk weight increased from 1day storage duration to 7days storage and took a downwards turn. According to Hamidu *et al*. (2010) as the embryo develops, it begins to utilize yolk as a feed source, by so doing weakening its membrane and causing cell‟s death as storage duration exceed 7days. The dry yolk weight reveals the significant changes that occur in the yolk when storage duration increases. The significant increase in yolk weight may be due to an increase in cell mass of the embryo. Although there appear to be relationship between yolk weight and albumen weight due

to the movement of moisture. It is not clear why the dry weight of the yolk was higher in 7days and 14days. Similar reports have been reported by Hagan *et al*. (2013), Ahn *et al*. (1997), Scott and Silversides (2000), Moula *et al*. (2009, Mahmoud *et al.* (2010), and Jin *et al* (2011). The storage temperature and interaction on storage temperature and storage duration did not influence (P>0.05) percentage yolk weight significantly (Table 4.2).

Table 4.2. The effect of ambient and coldroom storage temperature on internal egg quality for eggs stored for five different durations

Storage duration	**BD (mm)**	**AW(%)**	**WYW(%)**	**DYW(%)**
1day	4.40^{c}	64.06^{a}	52.10	15.20^{d}
3days	4.16^{c}	63.7^{a}	55.71	17.16^{d}
7days	4.66^{c}	63.36^{a}	56.12	20.04^{a}
10days	6.92^{b}	58.57^{b}	56.73	19.38^{b}
14days	10.79^{a}	59.28^{b}	54.49	19.04^{c}
SEM	0.2851	1.6147	1.3989	0.7885
P- value	<.0001	0.0302	0.1194	0.0003
Storage temperature				
Ambient (23- 31^{o}C)	6.96^{a}	59.99	55.45	18.44
Coldroom (18^{o}C)	5.41^{b}	61.97	54.61	17.89
SEM	0.935	1.0869	0.9269	0.5016
P- value	<.0001	0.2573	0.4849	0.4391
Interaction				
Ambient x 1day	4.17^{a}	64.06^{a}	50.22	14.63
Ambient x 3days	3.81^{a}	62.48^{a}	56.58	16.46
Ambient x 7days	4.61^{a}	58.31^{b}	57.06	21.38
Ambient x 10days	8.02^{b}	56.76^{c}	58.87	19.56
Ambient x 14days	14.19^{c}	58.31^{b}	54.50	20.16
Coldroom x 1day	4.63^{a}	64.06^{a}	53.97	15.78
Coldroom x 3days	4.51^{a}	64.91^{a}	54.84	17.85
Coldroom x 7days	4.71^{a}	63.12^{b}	55.17	18.69
Coldroom x 10days	5.81^{b}	60.2^{b}	54.59	19.21
Coldroom x 14days	7.38^{c}	60.25^{a}	54.48	17.92
SEM	0.3929	2.1395	1.9538	1.0143

P – value	<.0001	0.0288	0.2892	0.2415

Superscript [a-d] different letters indicate significant (P<0.05) differences among means in the same column. SEM =Standard Error of Mean, BD=blastoderm diameter, AW= albumen weight, WYW= wet yolk weight DYW = dry yolk weight

4.2 EXPERIMENT TWO

4.2.1 Fertility and Hatchability

4.2.1.1 Fertility and hatchability of eggs stored under ambient temperature

The percentage fertility was very low after 7 days of storage in the ambient temperature (Table 4.3). It is more likely that this loss of fertility to from 97% 23% at 10 days and 0% in 14day storage was due to increasing number of blastodermal deaths rather than actual infertility problems. We observed in the study that as the storage duration increased the yolk became pale and the blastoderm losing its symmetrical shape. In addition, the region of yolk from the blastoderm position appeared to be filled with water bubbles and had increased concentric dark around the balstoderms and continued outward (Plate 3.1c). According to Brake *et al*. (1993) early embryonic mortality in incubated eggs is normally due to long storage of fertile eggs. This is because when eggs are stored for long period of time, dehydration in the embryos occur. These eggs show no blood vessel during candling and are normally considered infertile although it might have been fertile (Brake *et al.*, 1993). As a result of the infertility observed, hatchability was zero in both 10 days and 14 days‟ storage treatment. The lower hatchability in eggs stored for 1 day compared to 4 and 7 days could result from many factors including smaller than normal air space to facilitate hatching, increased number of open blastodermal cells that leads to increased early embryonic mortality (Hamidu *et al*. 2010) and higher acidity because amount of CO_2 expected to move out

has not happened (Gavril and Usturoi, 2012). Therefore, hatchability tends to be low in eggs incubated fresh or at 1 day old.

