OPTIMUM INCUBATION TEMPERATURE DETERMINATION AND ALTERATION TO ENHANCE SEX REVERSAL, HATCHABILITY AND POST-HATCH PERFORMANCE OF JAPANESE QUAILS (*Coturnix coturnix japonica*)

BY

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CERTIFICATION

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DEDICATION

I dedicate this research work to Almighty God for His grace, will, mercy, protection, guidance, faithfulness and love for me and my family members.

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ABSTRACT

Japanese quails in domestication lack broody tendency hence adoption of artificial incubation for commercial production. Inappropriate Incubation Temperature-IT affects hatchability and sex determination in quails. Conventional IT and sexing techniques used for other poultry species cause low hatchability, survivability and inaccurate sexing. Information on optimum IT and *in ovo* sex predetermination in quails is inadequate. Therefore, optimum IT determination and effects of its alteration on sex reversal, hatchability and post-hatch performance of Japanese quails were assessed.

Clutch of 1,605 eggs were randomly allotted to incubators at 36°C (T_1), 37°C (T_2), 38°C (T_3), 39°C (T_4) and 40°C (T_5) for optimum IT determination. At hatch, incubation period and hatchability were recorded. Chicks were reared for six weeks, feed intake and survival rate were recorded. Six chicks/treatment were randomly selected and sacrificed to obtain carcass weight. Another clutch of 1,260 eggs were incubated at 38°C (T_A, Control) without pausing, 36°C (T_B), 37°C (T_C), 38°C (T_D), 39°C (T_E) and 40°C (T_F) paused for five hours on days 3, 4 and 5. Also, 612 eggs were set at 38°C (T_I, Control) without pausing, 36°C (T_{II}), 37°C (T_{III}), 38°C (T_{IV}), 39°C (T_V) and 40°C (T_{VI}) paused for five hours on days 11, 12 and 13. At hatch, hatchability was recorded. At week three, the chicks were sexed based on plumage pattern and reared separately. At week six, survival rate was determined, six birds per treatment were sacrificed and testes/ovaries were observed for sex confirmation. Non-conformity with the male/female plumage pattern was considered reversed sex. Data were subjected to descriptive statistics and ANOVA at $\alpha_{0.05}$. Hatching commenced on day 16 (T_4), 17 (T_3), 18 (T_2, T_5) and 20 (T_1). Hatchability was 36.9±13.8% (T_2), 36.6±9.8% (T_4), 35.9±28.7% (T_3), 30.6±19.9% (T_5) and 23.9±26.6% (T_1). Feed intake varied significantly and ranged from 10.2±5.29g (T_2) to 19.3±0.19g (T_1). Survival rate was significantly higher in T_5 (66.5±6.4%) than in T_4 (46.1±10.0%), T_2 (27.4±1.0%), T_3 (21.5±3.7%) and T_1 (14.5±0.0%). Carcass weight was significantly higher in T_1 (97.4±0.1g) than in T_3 (80.0±14.2g), T_5 (77.1±16.9g), T_4 (74.3±9.58) and T_2 (68.1±11.4g). Hatchability was higher in T_A (81.0±50.9%) than in T_C (46.0±17.7%), T_B (43.1±14.0%), T_E (12.5±9.2% and T_D (1.5±1.8%), while hatchability failure was recorded in T_F. Survival rate was similar in T_A (49.2±42.7%), T_C (38.9±1.6%) and T_B (22.2±14.1%), while none survived in T_D and T_E. Male/female ratio was 40:37 (T_A), 10:9 (T_B) and 10:11 (T_C) without sex reversal. Hatchability was 81.2±14.7%, 70.9±52.3%,

68.0±18.9% and 62.7±6.6% in T_I, T_{IV}, T_{III} and $T_{II,}$ respectively, while both T_V and T_{VI} had hatchability failure. Survival rate was similar in T_I (59.2±17.7%), T_{III} (55.9±5.2%) and T_{IV} (54.4±18.4%) but higher than 18.7±5.6% (T_{II}). Male/female ratio was 7:12 (T_I), 7:4 (T_{II}), 10:13 (T_{III}) and 0:10 (T_{IV}) without reversed sex.

Low temperature (36°C) prolonged incubation period, enhanced feed intake and carcass weight, while high temperature (40°C) enhanced survivability. However, 39°C at which hatching commenced on day 16 may be recommended as optimum incubation temperature for Japanese quails. Paused incubation temperature did not elicit sex reversal and caused hatchability failure.

Keywords: Japanese quails, Chick embryo, Chick survivability, Incubation temperature

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CHAPTER ONE
INTRODUCTION

1.1 Background of the study

Animal protein intake should not be less than 50g per caput in developing nations, but in Nigeria, only about 6–10g per caput is consumed (FAO, 2009). This could be one of the causes of malnutrition, stunted growth, early childhood death as well asthe low life expectancy of55years compared to75 years in the United States of America. This inadequacy of animal protein intake in the diets of most Nigerians is becoming more worrisome, as it poses a threat to national development because the wealth of a nation lies in the health of the citizenry. According to FAO (2013),malnutrition and undernutrition in sub-Saharan Africa and South Asia, were closely associated with poverty that resulted in depressed immunological status and high HIV/AIDS epidemic in the areas.

Inorder to ameliorate this problem, poultry production may be a better option because FAO (2013) reported that poultry products were the best sources of high quality proteins, vitamins and minerals needed by many people who live in abject poverty. It was emphasized that poultry meat is the healthiest and cheapest of all livestock meats with no major taboos on the consumption. Poultry production requires less space, water, starter-pack and has little or no detrimental effects on the environment yet, it provides employment and the products are widely acceptable, relatively inexpensive and available. Increased commercial poultry production becomes imperative,in order to boost the livestock subsector's contributions to national and economic growth, particularly in a developing nation like Nigeria. Poultry species include fowl, ducks, guinea fowl, geese and turkey. Others are pheasants, swan, ostriches, emu, pigeons and quails. Among these species,the Japanese quail has been singled out due to its short generation interval with the ability of the hen to start laying at about 40 days old, capability of producing about 250 – 320 eggs per year, while the cock matures at about 30 to 60 days old (Deveau, 2009). With these economic traits, Woodard*et al.* (1973) stated thatthree to fourgenerations

annually were possible in Japanese quail.Other attributes include requirements of little starter-pack, land area, labour and medical attention as well as high resistance to diseases. Essentially, Japanese quail has been reported to be a veritable source of nutrients (Randall, 2008) and the products especially egg has been speculated to be rich in minerals and vitamins but low in cholesterol (Fah, 2009). Hence, Japanese quail production is likely feasible in making the meat and eggs available at an affordable rate. Therefore, the best alternative to provide adequate animal protein all year round could be the large scaleproduction of the Japanese quail under an improved management system. More significantly, amongst all the avian species, Japanese quail is the most preferred research or laboratory birdin avian study(Perry, 1988; Naito *et al.*, 1990; Ono *et al.*, 1994a; Nakane and Tsudzuki, 1999; Ainsworth*et al*., 2010).According to Nakane and Tsudzuki (1999), whenavian embryos are needed in research, Japanese quail is always the option because of its superiority to fowl. Also, it was stressed that Japanese quail is more prolific, precocious and smaller in body size than the domestic fowl hence it will support efficient performance in experimental conditions.

Japanese quailhens in captivity hardly brood and incubate theireggs. Therefore, artificial incubators are used to simulate and mimic the role of a broody hen, in providing optimum environmental conditions that will stimulate embryonic development and maintain the growth until hatching (Wilson, 1991; French, 1997; Hill *et al.*, 2001; Lourens *et al.*, 2005). Such artificial incubators vary in sizes, materials used and the settings or arrangements within the incubators have resulted in manipulating the environmental conditions. Based on this, incubation temperatures of 37.2 to 37.8^{o}C (Wilson, 1999; Lourens *et al.*, 2007; Pam, 2015), as well as 38.9^{o}C to 39.4^{o}C (Musa *et al*., 2007; Mani *et al.*, 2008; ECQ, 2015)have been reported. These disparities may have resulted in hatchery failures, low chick yield, late hatching that often lead to high chick mortality rate and poor post-hatch chick performance. Consequently,a near constant incubation temperature with little or novariability should be maintained for optimum hatchability. Incubation temperature directly influenced hatchability, post-hatchhealth and performance in broiler chickens (Elmehdawi, 2013), Australian brush-turkeys (Göth and Booth, 2005), domestic fowls (Boerjan, 2002) and turkeys (Krischek *et al*., 2013). In each case, sex reversal was speculated and there is paucity of information on the influence of temperature on the

hatchlings' survival and reproductive potential of the sex-reversed chicks or poults were not provided. Meanwhile, Hamburger and Hamilton (1951) developed an atlas showing normal stages of fowl chick embryonic development and if such is developed in Japanese quail, *in ovo* studies may be eased up.

1.2 General objective of the study

The aim of this study was to know the required optimum incubation temperature for desirable egg hatchability, chick survivability and the effects of manipulating incubation temperature on sex reversal and post-hatch performance in Japanese quails.

1.2.1 Specific objectives of the study

The specific objectives were to:

1. Determine optimum incubation temperature in Japanese quails production.
2. Monitorthe embryonic development in Japanese quail eggs at varying incubation temperature.
3. Examine the role of temperature manipulation during egg incubation on Japanese quails sex reversal.
4. Evaluatepost-hatch performance of Japanese quails subjected to different incubation temperature regimes.

1.3 Justification of the study

The inability of Japanese quail hens to incubate their eggs as well as low chick yield, hatchability and chick survivability often recorded even when artificial incubators are used,warrants a determination of optimum artificial incubation temperature in order to boost productivity. Knowledge of the Japanese quail embryonic development will provide a clue to the stage of embryo death during residual egg breakout for better egg fertility determination, *in ovo*research, pharmacological operations and introduction of gene expression vectors into specific cell populations. Several undesired growers are often culled, following sex determination at about 3 to 6 weeks old in Japanese quail hence, the need to predetermine only the desired sex during eggs incubation. Various hormonal and physical manipulations can somewhat result in sex reversal, but no reliable method has been found in poultry production.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin of Japanese quails

Quails are members of *Phasianoidea*; Order *Galliformes*; Class *Aves* and Animal Kingdom just like fowls, pheasants and partridges (Fah, 2009). Species or subspecies of the Genus *Coturnix* are found in all the continents except the Americas. One of them *Coturnix coturnix* or common quail is a migratory bird in Asia, Africa and Europe. It was reported that many inter-crossing subspecies were known but the European quail (*Coturnix coturnix coturnix*) and Japanese quail (*Coturnix coturnix japonica*) are more important. Howes (1964) reported that one subspecies that regularly migratedfrom Europe to Asia was finally domesticated in China, where they were reared as companions and melodicanimals. The domesticated *Coturnix*wastaken to Japan across Korean bridge at about the eleventh century. It was emphasized that *Coturnix* was originallytamed in the Orient (Eastern part of the world: China and Japan) and not in the Middle East as being speculated (Fah, 2009). Also, it was stated that European *Coturnix*roaming toward thesouth inFall Season, through Mediterranean Sea, were in their tiredsituation caught and tamed. Egyptian and Biblical records of domesticated quails in Japan was in the 12^{th}century, wherethey were originallyreared for singing.

It was speculated that anEmperor in Japan was cured of tuberculosis whenhe ate quail meat, hencethe careful selection of quails for meat and egg production in Japan,during latter part of 19^{th}century. It was reported that in 1910,Japanesequails were extensivelyreared for meat and eggs.In 1941, the number of quailsimprovedswiftly in Japan particularly in Tokyo, Mishima, Nagoya, Gifu as well as Toyohashi. It was given that this era,signified a time of magnificentgrowth in Japanese quail domesticationin Korea, China, Taiwan and Hong Kong which advanced to Southeast Asia. Japanese quailwas called *Coturnix coturnix japonica* as well as Common quail, Eastern quail, Asiatic quail, Stubble quail or Pharaoh's quail. Other names were Red throat quail,

Japanese grey quail, Japanese migratory quail, King quail and Japanese King quail (Howes, 1964; Fah,2009). Meanwhile, the correct commonclassification of*Coturnix coturnixjaponica*(Temminck & Schlegel, 1849 as provided in Indreswari *et al*., 2019)was Japanese quail or*Coturnix*and not*Coturnix*quail because the Latin word "*Coturnix"*couldmean"quail"when interpreted.