Eggs stored for 3days recorded the highest percentage hatchability and eggs stored for 10 to 14days recorded no hatch egg. This is in agreement with Schmidt et al. (2009), Egbeyale *et al*. (2013) and Kirk *et al*. (1980) who recorded highest value for hatchability percentage for eggs stored for a period of 3days while the lowest hatchability value was recorded in eggs stored for a period of 12days. According to Scott and Silversides (2000), Romao *et al*. (2008) and Schmidt *et al*. (2009) long storage period could be detrimental to hatching egg quality and can also reduce hatchability.

Table 4.3: Effect of egg storage duration on fertility and hatchability of naked neck chickens' egg under ambient temperature

Parameters	Period of storage (days)				
	1	3	7	10	14
Fertility (%)	90.33	93.33	96.67	23.33	0
Hatchability (%)	64.29	85.18	84.61	0	0

Data was only expressed in percentages but was not analysed due to absence of replicates

4.2.1.2 Fertility and Hatchability of eggs stored under Coldroom Temperature

The fertility varied between storage durations but could not be dependent on storage periods since these were based on the total number of viable eggs that were incubated and determined at candling. From Table 4.4, it can be seen that when eggs are stored under coldroom temperature, the fertility is not much affected by increasing storage duration. The percentage hatchability also decreased when eggs were stored beyond 7days (Table 4.4). This suggest that

hatchability declines when storage duration is beyond 7days, irrespective of the storage temperature. However, eggs stored under ambient temperature are much more affected. According to Horbanczuk (2000), suitable storage temperature for eggs that are to be stored for a period not beyond a week (7days) may range from 12 to 18^{o}C.

Table 4.4: Effect of egg storage duration on fertility and hatchability of naked neck chickens" egg under coldroom temperature storage

	Period of storage (days)				
	1	3	7	10	14
Parameters					
Fertility (%)	93.33	100	100	86.67	80
Hatchability (%)	80.00	80.77	89.29	76.67	66.67

Data was only expressed in percentages but was not analysed due to absence of replicates

4.2.2 Chick Quality Characteristics of Naked Neck

There were no significant($P>0.05$) difference in chick weight for different storage durations however; different storage temperature conditions affected chick weight significantly ($P<0.01$). Eggs stored under coldroom temperature had significant higher chick weight than eggs stored under ambient temperature, (Table 4.3). Similar result was reported by Ruiz and Lunam (2002). They also observed significant difference in chick weight at hatch when eggs were stored under temperature of 10^{o}C and 16.5^{o}C with 10^{o}C recording higher chick weight. The interaction of storage duration and storage temperature also affected ($P<0.01$) chick weight significantly. In egg stored under ambient temperature, it was observed that as storage duration increased chick weight

decreased compared to eggs stored under coldroom chick weight was highest in 1day and 14days storage treatments. From Table 4.3, it can be concluded that not only high storage temperature (ambient) which reduces chick weight but the combined effect of storage duration and increased storage temperature can impact chick weight. Fasenko (2009), reported significant reduction in chick weight as storage duration and storage temperature increased.

Chick length was not affected($P>0.05$) by storage duration and storage temperature. Interaction on storage duration and storage temperature did not also had significant($P>0.05$) effect on chick length.

Chick shank length was significantly ($P<0.01$) affected by different storage durations and different storage temperature. As storage duration increased chick shank length decreased. Egg stored for one day had significant long chick shank length than the rest of the storage periods. Chick shank length from eggs stored for 3days, 7days, 10days and 14days was not significantly($P>0.05$) different from each other. This agrees with the work of Servet and Paul (2003) who also recorded significant changes in shank length in different storage durations.
Chick shank length decreased as storage duration increased. Storage temperature also affected chick shank length significantly. Eggs stored under coldroom temperature had significant higher chick shank length than those stored under ambient temperature. This suggest that when eggs are stored in a coldroom temperature chicks develop better when in ambient temperature and result in higher chick quality. The interaction between storage temperature and storage duration on chick shank length was also significantly ($P<0.01$) different. Chick shank length seems to decrease as storage duration increases, irrespective of the storage temperature. However, this is more obvious for eggs stored under cold room temperature (Table 4.3). The data for days 10 and 14 were missing

at ambient temperature because there were no single hatch of chicks following storage and incubation.

Table 4.5: Effect of ambient and coldroom storage temperature on chick quality for eggs stored under five different durations

Storage duration	**CW(g)**	**CL(cm)**	**CSL(cm)**
1day	35.21	15.74	1.66[a]
3days	36.47	15.34	1.60[b]
7days	35.3	15.99	1.61[b]
10days	35.32	15.43	1.59[b]
14days	36.59	15.34	1.56[b]
SEM	0.4798	0.249	0.0136
P- value	0.0825	0.3724	<.0001
Storage temperature			
Ambient (23-31^{o}C)	35.01[b]	15.81	1.59[a]
Coldroom(18^{o}C)	36.13[a]	15.5	1.63[b]
SEM	0.3228	0.1670	0.0086
P- value	0.0086	0.3106	0.0001
Interaction			
Ambient x 1day	33.1[c]	16.01	1.59[a]
Ambient x 3days	37.06[a]	15.02	1.58[a]
Ambient x 7days	34.44[b]	16.39	1.6[a]
Ambient x 10days	-	-	-
Ambient x 14days	-	-	-
Coldroom x 1day	36.79[a]	15.46	1.72[a]
Coldroom x 3days	35.93[b]	15.64	1.62[b]
Coldroom x 7days	36.19[b]	15.58	1.61[c]
Coldroom x 10days	35.32[b]	15.430	1.59[d]
Coldroom x 14days	36.59[a]	15.36	1.56[e]
SEM	0.6625	0.3563	0.0202
P – value	0.0013	0.0680	0.0062

Superscript [a-d] indicate significant (P< 0.05) differences among means in the same column. SEM= Standard Error of Mean, CW= chick weight, CL= chick length, CSL= chick shank length

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This research was undertaken to find out the impact of different storage duration and storage temperature on egg quality, fertility, hatchability and chick quality. There following conclusions were drawn after the study:

1. The study revealed that when eggs are stored beyond 7days, egg weight and shell weight decrease.
2. Storage temperature and interaction between storage duration and storage temperature have no significant effect on external egg quality.
3. Internal egg quality was significantly affected when storage duration increased. Blastoderm diameter and dry yolk weight increased whiles percentage albumen weight decreased with increasing storage duration.
4. Among the internal egg quality characteristics measured, only the blastoderm diameter was affected by storage temperature.
5. Blastoderm diameter and percentage albumen weight were significantly affected by interaction between storage duration and storage temperature. Blastoderm diameter increased with increasing storage duration while percentage albumen weight decreased

with increasing storage durations especially when the eggs are stored under ambient temperature.

6. Percentage fertility and hatchability decreased drastically after 7days of storage when eggs are stored under ambient temperature.
7. Chick shank length was the only chick quality parameters influenced($P<0.05$) by storage duration and interaction. Chick shank length decreased when storage duration increased irrespective of the storage temperature.
8. Storage temperature affected chick weight and chick shank length significantly. Eggs stored under coldroom temperature had higher chick weight and chick shank length than eggs stored under ambient temperature.