2.2 History of Japanese quails in Nigeria

According to Haruna *et al*. (1997), Japanese quail (*Coturnix coturnixjaponica*) was brought into Nigeria in 1992,through the Directorate of Food and Rural Infrastructure. At the moment, Japanese quail eggs and day-old chicks could be obtained from many farms within Nigeria but are better obtained from the Poultry Division, National Veterinary Research Institute, Vom Plateau State. Japanese quails are reared in most farms in Nigeria, just like other domesticated avian species, where either the litter or battery cage system is adopted (Mohammed and Ejiofor, 2015). The same routine management practices provided in other poultry species production should also be observed to the latter when rearing Japanese quail. Since thedomestication of Japanese quail in Nigeria, a lot of research findings using it as a laboratory bird have been reported (Sati *et al*., 2012; Amosun, 2014; Ojo, 2014; Adeyina *et al*., 2015; Makinde *et al*., 2015).

2.2.1 Performance of Japanese quails in Nigeria

Japanese quail performance characteristics are basically feed intake, weight gain, feed conversion ratio, protein efficiency ratio and age at first egg drop. Others are egg weight, length and width as well as carcass quality evaluation. Meanwhile, it was speculated that performance characteristics in poultry production could be divided into growth and production performance considering growth and output indices.However, genetic quality could be a major factor that influences the performance of Japanese quail. This is because thegenetic characteristics of the parentstock, will definitely determine the proficiency of the offspring. Such genetic characteristics include small body, small egg, growth rate or rate of weight gain and age at sexual maturity. Other factors that could affect performance in Japanese quail are production system, production cycle, stocking density, environmental conditions and nutritional plane (Edache *et al*., 2003; Babangida and Ubosi, 2006; Tuleun *et al*., 2013).

2.3 Japanese quail eggs

Fah (2009) stated that quail eggs are categorized by variation of colour forms ranging from snow white to absolutely brown. It was however stressed that the tan and dark brown speckled or mottled brown with a chalky blue layeris more common in Japanese quail.It was also stated that the normal egg from fully grown hen, weighs 10g on average (i.e. about 8% of its body weight unlike 3% in fowl). Japanese quail egg contains 158 calories, 74.6% water, 13.1% protein, 11.2% fat and 1.1% total ash. The mineral content was shown to be 0.59mg of calcium, 220mg of phosphorus and 3.8mg of iron. While the vitamin content was given as 300I.U of vitamin A, 0.12mg of vitamin B1, 0.85mg of vitamin B2 and 0.1mg nicotinic acid (Fah, 2009).

2.4 Japanese quail chicks

YoungJapanese quail chick has yellowwith brown stripes outlookthatsomewhat resembles young turkey if not forbody size (Fah, 2009). Japanese quailchick at day old weighsbetween6 and 7g yet,grows very quicklyin the first few days of life. At 72 hours old, flight plumagesare grown and complete feathers develop at 30 days old (Fah, 2009). At 21 days old, the sex could be identifiedpartiallyusing breast plumage pattern. In some cases however,certain birds may notexhibit this features even when fully grown thus, making this sex identification technique seemingly difficult (Cheng *et al.*, 1985).

2.5 Male Japanese quail

When fully grown, the rooster (maleJapanese quail)weighsbetween 100 and 140g and it could be known by the rusty-brown plumages at the neck and lower breast regions (Fah, 2009). In addition, the rooster has a cloacal-gland (a bulbous structure) found at the upper edge of the vent,whichdischarges a white-foamy secretions. This distinctive gland could be used in assessing the procreativecapability of the rooster (Cheng *et al*., 1985). At about 42 days of age,the rooster crows and Sanford (1957) defined the vocal soundlike a loud,castanet-like crow makinga sound as *pick-pera-wick* or *ko-turro-neex* and at the peak of normal reproductive cycle, the rooster crowseven at night.

2.6 Female Japanese quail

Fully grown Japanese quail henweighs between 120 and 160g,which make ita littleweightier than the rooster. According to Krishnan (2019), Japanese quail hens are heavier, grow faster and bigger than the rooster. The body colouration is comparable to

that of the roosterwith the exception ofthe plumage pattern at the neck and upper breast regions whichare longer, pointy and greatlybrighter cinnamon colour(Cheng *et al.*, 1985; Fah, 2009).

2.7 Uses of Japanese quail

2.7.1 Food

Japanese quail eggs could be utilised in a similarway as fowleggs, just that Japanese quail egg is so small that fivepieces are equivalent to one fowl egg (Fah, 2009). With respect to the smallish egg nature and their beautifullook, Japanese quail eggs are usually utilised in differentways. For instance, Japanese quailegg could be used whole or sliced in salads, casseroles or served boiled with a sauce (Fah, 2009).

Pickled Japanese quail eggs are special delicaciesprepared by placing the eggs in hot water and boiled for five minutes andstir occasionally. Then,the egg is drained, washed and put in commercial white vinegar overnight. Latter, the eggs arecleaned and the eggshell remnants, as well as the membrane are removed by hand. The eggs are packaged loosely in jars half-filled with a solution of vinegar, water with 75g salt per litre. Other flavouring ingredients may be added, then boil, cover the container and store at ambient temperature prior to consumption (Fah, 2009). Quail heart, liver, gizzard, testis and carcass are delicacies when fried or roasted or cooked in stew or fricassee for minimum yield. On average, quail dressed carcass could weigh as much as 125g (Fah, 2009).

2.7.2 Experimental/research birds

It has been established that because Japanese quail can reproduce up to 4 generations annually, it becomes an option for laboratory experimentation (Padgett and Ivey, 1959; Howes and Ivey, 1961; Wilson *et al*., 1961; Reese and Reese, 1962; Woodard *et al.,*1973). It was stated that if the day length is favourable, Japanese quail hens could commence laying at 5 weeks old (approximately 40 days) and would be at peak of layat 7 weeks old (Fah, 2009). Under favourable environmental conditions, Japanese quail can lay eggs for a very long period of time, producing approximately 250 eggs per year. More significantly, Japanese quail issomewhatcheap to keep and about 8 to 10 could live inequalland area meant for one fowl.

According to Woodard *et al.* (1973), the Department of Poultry, University of California, USA, incubated and hatched many Japanese quail eggs in 1957. Since then, several of

them have been conserved for experimental purposes.It was stressed that *Coturnix*couldcomparefavourably well with chickens and turkeys in some physiological featureshence,Japanese quail seems to be a respectedoptionin avian research (Reese and Reese, 1962; Woodard *et al.*1973). Earlier, Wilson *et al*. (1960) stated that the use of pilot animals in avianstudy was not a new idea, because research works in avian species were always limited bythe financial plan, timeand space.Thus, it wasopined that some of these problems may be eased up, if Japanese quail (*Coturnix coturnix japonica*) was used as a pilot animal in place of the more expensive chickens or turkeys experiments. Avian developmental biology experiment was reported to be a veritable tool for the study of embryogenesis in air space, to provide the support hardware needed for researchers to better understand and mitigate or nullify the forces of altered gravity on embryo development (NASA, 2017). It was further stressed that avian eggs were ideal for studying embryo development since they are self-contained, self-sustaining and can be nurtured without a maternal host. More so, it was expressed that the avian development facility, allows incubation of avian eggs under controlled conditions (humidity, temperature and gas environment), on orbit and the fixation of the eggs for research while minimizing the effects of launch and landing (NASA, 2017).

Ravinder *et al*. (2014) evaluated the effect of cell phone frequency electromagnetic field radiations on early development of chick embryos. It was concluded that exposure to electromagnetic field radiations, induced detrimental effects on embryo growth and development during early incubation period. Similarly, Tsybulin *et al*. (2012) reported that the effects of radiation from commercial GSM 900 MHz cell phone on developing quail embryos signified a possibility for the non-thermal impact of magnetic waves on embryogenesis. Thus, suggested that the facilitating effect of low doses of irradiation on embryo development could be explained by a hormesis effect induced by reactive oxygen species. Research findings have illustrated that electromagnetic field radiations were found to be responsible for various harmful effects on health, development, reproduction, immune system, growth, sleep, skin and brain (Batellier *et al*., 2008; Aziz *et al*., 2010; Bilgici *et al*., 2013). It was found out that electromagnetic field radiation adversely affected development, which was supported by the results showing additional chick embryo mortality, significantly delayed development and induced malformations in

electromagnetic field radiations-exposed group as compared to control (Batellier *et al*., 2008; Zareen and Khan, 2008). Various investigations have revealed the toxic effects of cellphone frequency electromagnetic field radiations, on brain cells of chick embryos including increased number of apoptotic cells, degeneration of brain tissues, severe haemorrhages and early embryo death.

2.8 Japanese quail eggs incubation

Incubation is the process of providing optimum temperature, air circulation and relative humidity suitable for embryo development, growth and emergence as chicks. This process could be natural, where the broody hen sits on the eggs and covers them with the feathers in order to provide suitable environmental conditions for hatching. Since Japanese quail hens do not naturally incubate and hatch their eggs, artificial incubators are used tosimulate environmental conditions required to stimulate embryonic development and growth until the emergence of chicks. The artificial incubator could be homemade or commercial but should typically have heating source,air circulator (fan), temperature regulator (thermostat) as well aswater trough and egg trays. Fertile eggs stored for as long as 7 to 10 days at room temperature (10 to 15°C) could be set for incubation (Woodard*et al.*, 1973). According to ISA (2016), eggs should not be incubated the same day it was laid, in order to avoid hatching failure. Woodard*et al.*, (1973) stated that on incubation days 0 – 12, the temperature should be adjusted to 37.5°C, 13 – 15 days(37.2°C) and on day 16 the temperature should be 37°C and increased to 37.6°C on day 17 when the chicks are expected to emerge. On the other hand, Musa *et al.* (2007) recommended 39.4°C in the tropics, whereas Ferguson (1994) gave a range of 37.5 – 38°C as optimum incubation temperature in poultry production.Late hatching, low chick yield, survivability, hatchery failure and poor post-hatch performance may have been recorded. Thus, it becomes difficult to establish the optimum temperature suitable for quail eggs incubation and would want to resort to natural incubation if only it is possible in commercial poultry production.

2.9 Incubation temperature and Japanese quail embryos development

In order to mimic natural incubation, artificial incubators are simulated to provide a temperature believed to be optimum for Japanese quail embryo development. French (2002) gave a range of 37 to 38°C as the standard incubation temperature which controls

avian embryos development.It was stated that temperature is a critical environmental factor that could accelerate or delay embryogenesis (Landauer, 1961; Freeman and Vince, 1974). In developmental biology experimentations, avian species (particularly Japanese quail) have been used as models in morphogenesis studies (Ainsworth *et al.,*2010).Some staging of Japanese quail embryo development has been attemptedbut incomplete and variations in descriptions, staging and incubation processes were always difficult. Itappeared to be a general agreement that at early stages of embryogenesis, there were some developmental differences between fowl embryo (Hamburger and Hamilton, 1951) and quail embryo (Ainsworth *et al.,* 2010). Yet, the basis for these differences has not been established experimentally hence,Ainsworth (2010) constructed a 46-stage series,irrespective of the enhanced ontogeny observed in the Japanese quail in order to make the staging series comparable. At the early stages of development (Stage 4 – 28), Japanese quail stage series was identical to the Hamburger and Hamilton (HH) stage in fowl chick series as the rate of development of both species was indistinguishable. At the mid stages (Stage 29 – 35), the descriptions of morphological changes of each stage were still comparable between fowl chick and Japanese quail chick series. At later stages of development (Stage 36 – 46), the HH stage fowl chick series was no longer comparable to the quail series with regard to incubation periods and morphological descriptions.

It has been reported that biological engineering in avian species has advanced.For example, artificial *in ovo* culture of 1-celled zygote of blastoderm stage, made the production of adult bird possible (Perry, 1988; Naito *et al.*, 1990; Ono *et al.*, 1994a). Similarly, some trials to produce transgenic of chimeric birds have been conducted (Etches *et al.*, 1993; Love *et al.*, 1994; Naito *et al.*, 1994; Ono *et al.*, 1994b) and the use of avian embryo in teratological test has been anticipated (Hashizume *et al.*, 1992; 1993). Nakane and Tsudzuki (1999)established that series of normal stages in the development of Japanese quail embryo skeleton composed of 15 stages. In that study, the time of chondrification and calcification of the skeleton were recorded every 24hours from incubation day 3 to 17 at 37.7°C. It was reported that the knowledge of skeletogenesis stages in Japanese quail embryo, will be useful as a normal control not only in experimental embryology, teratology and developmental engineering but also in identifying mutant embryos with skeletal abnormalities. Yet, the causes of

early,intermediate and late embryo death as well as hatching failure (Ramteke*et al.*, 2013) are not clear hence, the present study examined the effects of low and high incubation temperatures on Japanese quail embryogenesis.

2.10 Incubation temperature and Japanese quail hatchability

Myriads of incubation temperatures in some avian species have been given as shown intable 2.1.Incubation temperature has been described as the most critical environmental concern during hatchery operations. This is because the developing embryo can only withstand small fluctuations in the microclimate conditions. Fertile eggs begin to develop to the embryo when the temperature exceeds physiological zero temperature given as 26 – 36°C (Webb, 1987; Conway and Thomas, 2000). Below or within this range of temperature, embryonic growth is believed to be halted and at above it (i.e. 36 – 40.5°C), which is described as the lower limit of optimal development, growth is resumed. At above 40.5°C which is the upper lethal temperature, malformations of embryos or embryo death could occur. According to Boerjan (2016), avian embryonic growth could be halted or slowed down and eventually arrested, if the temperaturefalls below 'physiological zero'. That is the level at which incubation temperature is low enoughto keep embryonic cell activity at a greatly reduced rate but reversible level. Essentially, the embryo still has the potential to continue its development again if normal temperature is restored.That is why the term 'arrested development' should be preferred to 'stop development' that is commonly used.As a result, 'physiological zero' should not be restricted to a specific or particular set point temperature, instead to a range of temperature from 12 – 20°C), depending on the milieu of egg handling and storage duration. Hence, the reasons why different set points temperature for 'physiological zero' is defined in different ways depending on the situation being described.The definition of 'physiological zero' was first presented by Edwards (1902) as the set point temperature of about 21.0°C and below this value, there was no embryonic growth. The terms of reference for 'physiological zero' were reviewed by Proudfoot (1969) to include a storage temperature range of 11.5 – 21°C. More recently, Fasenko (2007) introduced the term 'embryonic diapause' as an alternative to the traditional 'physiological zero' temperature regime. This updated definition recognized that some cellular metabolic processes still continue, but gross morphological changes like shape and structure are arrested.

Table 2.1: Incubation period, transfer to hatcher day, temperature and relative humidity levels in some common birds

Common name	Incubation period (days)	Incubation conditions		Hatcher conditions		
		Temp (°F)	R/H (%)	Transfer day	Temp (°F)	R/H (%)
Canary	13–14	100.5	56–58	11	99	66–74
Chicken	21	99.5	58	18	98.5	66–75
Cockatiel	18–20	99.5	58–62	15–18	99	66–74
Cockatoo	22–30	99.5	58–62	20–27	99	66–74
Conure (sun)	28	99.5	58–62	25	99	66–74
Conure (various)	21–30	99.5	58–62	18–27	99	66–74
Dove	14	99.5	58	12	98.5	66–75
Duck	28	99.5	58–62	25	98.5	66–75
Muscovy duck	35–37	99.5	58–62	31–33	98.5	66–75
Finch	14	99.5	58–62	12	99	66–74
Domestic goose	30	99.5	62	27	98.5	66–75
Geese (various)	22–30	99.5	62	20–27	98.5	66–75
Grouse	24–25	99.5	54–58	22	99	66–74
Guinea	28	99.5	54–58	22	99	66–74
Lovebird	22–25	99.5	58–62	20–22	99	66–74
Macaw	26–28	99.5	58–62	23–25	99	66–74
Mynah	14	100.5	56–58	12	99	66–74
Parakeet	18–26	99.5	58–62	15–23	99	66–74
Budgerigar	18	99.5	58–62	15	99	66–74
Parrot (various)	18–28	99.5	58–62	15–25	99	66–74
Parrot (African grey)	28	99.5	58–62	25	99	66–74
Chukar partridge	23–24	99.5	62	20	99	66–74
Peafowl	28–29	99.5	58–62	25–26	98.5	66–75
Ptarmigan	21–23	99.5	58–62	18–20	99	66–74
Raven	20–21	99.5	58–62	17–18	99	66–74
Ring-neck pheasant	24–24	99.5	58–62	21	99	66–74
Pheasant	22–28	99.5	58–62	20–25	99	66–74
Pigeon	17–19	100.5	58	14	99	66–74
Bobwhite quail	23	99.5	54–58	21	99	66–74
Japanese quail	17–18	99.5	58–62	15	99	66–74
Swan	33–37	99.5	58–62	30–33	99	66–74
Turkey	28	99.5	54–58	25	98.5	66–75
Emu	49–50	97.5	32–40	47	97.5	69
Ostrich	42	97.5	32–40	39	97.5	69
Rhea	36–42	97.5	50	34–37	97.5	69

Temp: Temperature; R/H: Relative humidity

Source: Archer and Cartwright, 2018

‘Embryonic diapause’ has been described in many vertebrate species like turtles, marsupials and even mammals such as Roe deer. ‘Embryonic diapause’ or ‘embryonic dormancy’ describes a stage at which metabolic activity and cell division is down-regulated or arrested and can be regarded as a strategy for coping with temporarily unfavourable environmental conditions.In avian species, embryonic development could be arrested after laying and cooling the eggs down to room temperature of between 22 – 25°C. During this cooling period under optimal conditions, the embryo develops from gastrula stage IX – X as described by Eyal-Giladi and Kochav (1976) to stage XII – XIII reported by Gilbert*et al*.(2006).The definition of physiological zero temperature was restricted specifically to stages XII – XIII of development. If the embryo has developed beyond this stage and primitive streak development has started, reduced temperatures will slow down development and finally result inearly mortality of the embryo. This may explain the higher rates of early mortality often recorded, when eggs are kept too long in the nests and when egg cooling is too slow (Fasenko, 1991; 1999).Therefore, optimum incubation temperature could be given as 37.5 to 37.6°C but should be reduced to 36.9°C during the last 3 days of the incubation period (Woodard *et al*., 1973; Scott and Steven, 1999; Lourens*et al*., 2005;Harb *et al*., 2010). According to Tullett and Burton (1982), incubation temperature has a major impact on chick yield and low incubation temperature (36.6°C) during the first 10 days of fowl embryogenesis increased body weight at hatch. Whereas high incubation temperature (39.5°C) during the same period, reduced chick weight compared to those incubated at 37.8°C. It has been established that avian embryos lose 12 – 14% moisture at the pipping stage, in order to hatch successfully and survive (Meir and Ar, 1991; French, 2009). Wilson (1991) gave the optimum temperature for fowl egg incubation as 37.8°C and should not vary more than ±0.3^{o}C. Yalcin and Siegel (2003) stated that because the developing embryo is poikilothermic, any changes in the incubation temperature may affect embryo size, organogenesis, metabolic rate, physiological development and hatching success. However, temperature during incubation may influence thermoregulation in avian species after hatching thus, Nichelmann and Tzschentke (2002) suggested that epigenetic adaptation occurrence during earlyor postnatal ontogeny may contribute to thermoregulation control mechanism. Halberslaben and Mussehl (1922) reported that normal weight of hatched chicks ranged from 62 to

76%of initial egg weight. But Skewes *et al.* (1988) stated that chicks may hatch with the same initial egg weight, though the residual yolk sac weight will differ due to greater development during incubation or the chick may weigh less with larger residual yolk sac and can survive longer without feed. According to Joseph *et al*. (2006), chicks that hatched at low incubation temperature during embryogenesis differed in body weight due to differences in yolk sac weight. Whereas, those that hatched from high incubation temperature during late embryogenesis, varied in body weight due to differences in free-yolk body weight.Avian egg hatchability is determined by the total number of hatched chicks divided by the total number of set eggs or fertile eggs multiplied by 100. Deviation from the optimum incubation temperature (37.5°C) may influence avian egg hatchability, depending on the magnitude of temperature deviation, duration of deviation and embryo stage during the period.In a study where broiler eggs were incubated at 36.6°C, it was reported that hatchability was depressed, but chick yield and body weight were increased compared to the control birds (Joseph *et al*., 2006).

Subsequently, an increase in incubation temperature to 39.5°C resulted in improved hatchabilitybut was observed to reduce chick yield and body weight. Incubation temperature manipulations during embryo development have been reported to enhance hatchability (Elmehdawi, 2013). Collin *et al*. (2007) increasedoptimum incubation temperature by 1.7°C for 3 hours per day (on days 8,10,16and 18 of incubation) and recorded improvement in hatchability. Similarly, when the optimum incubation temperature was increased by 1°C for 2hours onincubation days 18, 19, 20 and 21, improved hatchability, body weight and feed conversion were recorded (Tzschentke and Halle, 2009; Halle and Tzschentke, 2011). Piestun *et al*. (2013a) reported that thermal stimulation above the normal temperature during pre-incubation and first 5days of embryogenesis in broilers, improved hatchability. Unfortunately, there is seemingly little information on the causes of late hatching and hatching failure in poultry species. Thus, in the present study, Japanese quail eggs were subjectedto low, moderate and high temperatures, in order to determine the optimum incubation temperature.

2.10.1 Factors affecting Japanese quail eggs hatchability

Age of male and female Japanese quail in a convoy was reported to affect hatchability (Rogue and Soares, 1994; Buhr, 1995). While Gebhardt-Henrich and Merik (1991) stated

that the mating system adopted could affect Japanese quail hatchability, Weis (1991) observed that the husbandry system and rearing technologygreatly influenced Japanese quail hatchability. Brah and Sandhu (1989) as well as Tarongoy *et al.* (1990) in separate findings, adduced low hatchability to nature of eggs set and storage conditions. More significantly, incubation temperature, ventilation, relative humidity and egg turning angle were reported as a major determinant of Japanese quail eggs hatchability (Permsak, 1996; Hill, 2001; Lourens *et al.*, 2005).

2.11 Incubation temperature and avian sex determination

It has been reported that in some lower vertebratessuch as reptiles, amphibians and fish, environmental factors particularly incubation temperature influenced the sex of the hatchlings (Deeming and Ferguson, 1989; Strussmann and Patino, 1999; Selim *et al*., 2009). This phenomenon may be adopted in avian species but Pike and Petrie (2003) stated that no convincing evidence of temperature-dependent sex determination or manipulation of temperature to determine sex in avian has been recorded. Meanwhile, it has been speculated that in avian species, the primary sex ratio at the time of fertilization was almost equal (1 male: 1 female), but one sex was somewhat favoured when the environmental temperature was adjusted during embryogenesis. For instance, in Australian brush turkey (*Alectura lathami*), Göth and Booth (2005) observed that more males hatched at 31^{o}C, more females hatched at 36^{o}C and the sex ratio was 1:1 at 34^{o}C, which was the natural (control) mounds incubation temperature.

Meanwhile, Eiby *et al*. (2008) adduced sex ratio skewness in *A. lathami* to embryonic mortality and speculated that more male embryo mortality was recorded at higher incubation temperature that favoured the females. On the other hand, more female embryo mortality was observed at lower incubation temperature whereas, the embryonic mortality at the natural (control) mounds incubation temperature was equal (1 male: 1 female). Furthermore, Tzschentke and Halle (2010) reported a possibility of temperature-dependent sex determination in fowl, when a higher proportion of male chicks hatched at 1^{o}C above optimum incubation temperature (37.2°C). In a similar trend, Halle *et al*. (2012) observed sex reversal in Pekin ducks, when their eggs were subjected to lower incubation temperature (-1°C) for 2 hours per day during the last 6 days of incubation period with more female ducklings emerging. At day old, quail sex determination is nearly impossible

therefore incubation temperature manipulation in order to favour the desired sex may be appreciated in commercial quail productivity.

2.12 Incubation temperature and avian post-hatch performance

Elmehdawi (2013) stated that the goal of hatchery operation should be to produce healthy and marketable chicks from fertile eggs. However, it was reported that high hatchability did not often correlate positively with best post-hatchviability and performance of the chicks. At day old, chick quality characteristics should express good health indices for potential optimum post-hatchperformance. Therefore, a good quality chick should be clean, fluffy-dry, dirt-free, uncontaminated with clear and bright eyes and should not be deformed or have skin lesions. Also, the chick should have normal leg conformation, well-formed and firm beak, straight toes, completely sealed and clean navel that is free of the yolk sac or dried membrane. More importantly, the chick should be alert, inquisitive and explore the environment as well as respond to sounds (Decuypere *et al*., 2001; Meijerhof, 2005; Tona *et al*., 2005).