5.2 Recommendation

From the result obtained the following recommendation can be made:

1. Naked neck eggs should not be stored for more than a week for this could detrimental to the internal egg quality. For maximum hatchability, eggs should be stored under coldroom($18^{o}C$) temperature conditions and should not be stored beyond 7days.
2. Further research should be carried out to include the effect of turning frequency, temperature of incubation after 18 days, egg shell conductivity and the physiology of the embryo during hatching on naked neck eggs.

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APPENDIX

ANOVA FOR EGG WEIGHT BEFORE STORAGE

Effect	Num DF	Den DF	F Value	Pr > F
Duration	4	70.5	2.33	0.0642
Temperature	1	70.6	0.01	0.9364
Duration*Temperature	4	70	1.80	0.1391

ANOVA FOR EGG WEIGT AFTER STORAGE

Effect	Num DF	Den DF	F Value	Pr > F
Duration	4	70.3	2.07	0.0937
Temperature	1	70.4	0.01	0.9063
Duration*Temperature	4	69.9	1.83	0.1321

ANOVA FOR EGG WEIGHT LOSS

Effect	Num DF	Den DF	F Value	Pr > F
Duration	4	247	79.24	<.0001
Temperature	1	247	1.78	0.1832
Duration*Temperature	4	247	2.70	0.0312

ANOVA FOR EGG SHELL THICKNESS

Effect	Num DF	Den DF	F Value	Pr > F
Duration	4	79	7.57	<.0001
Temperature	1	79	0.68	0.4124
Duration*Temperature	4	79	1.91	0.1163

Effect **F**

ANOVA FOR WET SHELL WEIGHT

	Num DF	Den DF	F Value	Pr >
Duration	4	71.3	4.16	0.0044
Temperature	1	71.5	1.58	0.2134
Duration*Temperature	4	70.7	0.32	0.8662

ANOVA FOR RY SHELL WEIGHT

Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Duration	4	70.9	5.33	0.0008
Temperature	1	71	2.22	0.1409
Duration*Temperature	4	70.4	0.46	0.7665

ANOVA FOR BLASTODERM DIAMETER

Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Duration	4	36	107.23	<.0001
Temperature	1	36	41.14	<.0001
Duration*Temperature	4	36	34.14	<.0001

ANOVA FOR PERCENTAGE ALBUMEN WEIGHT

Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Duration	4	70.2	2.24	0.0302
Temperature	1	71.5	0.14	0.2573

Effect			F
Duration*Temperature	4	70	1.14 0.0288

ANOVA FOR PERCENTAGE WET YOLK WEIGHT

	Num DF	Den DF	F Value Pr >
Duration	4	70.1	1.90 0.1194
Temperature	1	71.4	0.49 0.4849
Duration*Temperature	4	69.8	1.27 0.2892

ANOVA FOR PERCENTAGE DRY YOLK WEIGHT

Effect	Num DF	Den DF	F Value Pr > F
Duration	4	71	6.11 0.0003
Temperature	1	71.2	0.61 0.4391
Duration*Temperature	4	70.3	1.40 0.2415

ANOVA FOR CHICK WEIGHT

Effect	Num DF	Den DF	F Value Pr > F
Duration	4	144	2.11 0.0825
Temperature	1	143	7.10 0.0086
Duration*Temperature	2	143	6.93 0.0013

Effect **F**

ANOVA FOR CHICK LENGTH

Effect	Num DF	Den DF	F Value	Pr > F
Duration	4	143	1.07	0.3724
Temperature	1	142	1.04	0.3106
Duration*Temperature	2	142	2.74	0.0680

ANOVA FOR CHICK SHANK LENGTH

	Num DF	Den DF	F Value	Pr >
Duration	4	163	6.87	<.0001
Temperature	1	163	15.80	0.0001
Duration*Temperature	2	163	5.25	0.0062

ANOVA FOR PERCENTAGE FERTILITY

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Duration	4	4571.102260	1142.775565	1.35	0.3894
Temperature	1	2777.888890	2777.888890	3.28	0.1444

ANOVA FOR PERCENTAGE HATCHABILITY

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Duration	4	4555.752460	1138.938115	2.09	0.2457

Effect				F	
Temperature	1	2240.110890	2240.110890	4.12	0.1122