Table 2.2 shows the recommended incubation temperature in some avian species.Incubation temperature is the most important factor that affects chick quality.For example, chicks hatched at high temperature, had pale colour, exhibited short feathers, unhealed navel, cross beaks, weakness and unsteady gait (Leksrisompong *et al*., 2007). However, Boerjan (2002) exposedbroilers to optimum incubation temperature increased by 0.56^{o}C and recorded improvement in chick quality. Increase in incubation temperature during late embryogenesis was observed to elevate plasma blood glucose concentrations (Christensen *et al*., 2003; Willemsen *et al*., 2011). According to Tzschentke and Halle (2010), broiler eggs exposed to increased incubation temperature by 1^{o}C for 2 hours per day (on days 18 to 21 of incubation), resulted in improved hatchability and high chick quality score. Interestingly, French (2009) stated that many researchers have attempted to investigate if incubation temperature alteration during embryogenesis will have long-living effects on growth and post-hatch performance.Also, Joseph *et al*. (2006) observed negative effects on chick weight when broiler eggs were exposed to low incubation temperature during early embryogenesis.

Table 2.2: Recommendedoptimum incubation temperature in some poultry species

Avian species	Range of temperature		Typical incubation period (days)
	Celsius (^{o}C)	Fahrenheit (^{o}F)	
Fowl	37.4 – 37.6	99.3 – 99.6	21
Guinea Fowl	37.5	99.5	28
Turkey	37.2 – 37.5	99 – 99.5	28
Pheasant	37.6 – 37.8	99.6 – 100	23 – 27
Chukar Partridge	37.5	99.5	23
Japanese Quail	37.6 – 37.8	99.6 – 100	16 – 18
Bobwhite Quail	37.5	99.5	22 – 23
Ducks	37.4 – 37.6	99.3 – 99.6	28
Indian Runner Duck	37.5	99.5	28 – 30
Mallard	37.5	99.5	28 – 30
Muscovy Duck	37.5	99.5	35 – 37
Swan	37.5	99.5	30 – 37
Geese	37.4 – 37.6	99.3 – 99.6	28 – 30
Ostrich	35.8 – 36.4	96.5 – 97.5	42
Canada Goose	37.5	99.5	28 - 30
Egyptian Goose	37.5	99.5	28 - 30
Emu	35.8 – 36.1	96.5 – 97	50 - 56
Grouse	37.5	99.5	25
Amazons	36.8 – 37.0	98.3 – 98.6	24 – 29
Macaws	36.8 – 37.0	98.3 – 98.6	26 – 28
Love Birds	36.8 – 37.0	98.3 – 98.6	22 – 24
Peafowl	37.5	99.5	26 - 29
Pigeon	37.5 – 38.2	99.5-100.5	17
Rheas	35.8 – 36.4	96.5-97.5	35 – 40

Sources: Sartell, 2018

Similarly, Krischek *et al*., (2013) recorded negative influence on post-hatch performance on turkey embryogenesis exposed to manipulated incubation temperature.Evidence abounds that temperature manipulation during embryogenesis resulted in long-lasting changes in perinatal epigenetic programming of body functions such as improved thermo-tolerance, meat quality and body weight (Moraes *et al*., 2003; Collin *et al*., 2005; Tzschentke and Plagemann, 2006). However, Piestun *et al*. (2011) reported better post-hatchperformance in broiler eggs exposed to high incubation temperature, confirming higher breast meat yield in treated broilers reported by Collin*et al*. (2007). Increased myo-fibre diameter, body weight and absolute pectoral muscle growth have also been reported in increased incubation temperature treated avian species (Collins *et al*., 2007; Tzschentke and Halle, 2009; Piestun *et al*., 2009; 2011).

Nonetheless, Elmehdawi (2013) noted that some avian species showed better post-hatch performance, when exposed to high temperature during the late phase of embryogenesis and others showed better post-hatch performance when exposed to low incubation temperature at the same phase of embryogenesis. For example, Halle *et al*. (2012) observed reduced body weight and feed conversion in Pekin ducks, when the eggs were exposed to high incubation temperature whereas, improvement in body weight and feed conversion was recorded when the incubation temperature was reduced by 1^{o}C. With all these beneficial effects of manipulating incubation temperature on avian embryology, little is known about the causes of poor growth rate, high mortality, late sexual maturity or age at the point of lay in day-old chicks acquired from some commercial hatcheries. Hence, the present study was targeted at evaluating the effects of low, moderate and high incubation temperatures on Japanese quail feed intake, growth rate, mortality rate and age at point of lay.

2.13 Avian haematology

Haematology is the study of blood and blood-forming tissues and should be an integral part of clinical laboratory diagnostics in avian medicine (Bickford, 2007). Its assays rarely provide etiological diagnosis yet, indispensable in evaluating health and disease status of avian species. Avianspecies are well-known for their disguising clinical signs and they often show only subtle changes to indicate illness (Samour, 2006; Bickford, 2007). Improvements in the use of haematological assays in the differential diagnosis of

pathologic conditions in avian species have been reported (Samour, 2006).Before now, analysis of avian blood samples was restricted to red blood cell counts but recently, it has attracted more attention with more comprehensive and accurate high-tech facilities (Samour, 2006). In avian, blood samples could be collected through the jugular veins, basilica vein or caudal tibial vein but in most cases, the medial metatarsal and jugular veins are used (Fudge, 2000; Samour, 2006; Campbelland Ellis, 2007). In blood collection for haematological assay, anticoagulants like ethylenediaminetetraacetic acid (EDTA)orheparin capillary tubes could be used in order to keep the blood tissues integrity. However, it was reported that improper use of heparin as an anticoagulant, may cause clumping and poor cellular morphologic features, leading to inaccurate cell counts (Bickford, 2007; Campbell and Ellis, 2007). Although EDTA has been used successfully in avian blood collection, Latimer and Beinzle (2000) reported that it could alter cell morphology, cause erythrolysis and viscosity changes. Generally, haematological parameters that could be estimated in avian species include the following as given by Lucas and Jamroz (1961), Fudge(2000)and Samour(2000).

- Total red blood cell (x 10^{12}/L).
- Haemoglobin (g/dL).
- Packed cell volume (%) or Haematocrit (L/L).
- Total white blood cells (x 10^{9}/L) and the differential counts.
- Fibrinogen (g/L).

According to Bickford (2007), heterophil is the avian equivalent of the mammalian neutrophil, though the functions of the two cell types are thought to be similar. In most avian species, the heterophil is the most commonly seen white blood cell components.

2.13.1 Avian serum biochemistry determination

Unlike haematological assays where blood tissues integrity must be maintained, blood samples meant for serum biochemical evaluation should be collected without anticoagulant, in order to separate the plasma from the serum(Sakas, 2002). This is generally preferred to whole blood or plasma for biochemical analysis. Meanwhile, some commercial laboratories prefer to run biochemistry on plasma. According to Sakas(2002), serology is the evaluation of the serum portion of the blood, which includes chemical test or other specialized tests. It was stated that blood collection and storage should be done

carefully, in order to avoid inaccurate results. Serum biochemical profile tests are recommended for any avian species, to provide a better assessment of the health status. Such basic tests according to Sakas (2002) include the following:

- Serum protein whose normal value in avianspecies should range between 3.5 and5.5mg with a lower value indicating stress conditions, low nutritional plane or disease conditions.
- Creatinine with a normal value of 100 – 300IU/L in avian species.
- Albumin which was reported to be the largest protein fraction, constituting up to 40% of the total serum protein. A decrease in albumin level could be due to renal dysfunction, parasitism and disease as well as overhydration.
- Cholesterol whose normal value in avian species has not been well documented seemingly range from 100to300mg. Lower value in avian species could be an indicator of liver and kidney dysfunction and higher level could be due to high-fat diets, obesity and hypothyroidism.
- Glucose whose normal value could be as high as 700 to 1,000mg.
- Aspartate aminotransferase which is considered as the most reliable indicator of liver dysfunction in caged birds. Serum value larger than 350IU/L is somewhat abnormal and the upsurge could be due to liver, heart or muscle damage.
- Uric acid which is the primary nitrogenous waste product of the avian kidney and its serum level is an excellent indicator of renal function. Normal value varied from 2 to 15mg depending on the avian species.
- Alanine aminotransferase (ALT) level in the serum shows the efficiency of the liver as well as the level of muscle and heart tissues damage. Meanwhile, Lewandowski *et al.* (1986) speculated that published reference values vary, depending on the breeding activity, sex, age and time of the year. Meanwhile, Kraft and Durr (1999) described ALT as a cytoplasmic enzyme that catalyses transamination biochemical pathways.

2.14 Carcass quality determination in avian species

Adult birds proposed for quality evaluation should be weighed, slaughtered and bled properly. The dead bird should be dipped in water for scalding at about 55^{o}C for 2 minutes to ease defeathering (Alkan *et al.,* 2010). The water should not be too hot to prevent

carcass disintegration hence automated defeathering machine may be a better option if available and affordable. Thereafter, the carcasses should be carefully eviscerated to empty the visceral contents. Carcass weight value and the initial live weight are used to determine carcass yield (or dressing percentage). The in-depth evaluation includes cut parts weight like breast, drumstick, wing, neck and back (Alkan *et al.,* 2010). Carcass characteristics maybe influenced by age (Yannakopoulos and Tserveni-Gousi, 1986), diets (Florou-Paneri, 1989) or sex (Alamuoye and Ojo, 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of study area

The study was conducted at the National Veterinary Research Institute (NVRI), Vom, Jos North, Plateau State,Nigeria located on latitude 9° 44' 0"N,longitude 8° 47' 0"Ewith a population of about 900,000 residents (NPC, 2006).The altitude of Jos is about 1,264.5m above sea level with about 1,400 millimetres (55 inches) of rainfall annually. Jos enjoys a more temperate climate than the rest parts of Nigeria. Average monthly temperature range from 21 – 25°C (70– 77°F) and from mid-November to late January, night-time temperature drops to as low as 11°C (52°F). Hails sometimes fall during the rainy season because of the cooler temperature at high altitude. This cooler temperature has from colonial times until the present day, made Jos a favourite holiday location for both tourists and expatriates based in Nigeria (Webster, 1983). The location of Vom as shown in map of Nigeria is presented in figure 1.

3.2 Animal use ethical clearance

Application to use Japanese quails as subjects in this research was submitted to Animal Use and Care Committee, National Veterinary Research Institute, Vom and was approved with Research Project Number: AEC/02/41/17 (See appendix 1).

3.3 Fabrication of incubators

Five electric incubators were designed and fabricated with the capacity to hold about 1,500 Japanese quail eggs, fitted with sensitive thermoregulatory devices to switch off/on at appropriate intervals yet, maintained temperature of as low as 1^{o}C and as high as 150^{o}C. The fabrication of the electric incubators was done at the Fabrication Division, Dagwom Farm, NVRI, Vom, Plateau State. The needed materials were sourced locally and used in fabricating five units of electric incubators strictly according to the designs and specifications.

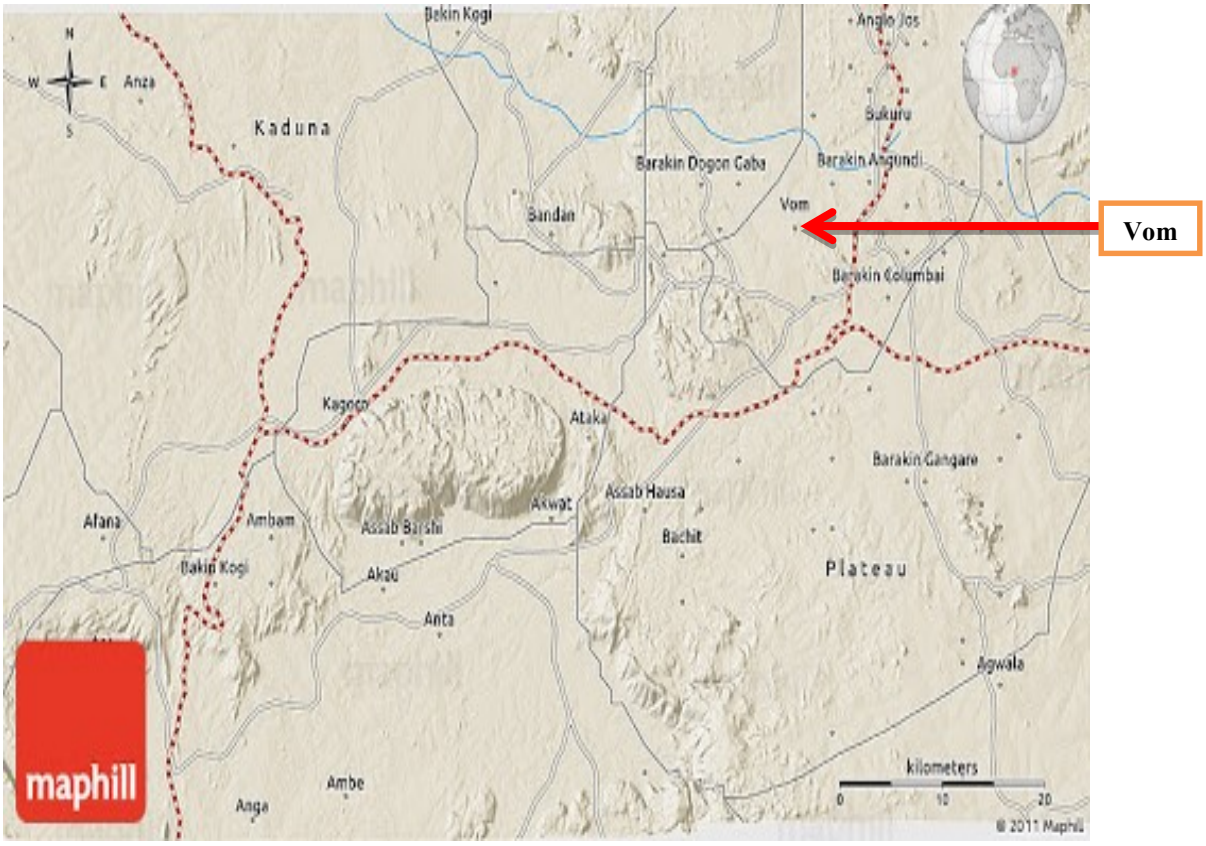


Figure 1: Map of Nigeria showing Vom

Source: https://www.europa.uk.com/global-1000-atlas/map/?pid=107247

The materials utilized included high density fibre plywood for the body and outer door, acrylic glass for inner door to reduce heat loss during data collection and a 315W heater inserted on the side opposite the door to generate heat. Others were three-tier wire gauze egg-tray that was turned together at 180 degreeswith a pedal outside the incubator. Two electric bulbs (40W and 100W) were positioned strategically for lighting system as well as to be used as a heat source when the heater is not working and a fan was installed on the inner top to facilitate even warm air circulation. A hygrothermometer with a sensitive probe capable of detecting inner and outer temperature and relative humidity was inserted at the middle of the incubator. Thermostat and relay devices were connected to the heater for temperature regulation and four rollers were fixed on the base to ease movement. The incubators were perforated at the top and the two opposite sides adjacent to the door for inlet and outlet of air. A switch was connected to power the two bulbs and another switch to operate the incubator with a plug to be inserted on the main, to power the entire incubator's electrical system.

3.3.1 Test run of the fabricated incubators

The fabricated incubators were test run, adjusted and amended where necessary until they were observed to be efficient and switched off at the calibrated temperatures (36^{o}C, 37^{o}C, 38^{o}C, 39^{o}C and 40^{o}C).The fabricated incubators were able to switch on or off automatically, whenever the temperature was increasedor dropped by ±0.26 – 0.97^{o}C or when it reached the calibrated temperaturewithin 2 – 5minutes, depending on the ambient temperature and rate of heat loss through the perforations in the incubators.

3.3.2 Limitations of the fabricated incubators

The fabricated incubators were observed to be poor in conserving heat whenever there was power outage.This shortcoming would have disrupted the study because, at physiological zero temperature (26 – 36°C), embryogenesis may be halted.However,there was a standby generating set that was promptly switched over to during the study. Also, the incubation temperature values were fluctuating and did not give the exact treatment temperature desired. Fortunately, this was similar to the imported incubator (used as control in the study)that was not able to maintain exactly the calibrated temperature too. However, there was a wider range of about ±0.26 – 0.97^{o}C in the fabricated incubators, compared to maximum fluctuation range of ±0.3^{o}C in the imported incubator.

3.3.3 Maintenance of the incubators

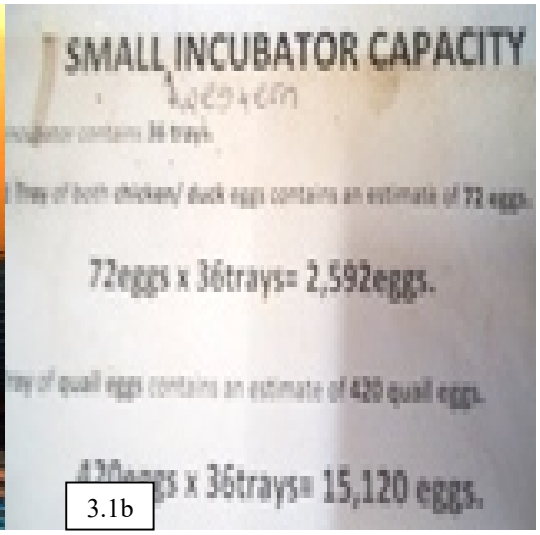
Plastic trays were used to keep water inside the incubators to provide moisture for the developing embryos. The egg trays were turned mechanically by pedalling up or down (within 180 degrees)at regular intervals daily, until when the eggs were transferred to the hatchery compartment of the incubators. After each study, the incubators were switched off, cleaned and turned on for 24hours prior to another round of incubation. Minor repairs and replacement of some components were done as deemed necessary.

3.4 Experimental design and layout

All the eggs were weighed in batches using a sensitive weighing scale and allotted in a completely randomised design to each of the three egg trays in each incubator. The three egg trays in the incubatorsserved as replicates in each treatment. An automated commercial incubator with egg holding capacity of about 15,120 Japanese quail eggs was used as the control with the temperature adjusted to 38^{o}C which was the standard optimum temperature adopted at the National Veterinary Research Institute. The fabricated incubators with egg holding capacity of about 1,500 Japanese quail eggs, were adjusted to maintain the calibrated temperatures of 36^{o}C, 37^{o}C, 38^{o}C, 39^{o}C and 40^{o}C representing the treatments during the study.The treatments were Control: 38^{o}C (imported incubator); Very low incubation temperature: 36^{o}C; Low incubation temperature: 37^{o}C; Medium incubation temperature: 38^{o}C; High incubation temperature: 39^{o}C and Very high incubation temperature: 40^{o}C. However, in experiment 1, imported incubatorwas not included to determine the optimum incubation temperature in Japanese quails.

3.5 Egg fumigation

All the egg trays with the weighed eggs were always placed on the floor, with the windows and door closed for fumigation using 30ml of formalin and 10g of $KMnO_4$ mixture at room temperature for 10 minutes as described by Cadirci (2009). Thereafter, the egg trays holding the eggs were placed in their respective positions in the incubators and fumigated again, using 30ml of formalin and 10g of $KMnO_4$ mixture for 10 minutes. The fumigation was done carefully and in two batches in order to avert the negative consequences of using formaldehyde as fumigant reported by Banwell (2013) in poultry eggs incubation.



Plates 3.1a – d: Features of the imported incubator used as control with temperature calibrated at 38^{o}C

3.2

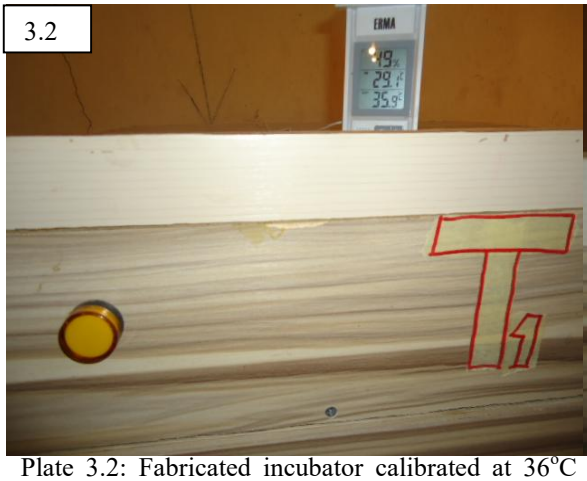


Plate 3.2: Fabricated incubator calibrated at 36°C representing Very low incubation temperature

3.3

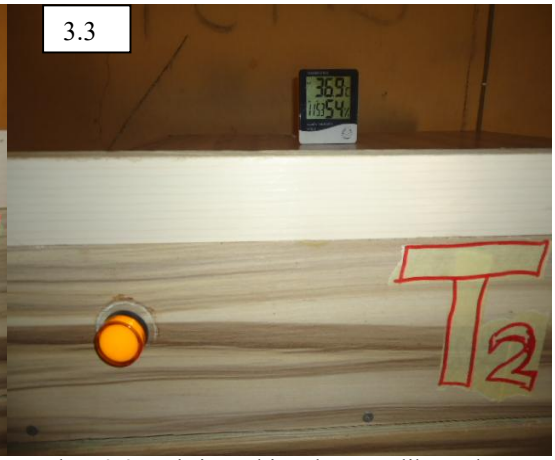


Plate 3.3: Fabricated incubator calibrated at 37°C representing Low incubation temperature

3.4

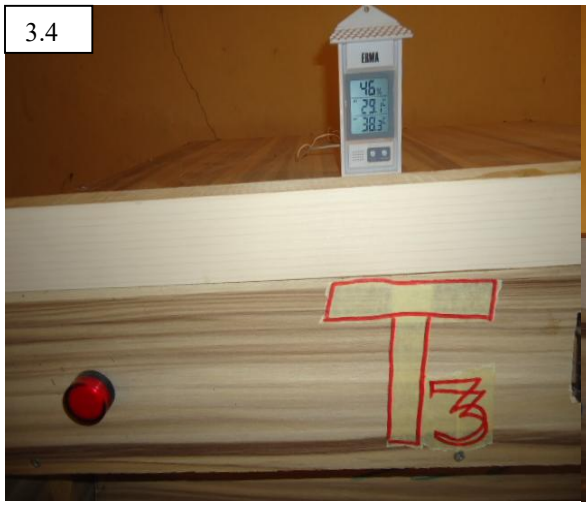


Plate 3.4: Fabricated incubator calibrated at 38°C representing Medium incubation temperature

3.5

Plate 3.5: Fabricated incubator calibrated at 39°C representing High incubation temperature

3.6

Plate 3.6: Fabricated incubator calibrated at 40°C representing Very high incubation temperature

3.6 Experiment 1: Determination of optimum incubation temperature

A total of 1,605 Japanese quail eggs were collected from a flock in deep litter systemat peak of egg production, over a period of 5 days but were set on the 6th day for the last clutch to cool overnight, according to the recommendation of ISA (2016).The eggs were randomly allotted based on weight such that 107 eggs each were set in the upper, middle and bottom egg trays that served as the replicates in each incubator.The egg trays were turned every hour and the water troughs inside the incubators were refilled every morning.

3.6.1 Data collection

3.6.1.1 Temperature, relative humidity and hatchability parameters

All the eggs per each egg tray were weighed together to obtain egg weight using sensitive weighing scale and at hatch, all the chicks per each egg tray were weighed togetherin a group of five birds to obtain chicks' weight per group.The group weight values obtained werelater divided by the total number of chicks per group (maximum of 5) to obtain individual chick weight. Meanwhile, because all the eggs did not hatch, an equivalent number of hatched eggs were used and the average egg weight of that equivalent number and chicks' weight were used to obtain chick yield value. Incubationand ambient temperature as well as relative humidity values were recorded daily at 9:00am, 12:00noon and 3:00pm using hygrothermometer during the incubation periods. The time between egg incubation and the emergence of the first chick was considered as the incubation period. After the emergence of the first group of chicks, other eggs were still left in the incubators for an extra 5 days, in case of possible late hatching. Thereafter, all the unhatched eggs were carefully windowed to evaluate the physiological status of the developing embryos that could not emerge as chicks. Such physiological statuses wereeggs without any obvious signs of growth, rather intact yolk and albumen were still in a fluid formand such eggs wereconsidered as "infertile eggs". Eggs with intact yolk and albumen but in a dried form were designated as "caked eggs"and those with intact yolk and albumen but with blood spot, blacken spot and partially dried yolk and albumen were considered as "not clear whether fertileor not". Developed embryos with undifferentiated body parts but not alivewere termed "early dead embryos whereas, those with defined body parts and downy feathers without pipping were referred to as "fully developed but dead embryos". The fully developed embryos that pipped and died in the process were called "pipped and dead

embryos" while those that were alive andpippingbut could not complete the pipping process were regarded to as "pipped and alive but not able to emerge embryos". Those that were fully developed, pipped and emergedalive were considered as "hatched chicks".The hatched chicks were allowed to remain in the incubators until fluffy dried, weighed to obtain chick weight value and were transferred to the Brooding Unit, Poultry Division, NVRI. The egg hatchability, fertility and chick yield values were calculated using equations I, II and III respectively.

$$\text{Egg hatchability} = \frac{\text{Total number of hatchlings}}{\text{Total number of fertile eggs}} \text{ X } \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{equation I}$$

$$\text{Egg fertility} = \frac{\text{Total number of fertile eggs}}{\text{Total number of eggs set}} \text{ X } \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{equation II}$$

$$\text{Chick yield} = \frac{\text{Total chicks}'\text{weight at day old}}{\text{Total weight of eggs at setting}} \text{ X } \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{equation III}$$

Where:Fertile eggs = the summation of "early dead embryos", "fully developed but dead embryos", "pipped and dead embryos", "pipped and alive but not able to emerge embryos" and "hatched chicks".

3.6.1.2 Brooding, sex identification, feeding and growth parameters

The brooding unit was properly covered with thick cellophane to conserve heat that was generated with electric bulbs placed in strategic locations. The temperature and relative humidity in the brooding unit were monitored daily at 9:00 am, 12:00 noon and 3:00 pm with two different hygrothermometers hung in strategic positions throughout the brooding periods. The brooding unit was partitioned into 18 separate cubicles (i.e. three replicates in six treatments), using hard cartons with good lighting system to enhance agility, drinking and eating. Hatched chicks fromeach of the egg trays that represented the treatment replicates in each of the treatments were housed together in each of the 18 cubicles in the brooding unit. At the end of the brooding phase (i. e. 3 weeks old), the sex of the growers was identified using breast plumage and were transferred to the growers unit and housed separately based on sex and treatments. Anti-stress and other veterinary drugs were administered by a veterinarian only when it was deemed necessary and not on a routine schedule. The experimental birds were offered chicks, growers and layersfeedduring the respective growth phases. The feed were supplied by the Feed Mill Unit, Dagwom Farm,

National Veterinary Research Institute, Vom. Pebbles were placed in the drinkers to prevent the chicks from drowningand clean drinking water was offered *ad libitum*. In each growth phase, the quantity of feed offered was weighed using sensitive weighing scale and the weight of the remnant was deducted to obtain feed intake. All the chicks were weighed weekly in groups of a maximum of 5 birds and the recorded values were used to obtainrelative and absolute growth rate using equations IV and V.The group weight values obtained were later divided by the total number of chicks per group (a maximum of 5) to obtain individual chick weight.

$$\text{Relative growth rate} = \frac{\text{Total chicks}'\text{weight}}{\text{No. of chicks}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{equation IV}$$

$$\text{Absolute growth rate} = Wf - Wi \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{equation V}$$

Where: Wf = Final weight value; Wi = Initial weight value.

3.6.1.3Point of lay, blood profiles determination, carcass evaluation, sex re-examination, mortality and survival rates

The first egg drop was recorded as "the point of lay" representing sexual maturity. A week allowance was given inorder for the late hatched chicks to equally attain sexual maturity. At sexual maturity, 8 birds(1:1 sex ratio) were randomly picked per treatment, weighed using a sensitive scale to obtain live weight value and tagged for sex identification. After fasting them overnight as reported by Raji*et al.* (2015) and Abang *et al.*(2016), the selected birds were taken to the Clinical Laboratory,Biochemistry Division NVRI Vom. The jugular veins of the birds were carefully cut with a razor blade and blood samples were collected in bottles with ethylenediaminetetraacetic acid (EDTA).The samples were processed according to standard operating procedures in haematology (Fudge, 2000; Samour, 2006; Campbell and Ellis, 2007) to obtain white bloodcells, neutrophils, lymphocytes, monocytes, eosinophil, red blood cells, haemoglobin and packed cell volume. Also, blood samples were collected in bottles without EDTA and left on the racks in a slant position for anhour. Thereafter, the serum was carefully separated and processed following Randox procedures to obtain Alanine aminotransferase (ALT), Total protein(TP), Albumin (ALM), Creatinine (CREA) and Cholesterol (CHO) values as given byFudge (2000) and Sakas (2002).After bleeding, the carcasseswere left for an hour before weighing again to obtain bled weight value. Thereafter, they were all immersed in

hot water for two minutes to ease defeathering (Alkan *et al.,* 2010). Then the carcasses were weighed without the heads and shanks to obtain defeathered weight. The carcasses were cut open from the breast region through the abdomen to collect the kidneys, gizzards, livers, heartsand testes(Yannakopoulos and Tserveni-Gousi, 1986; Florou-Paneri, 1989; Alkan *et al.,* 2010). Also, the developing yolk and eggs were carefully removed and counted to obtain number of yolk and formed eggs. Thereafter, all theorgans were weighed to obtain organ weights (Alamuoye and Ojo, 2015). The carcasses were individually weighed to obtain carcass weight and the dressingpercentage value was determined using equation VI.The sex was reaffirmed at slaughter by carefully observing the testes/ovaries in the eviscerated carcasses and nonconformity with the identified sex was considered reversed sex. Throughout the study period, every dead bird was recorded as mortality and the mortality rate was calculated using equation VII and the survival rate was determined using equation VIII.

$$\text{Dressing per centage} = \frac{\text{Carcass weight}}{\text{Live weight}} \text{ X } \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{equation VI}$$

$$\text{Mortality rate} = \frac{\text{Total number of dead birds}}{\text{Total number of chicks hatched}} \text{ X } \frac{100}{1} \ldots\ldots \text{equation VII}$$

Survival rate = 100 – mortality rate……..equation VIII

3.6.1.4Data analysis

Data collected were subjected to analysis of variance procedure of SPSS (2010) and mean values were separated using Duncan Multiple Range Test of the same software package.

3.6.2 Experiment 2: Morphological examination of embryos in Japanese quail eggs subjectedto different incubation temperatures

A clutch of 504 eggs was collected from Japanese quail layers in a deep litter system at the Breeders Unit, Poultry Division, NVRI, Vom, left overnight at room temperature, weighed individually and labelled using permanent marker for easy identification. In each egg tray, 28 eggs were setamounting to 84 eggs per incubator. The egg trays were turned every hour and the water troughs inside the incubators were refilled every morning.

3.6.2.1 Data collection and analysis

The incubation and ambient temperature, as well as relative humidity were monitored using hygrothermometer. Eggs with evidence of embryogenesis were used and those with

dismembered parts orsuspected to be infertile were discarded.The eggs were carefully windowed daily, observations on embryo growth and development were recordedand pictures of the embryo features were taken.Data collection was terminated with the emergence of a chick in a treatment, while it continued in others until chicks emerged in all the treatments.Data on temperature and relative humidity were subjected to analysis of variance procedure of SPSS (2010) and meanvalues were separated using Duncan Multiple Range Test of the same software package whereas, the observed embryo growth was presented pictorially.

3.6.3 Experiment 3: Effects of incubation temperature manipulation during early embryogenesis on Japanese quail sex

A total of 1,260 Japanese quail eggs were gathered within a week from layers in a deep litter system at their peak of egg production and were stored at room temperature before setting on the 8thday. Two hundred and ten eggs were set in each incubator such that each egg tray held 70 eggs. The incubation temperature was paused for 5 hours between 9:00am and 2:00pm on incubation days 3, 4 and 5 by switching off the main, in order to alter the sex of the developing embryo. The egg trays were turned every hour and the water troughs inside the incubators were refilled every morning.

3.6.3.1 Data collection

3.6.3.1.1 Temperature, relative humidity and hatchability parameters

All the eggs per each egg tray were weighed together to obtain egg weight using sensitive weighing scale and at hatch, all the chicks per each egg tray were weighed together (five per group) to obtain chicks' weight per group. The values obtained were divided by the total number of chicks to obtain individual chick weight. Meanwhile, because all the eggs did not hatch, an equivalent number of hatched eggs were used and the average egg weight of that equivalent number and chicks' weight were used to obtain chick yield value. Incubation and ambient temperature as well as relative humidity values were recorded daily at 9:00 am, 12:00 noon and 3:00 pm using hygrothermometer during the incubation periods.The time between egg incubation and the emergence of the first chick was considered as the incubation period. After the emergence of the first group of chicks, other eggs were still left in the incubators for an extra 5 days, in case of possible late hatching. Thereafter, all the unhatched eggs were carefully windowed to evaluate the

physiological status of the developing embryos that could not emerge as chicks. Such physiological statuses were eggs without any obvious signs of growth, rather intact yolk and albumen were still in a fluid form and such eggs were considered as “infertile eggs”. Eggs with intact yolk and albumen but in a dried form were designated as “caked eggs” and those with intact yolk and albumen but with blood spot, blacken spot and partially dried yolk and albumen were considered as “not clear whether fertile or not”. Developed embryos with undifferentiated body parts but not alive were termed “early dead embryos whereas, those with defined body parts and downy feathers without pipping were referred to as “fully developed but dead embryos”. The fully developed embryos that pipped and died in the process were called “pipped and dead embryos” while those that were alive and pipping but could not complete the pipping process were regarded to as “pipped and alive but not able to emerge embryos”. Those that were fully developed, pipped and emerged alivewere considered as “hatched chicks”. The hatched chicks were allowed to remain in the incubators until fluffy dried, weighed to obtain chick weight value and were transferred to the Brooding Unit, Poultry Division, NVRI. The egg hatchability, fertility and chick yield values were calculated using equations I, II and III respectively.

$$\text{Egg hatchability} = \frac{\text{Total number of hatchlings}}{\text{Total number of fertile eggs}} \text{ X } \frac{100}{1} \ldots\ldots\ldots\ldots \text{equation I}$$

$$\text{Egg fertility} = \frac{\text{Total number of fertile eggs}}{\text{Total number of eggs set}} \text{ X } \frac{100}{1} \ldots\ldots\ldots\ldots\ldots \text{equation II}$$

$$\text{Chick yield} = \frac{\text{Total chicks'weight at day old}}{\text{Total weight of eggs at setting}} \text{ X } \frac{100}{1} \ldots\ldots\ldots\ldots \text{equation III}$$

Where:Fertile eggs = the summation of “early dead embryos”, “fully developed but dead embryos”, “pipped and dead embryos”, “pipped and alive but not able to emerge embryos” and “hatched chicks”.

3.6.3.1.2 Brooding, sex identification, feeding and growth parameters

The brooding unit was properly covered with thick cellophane to conserve heat that was generated with electric bulbs placed in strategic locations. The temperature and relative humidity in the brooding unit were monitored daily at 9:00 am, 12:00 noon and 3:00 pm with two different hygrothermometers hung in strategic positions throughout the brooding periods.The brooding unit was partitioned into 18 separate cubicles (i.e. three replicates in

six treatments), using hard cartons with a good lighting system to enhance agility, drinking and eating. Hatched chicks from each of the egg trays that represented the treatment replicates in each of the treatments were housed together in each of the 18 cubicles in the brooding unit. At the end of the brooding phase (i. e. 3 weeks old), the sex of the growers was identified using breast plumage and were transferred to the growers unit and housed separately based on sex and treatments. Anti-stress and other veterinary drugs were administered by a veterinarian only when it was deemed necessary and not on a routine schedule. The experimental birds were offered chicks, growers and layers feed during the respective growth phases. The feed were supplied by the Feed Mill Unit, Dagwom Farm, National Veterinary Research Institute, Vom. Pebbles were placed in the drinkers to prevent the chicks from drowning and clean drinking water was offered *ad libitum*.

In each growth phase, the quantity of feed offered was weighed using a sensitive weighing scale and the weight of the remnant was deducted to obtain feed intake. All the chicks were weighed weekly in groups of a maximum of 5 birds and the recorded values were used to obtain relative and absolute growth rate using equations IV and V. The group weight values obtained were later divided by the total number of chicks per group (a maximum of 5) to obtain individual chick weight.

$$\text{Relative growth rate} = \frac{\text{Total chicks}'\text{weight}}{\text{No. of chicks}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{equation IV}$$

$$\text{Absolute growth rate} = Wf - Wi \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{equation V}$$

Where: Wf = Final weight value; Wi = Initial weight value.

3.6.3.1.3 Point of Lay, blood profiles determination, carcass evaluation, sex re-examination, mortality and survival rates

The first egg drop was recorded as a point of lay representing sexual maturity. A week allowance was given in order for the late hatched chicks to equally attain sexual maturity. At sexual maturity, 8 birds (1:1 sex ratio) were randomly picked per treatment, weighed using a sensitive scale to obtain live weight value and tagged for sex identification. After fasting them overnight as reported by Raji *et al.*(2015) and Abang *et al.*(2016), the selected birds were taken to the Clinical Laboratory, Biochemistry Division NVRIVom. The jugular veins of the birds were carefully cut with a razor blade and blood samples

were collected in bottles with EDTA. The samples were processed according to standard operating procedures in haematology (Fudge, 2000; Samour, 2006; Campbell and Ellis, 2007) to obtain white blood cells, neutrophils, lymphocytes, monocytes, eosinophil, red blood cells, haemoglobin and packed cell volume. Also, blood samples were collected in bottles without EDTA and left on the racks in a slant position for 1 hour. Thereafter, the serum was carefully separated and processed following Randox procedures to obtain Alanine aminotransferase (ALT), Total protein (TP), Albumin (ALM), Creatinine (CREA) and Cholesterol (CHO) values as given by Fudge (2000) and Sakas (2002).After bleeding, the carcasses were left for an hour before weighing again to obtain bled weight value. Thereafter, they were all immersed in hot water for two minutes to ease defeathering (Alkan *et al.,* 2010). Then the carcasses were weighed without the heads and shanks to obtain defeathered weight. The carcasses were cut open from the breast region through the abdomen to collect the kidneys, gizzards, livers, hearts and testes (Yannakopoulos and Tserveni-Gousi, 1986; Florou-Paneri, 1989; Alkan *et al.,* 2010).

Also, the developing yolk and eggs were carefully removed and counted to obtain number of yolk and formed eggs. Thereafter, all the organs were weighed to obtain organ weights (Alamuoye and Ojo, 2015). The carcasses were individually weighed to obtain carcass weight and the dressing percentage value was determined using equation VI.The sex was reaffirmed at slaughter by carefully observing the testes/ovaries in the eviscerated carcasses and nonconformity with the identified sex was considered reversed sex. Throughout the study period, every dead bird was recorded and the mortality rate was calculated using equation VII and the survival rate was determined using equation VIII.

$$\text{Dressing percentage} = \frac{\text{Carcass weight}}{\text{Live weight}} \text{ X } \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{equation VI}$$

$$\text{Mortality rate} = \frac{\text{Total number of dead birds}}{\text{Total number of chicks hatched}} \text{ X } \frac{100}{1} \ldots\ldots\ldots\ldots \text{equation VII}$$

Survival rate = 100 – mortality rate……equation VIII

3.6.3.2Data analysis

Data collected were subjected to analysis of variance procedure of SPSS (2010) and mean values were separated using Duncan Multiple Range Test of the same software package.

3.6.4 Experiment 4: Effects of incubation temperature alteration during late embryogenesis on Japanese quail sex

Six hundred and twelve Japanese quail eggs were collected from layers reared in a deep litter system over 4 days period and were cooled at room temperature before setting on the 5th day. In each incubator, 102 eggs were randomly distributed based on weight such that each egg tray in the incubators held 34 eggs. The incubation temperature was paused on incubation days 11, 12 and 13 for 5 hours each day by switching off the main in order to alter the embryo sex.The egg trays were turned every hour and the water troughs inside the incubators were refilled every morning.

3.6.4.1 Data collection

3.6.4.1.1 Temperature, relative humidity and hatchability parameters

All the eggs per each egg tray were weighed together to obtain egg weight using sensitive weighing scale and at hatch, all the chicks per each egg tray were weighed together (five per group) to obtain chicks' weight per group. The values obtained were divided by the total number of chicks to obtain individual chick weight. Meanwhile, because all the eggs did not hatch, an equivalent number of hatched eggs were used and the average egg weight of that equivalent number and chicks' weight were used to obtain chick yield value. Incubation and ambient temperature as well as relative humidity values were recorded daily at 9:00 am, 12:00 noon and 3:00 pm using hygrothermometer during the incubation periods. The time between egg incubation and the emergence of the first chick was considered as the incubation period.

After the emergence of the first group of chicks, other eggs were still left in the incubators for an extra 5 days, in case of possible late hatching. Thereafter, all the unhatched eggs were carefully windowed to evaluate the physiological status of the developing embryos that could not emerge as chicks. Such physiological statuses were eggs without any obvious signs of growth, rather intact yolk and albumen were still in a fluid form and such eggs were considered as "infertile eggs". Eggs with intact yolk and albumen but in a dried form were designated as "caked eggs" and those with intact yolk and albumen but with blood spot, blacken spot and partially dried yolk and albumen were considered as "not clear whether fertile or not". Developed embryos with undifferentiated body parts but not alive were termed "early dead embryos whereas, those with defined body parts and downy

feathers without pipping were referred to as “fully developed but dead embryos”. The fully developed embryos that pipped and died in the process were called “pipped and dead embryos” while those that were alive and pipping but could not complete the pipping process were regarded to as “pipped and alive but not able to emerge embryos”. Those that were fully developed, pipped and emerged alive were considered as “hatched chicks”. The hatched chicks were allowed to remain in the incubators until fluffy dried, weighed to obtain chick weight value and were transferred to the Brooding Unit, Poultry Division, NVRI. The egg hatchability, fertility and chick yield values were calculated using equations I, II and III respectively.

$$\text{Egg hatchability} = \frac{\text{Total number of hatchlings}}{\text{Total number of fertile eggs}} \text{ X } \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots \text{equation I}$$

$$\text{Egg fertility} = \frac{\text{Total number of fertile eggs}}{\text{Total number of eggs set}} \text{ X } \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots \text{equation II}$$

$$\text{Chick yield} = \frac{\text{Total chicks'weight at day old}}{\text{Total weight of eggs at setting}} \text{ X } \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots \text{equation III}$$

Where:Fertile eggs = the summation of “early dead embryos”, “fully developed but dead embryos”, “pipped and dead embryos”, “pipped and alive but not able to emerge embryos” and “hatched chicks”.

3.6.4.1.2 Brooding, sex identification, feeding and growth parameters

The brooding unit was properly covered with thick cellophane to conserve heat that was generated with electric bulbs placed in strategic locations. The temperature and relative humidity in the brooding unit were monitored daily at 9:00 am, 12:00 noon and 3:00 pm with two different hygrothermometers hung in strategic positions throughout the brooding periods. The brooding unit was partitioned into 18 separate cubicles (i.e. three replicates in six treatments), using hard cartons with a good lighting system to enhance agility, drinking and eating. Hatched chicks from each of the egg trays that represented the treatment replicates in each of the treatments were housed together in each of the 18 cubicles in the brooding unit. At the end of the brooding phase (i. e. 3 weeks old), the sex of the growers was identified using breast plumage and were transferred to the growers unit and housed separately based on sex and treatments. Anti-stress and other veterinary drugs were administered by a veterinarian only when it was deemed necessary and not on

a routine schedule. The experimental birds were offered chicks, growers and layers feed during the respective growth phases. The feed were supplied by the Feed Mill Unit, Dagwom Farm, National Veterinary Research Institute, Vom. Pebbles were placed in the drinkers to prevent the chicks from drowning and clean drinking water was offered *ad libitum*. In each growth phase,the quantity of feed offered was weighed using a sensitive weighing scale and the weight of the remnant was deducted to obtain feed intake. All the chicks were weighed weekly in groups of a maximum of 5 birds and the recorded values were used to obtain relative and absolute growth rate using equations IV and V. The group weight values obtained were later divided by the total number of chicks per group (a maximum of 5) to obtain individual chick weight.

$$\text{Relative growth rate} = \frac{\text{Total chicks}'\text{weight}}{\text{No. of chicks}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{equation IV}$$

$$\text{Absolute growth rate} = Wc - Wp \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{equation V}$$

Where: Wf = Final weight value; Wi = Initial weight value.

3.6.4.1.3 Point of lay, blood profiles determination, carcass evaluation, sex re-examination, mortality and survival rates

The first egg drop was recorded as point of lay representing sexual maturity. A week allowance was given in order for the late hatched chicks to equally attain sexual maturity. At sexual maturity, 8 birds (1:1 sex ratio) were randomly picked per treatment, weighed using a sensitive scale to obtain live weight value and tagged for sex identification. After fasting them overnight as reported by Raji *et al.*(2015) and Abang *et al.*(2016), the selected birds were taken to the Clinical Laboratory, Biochemistry Division NVRI Vom. The jugular veins of the birds were carefully cut with a razor blade and blood samples were collected in bottles with EDTA. The samples were processed according to standard operating procedures in haematology (Fudge, 2000; Samour, 2006; Campbell and Ellis, 2007) to obtain white blood cells, neutrophils, lymphocytes, monocytes, eosinophil, red blood cells, haemoglobin and packed cell volume. Also, blood samples were collected in bottles without EDTA and left on the racks in a slant position for 1 hour. Thereafter, the serum was carefully separated and processed following Randox procedures to obtain Alanine aminotransferase (ALT), Total protein (TP), Albumin (ALM), Creatinine (CREA) and Cholesterol (CHO) values as given by Fudge (2000) and Sakas (2002).After bleeding,

the carcasses were left for an hour before weighing again to obtain bled weight value. Thereafter, they were all immersed in hot water for two minutes to ease defeathering (Alkan *et al.,* 2010). Then the carcasses were weighed without the heads and shanks to obtain defeathered weight. The carcasses were cut open from the breast region through the abdomen to collect the kidneys, gizzards, livers, hearts and testes (Yannakopoulos and Tserveni-Gousi, 1986; Florou-Paneri, 1989; Alkan *et al.,* 2010). Also, the developing yolk and eggs were carefully removed and counted to obtain number of yolk and formed eggs. Thereafter, all the organs were weighed to obtain organ weights (Alamuoye and Ojo, 2015). The carcasses were individually weighed to obtain carcass weight and the dressing percentage value was determined using equation VI.The sex was reaffirmed at slaughter by carefully observing the testes/ovaries in the eviscerated carcasses and nonconformity with the identified sex was considered reversed sex. Throughout the study period, every dead bird was recorded as mortality and the mortality rate was calculated using equation VII and the survival rate was determined using equation VIII.

$$\text{Dressing per centage} = \frac{\text{Carcass weight}}{\text{Live weight}} \text{ X } \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{equation VI}$$

$$\text{Mortality rate} = \frac{\text{Total number of dead birds}}{\text{Total number of chicks hatched}} \text{ X } \frac{100}{1} \ldots\ldots\ldots \text{equation VII}$$

Survival rate = 100 – mortality rate……...……equation VIII

3.6.4.2 Data analysis

Data collected were subjected to analysis of variance procedure of SPSS (2010) and mean values were separated using Duncan Multiple Range Test of the same software package.

3.6.5 Experiment 5: Efficiency test of the fabricated incubators

A total of six hundred and forty-eight Japanese quail eggs were gathered in 3 days from layers reared ina deep litter system at the middle of egg production cycle. The eggs were kept in the pen to cool at room temperature before setting on the 4^{th} day. One hundred and eight eggs were randomly assigned to each incubator based on weight such that each egg tray in the incubators held 36 eggs. The egg trays were turned every hour and the water troughs inside the incubators were refilled every morning.

3.6.5.1 Data collection

3.6.5.1.1 Temperature, relative humidity, hatchability parameters and chick mortality

All the eggs per each egg tray were weighed together to obtain egg weight using a sensitive weighing scale and at hatch, all the chicks per each egg tray were weighed together (five per group) to obtain chicks' weight per group. The values obtained were divided by the total number of chicks to obtain individual chick weight. Meanwhile, because all the eggs did not hatch, an equivalent number of hatched eggs were used and the average egg weight of that equivalent number and chicks' weight were used to obtain chick yield value. Incubation and ambient temperature as well as relative humidity values were recorded daily at 9:00am, 12:00noon and 3:00pm using hygrothermometer during the incubation periods. The time between egg incubation and the emergence of the first chick was considered as the incubation period. After the emergence of the first group of chicks, other eggs were still left in the incubators for an extra 5 days, in case of possible late hatching. Thereafter, all the unhatched eggs were carefully windowed to evaluate the physiological status of the developing embryos that could not emerge as chicks. Such physiological statuses were eggs without any obvious signs of growth, rather intact yolk and albumen were still in a fluid form and such eggs were considered as "infertile eggs". Eggs with intact yolk and albumen but in a dried form were designated as "caked eggs" and those with intact yolk and albumen but with blood spot, blacken spot and partially dried yolk and albumen were considered as "not clear whether fertile or not". Developed embryos with undifferentiated body parts but not alive were termed "early dead embryos whereas, those with defined body parts and downy feathers without pipping were referred to as "fully developed but dead embryos". The fully developed embryos that pipped and died in the process were called "pipped and dead embryos" while those that were alive and pipping but could not complete the pipping process were regarded to as "pipped and alive but not able to emerge embryos". Those that were fully developed, pipped and emerged alive were considered as "hatched chicks". The hatched chicks were allowed to remain in the incubators until fluffy dried, weighed to obtain chick weight value and were transferred to the Brooding Unit, Poultry Division, NVRI. The egg hatchability, fertility and chick yield values were calculated using equations I, II and III respectively. Every dead bird was recorded and the mortality rate was calculated using equation IV.

$$\text{Egg hatchability} = \frac{\text{Total number of hatchlings}}{\text{Total number of fertile eggs}} \text{ X } \frac{100}{1} \ldots\ldots\ldots\ldots\ldots \text{equation I}$$

$$\text{Egg fertility} = \frac{\text{Total number of fertile eggs}}{\text{Total number of eggs set}} \text{ X } \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots \text{equation II}$$

$$\text{Chick yield} = \frac{\text{Total chicks' weight at dayold}}{\text{Total weight of eggs at setting}} \text{ X } \frac{100}{1} \ldots\ldots\ldots\ldots\ldots \text{equation III}$$

$$\text{Mortality rate} = \frac{\text{Total number of dead birds}}{\text{Total number of chicks hatched}} \text{ X } \frac{100}{1} \ldots\ldots\ldots\ldots \text{equation IV}$$

Where:Fertile eggs = the summation of "early dead embryos", "fully developed but dead embryos", "pipped and dead embryos", "pipped and alive but not able to emerge embryos" and "hatched chicks".

3.6.5.2 Data analysis

Data collected were subjected to analysis of variance procedure of SPSS (2010) and mean values were separated using Duncan Multiple Range Test of the same software package.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Results

4.1.1 Optimum incubation temperature determination in Japanese quail

4.1.2: Japanese quail eggs incubation temperature and relative humidity

Table 4.1 presents the incubation and ambient temperature as well as relative humidity valuesrecorded during Japanese quail eggs incubation. There were significant differences ($P<0.05$) in all the parameters monitored across the treatments except in incubation relative humidity. It was shown that the incubation temperature was within the desired values calibrated to represent the treatments: Very low incubation temperature(36^{o}C), Low incubation temperature(37^{o}C), Medium incubation temperature(38^{o}C), High incubation temperature(39^{o}C) and Very high incubation temperature(40^{o}C), respectively. However, fluctuation of additional range of 0.11 to 0.27^{o}C was recorded with a minimum temperature of 34.45^{o}C and maximum of 41.06^{o}C during the period. While the ambient temperature values did not differ significantly ($P>0.05$) between T_3 (28.82^{o}C) and T_4 (29.00^{o}C), they were statistically different ($P<0.05$) from T_1 (27.67^{o}C) and T_2 (27.61^{o}C) that were similar and T_5 (28.3^{o}C) was somewhat similar to all the observed values in other treatments. Incubation relative humidity values ranged from 27.47% (T_1) to 27.48% (T_5) and ambient relative humidity was least (10.04%) in T_1 and highest (20.14%) in T_3 and T_5.

Table 4.1: Temperature and relative humidity during Japanese quail eggs incubation

	Treatments					Statistics			
	T_1	T_2	T_3	T_4	T_5			Overall	
Parameters	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**Min**	**Max**	**mean**	**SEM**
IT (°C)	36.11^{e}	37.20^{d}	38.22^{c}	39.27^{b}	40.12^{a}	34.45	41.06	38.37	0.08
AT (°C)	27.67^{b}	27.61^{b}	28.82^{a}	29.00^{a}	28.30^{ab}	20.52	38.28	28.43	0.13
IRH (%)	27.47	27.30	27.44	27.35	27.48	27.04	38.02	26.44	0.26
ARH (%)	10.04^{d}	10.89^{c}	20.14^{a}	12.90^{b}	20.14^{a}	10.04	22.06	14.74	0.22

abcde: Mean values on the same row with different superscript differ statistically at 5% probability test; SEM: Standard error of means; IT: Incubation temperature; AT: Ambient temperature; IRH: Incubation relative humidity; ARH Ambient relative humidity; Min: Minimum value; Max: Maximum value.

4.1.3: Japanese quaileggs hatchability

Physiological status of Japanese quail eggs incubated at 36 to 40^{o}C, hatchabilityand chick yieldare given in table 4.2. There wereno statistical differences (P>0.05) in total egg weight, average egg weight, infertile eggs, hatched chicks, fertile eggs, averagechick weight, hatchability,fertility and chick yield. While the average egg weight value was approximately 10g across the treatments, the incubation period was observed to be shortest (16 days) in T_4 (39^{o}C) and as long as 20 days in T_1.

Infertile eggs ranged from 7.64(T_4) to 12.31 (T_5), more early dead embryos were recorded in T_2 (27.70) and T_5 (21.67) but these values were statistically similar (P>0.05) to 18.32 recorded in T_4whose value wassimilar to 15.13 (T_3) and 12.03 (T_1).Fertile eggs ranged from 61.00(T_4) to 66.80 (T_3), hatched chicks were more in T_3 (24.00) slightly followed by T_2 (23.00),T4 (22.34) and fewer(14.67) in T_1. Average chick weight value varied from 5.45g (T_2) to 6.67g (T_1), hatchability varied from 23.93% in $T_1$to 36.90%in $T_2$and fertility rate value ranged from 57.09% (T_4) to 62.43% (T_3). Chick yield value was lowest (53.02%) in T_2 and was as high as 66.60% in T_4.

Table 4.2: Physiological status of Japanese quaileggs incubated at 36 – 40°C,hatchability and chick yield

	Treatments					
	T_1	T_2	T_3	T_4	T_5	
Parameters	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**SEM**
Total eggs set	321	321	321	321	321	-
TEW (g)	3361.18	3306.68	3238.87	3222.82	3352.13	3.11
AEW (g)	10.34	10.28	10.14	10.00	10.42	0.04
IP (days)	20	18	17	16	18	-
Unfertile eggs: No evidence of embryo growth						
Infertile eggs	10.68	8.30	7.67	7.64	12.31	1.22
Caked eggs	19.78^{ab}	25.70^{a}	16.53^{b}	22.64^{ab}	15.71^{b}	1.64
NCWFN	15.24^{a}	10.67^{b}	16.00^{a}	15.72^{a}	12.51^{b}	1.35
Fertile eggs: Evidence of embryo growth						
EDE	12.03^{b}	27.70^{a}	15.13^{b}	18.32^{ab}	21.67^{a}	7.68
FDDE	28.60^{a}	8.06^{c}	14.67^{b}	12.67^{b}	14.33^{b}	2.37
PDE	4.67^{c}	3.57^{c}	13.00^{a}	7.67^{b}	10.13^{a}	0.48
PAE	1.33^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.17
Hatched chicks	14.67	23.00	24.00	22.34	20.34	3.05
Fertile eggs	61.30	62.33	66.80	61.00	66.47	4.83
Fertility (%)	57.29	58.25	62.43	57.09	62.12	2.64
Hatchability and chick yield						
Hatchability (%)	23.93	36.90	35.93	36.62	30.60	4.15
TCW (g)	96.89^{b}	125.78^{ab}	183.02^{a}	147.22^{ab}	122.89^{ab}	20.31
ACW(g)	6.67	5.45	6.64	6.66	6.10	0.17
Chick yield (%)	64.50	53.02	65.48	66.60	58.54	3.76

abcde: Mean values on the same row with different superscript differ statistically at 5% probability test;SEM: Standard error of means; TEW: Total egg weight; AEW: Average egg weight; IP: Incubation period; NCWFN: Not clear whether fertile eggs or not; EDE: Early dead embryos; FDDE: Fully developed but dead embryos; PDE: Pipped and dead embryos; PAE: Pipped and alive but not able to emerge embryos; TCW: Total chick weight; ACW: Average chick weight.

4.1.4: Post-hatchperformance of Japanese quail chicks subjected to varied incubation temperature

4.1.4.1: Brooding of Japanese quail chicks hatched at varied incubation temperature

Table 4.3 shows the temperature and relative humidity during brooding of Japanese quail chicks. There were no significant differences (P>0.05) in all the parameters across the treatments. While the brooding pen mean temperaturevaried between 34.61^{o}C (week 2) and 34.81^{o}C (week 1), the ambient mean temperature ranged from 26.69^{o}C (week 3) to 27.16^{o}C (week 2).The brooding pen relative humidity value varied from 69.98 to 70.87%,similar to a range of 67.97 to 69.44% recorded as ambientrelative humidity throughout the study period.

Table 4.3: Brooding temperature and relative humidity of Japanese quail chicks

	Brooding period											
	Week 1				Week 2				Week 3			
Parameters	**Mean**	**Min**	**Max**	**SEM**	**Mean**	**Min**	**Max**	**SEM**	**Mean**	**Min**	**Max**	**SEM**
Temperature (^{o}C)												
Brooding pen	34.81	30.50	34.92	0.43	34.61	30.50	34.84	0.41	34.74	30.50	34.80	0.42
Ambient	27.12	24.00	30.80	0.35	27.16	24.00	29.40	0.35	26.69	24.00	29.40	0.33
Relative humidity (%)												
Brooding pen	69.98	54.00	79.00	0.88	70.82	61.00	79.00	0.76	70.87	61.00	79.00	0.70
Ambient	67.97	50.00	79.00	1.19	68.55	50.00	79.00	1.17	69.44	50.00	79.00	1.18

SEM: Standard error of means; Min: Minimum value; Max: Maximum value

4.1.4.2: Feed intake of Japanese quails hatched at varied incubation temperature

Daily feed intake by Japanese quail chicks hatched at 36^{o}C, 37^{o}C, 38^{o}C, 39^{o}C and 40^{o}C is provided in table 4.4. There were significant differences ($P<0.05$) in the feed intake values across the treatments during the 6 weeks study. It was observed that the population size was not consistent, yet feed intake was increasing with age among all the treated birds except, in cases where population size reduced due to mortality.

It was observed that in week 2, feed intake per chicks ranged from 7.67g (T_1) to 11.75g (T_5), week 3 (7.26 to 13.95g), week 4 (10.61 to 16.83g), week 5 (9.34 to 14.64g) and in week 6, it was highest (19.30g) in T_1, followed by 14.85g (T_3, T_4), 13.68g (T_5) and10.18g (T_2), accordingly. Feed intake per group of chicks in week 2,ranged from as low as 24.67g (T_1) to as high as 553.09g(T_5), week 3 (29.14 to 632.07g), week 4 (25.28 to 540.28g), week 5 (20.14 to 623.21g) and in week 6, it was highest (597.00g) in T_5, followed by 512.90g (T_4), 386.70g (T_3), 172.28g (T_2) and 19.30g (T_1),accordingly.

Table 4.4: Weekly feed intake by Japanese quail chicks hatched at 36 – 40°C

		Treatments					
		T_1	T_2	T_3	T_4	T_5	
Week	**Parameters**	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**SEM**
1	No. of chicks	44	69	72	67	61	-
	Feed intake/group (g)	ND	ND	ND	ND	ND	-
	Feed intake/chick (g)	ND	ND	ND	ND	ND	-
2	No. of chicks	3	20	26	34	44	-
	Feed intake/group (g)	24.67^{d}	209.86^{c}	288.57^{b}	283.43^{b}	553.09^{a}	8.65
	Feed intake/chick (g)	7.67^{b}	9.94^{a}	10.84^{a}	8.82^{ab}	11.75^{a}	0.56
3	No. of chicks	3	18	26	34	44	-
	Feed intake/group (g)	29.14^{e}	128.93^{d}	380.07^{c}	444.00^{b}	632.07^{a}	13.40
	Feed intake/chick (g)	9.71^{b}	7.26^{c}	13.93^{a}	13.35^{a}	13.95^{a}	1.31
4	No. of chicks	2	16	26	33	44	-
	Feed intake/group (g)	25.28^{d}	171.71^{c}	443.36^{b}	453.93^{b}	540.28^{a}	11.88
	Feed intake/chick (g)	12.84^{b}	10.61^{c}	16.83^{a}	12.92^{b}	12.30^{b}	1.01
5	No. of chicks	2	16	26	33	44	-
	Feed intake/group (g)	20.14^{e}	147.30^{d}	369.00^{c}	520.86^{b}	623.21^{a}	13.43
	Feed intake/chick (g)	9.78^{b}	9.34^{b}	14.20^{a}	14.64^{a}	13.87^{a}	0.89
6	No. of chicks	1	16	26	33	43	-
	Feed intake/group (g)	19.30^{e}	172.28^{d}	386.70^{c}	512.90^{b}	597.00^{a}	13.09
	Feed intake/chick (g)	19.30^{a}	10.18^{c}	14.85^{b}	14.85^{b}	13.68^{b}	1.15

abcde: Mean values on the same row with different superscript differ statistically at 5% probability test; SEM: Standard error of means, ND: Not determined due to irregular incubation periods;Group: Represent total number of birds per treatments.

4.1.4.3: Growth of Japanese quail chicks hatched at varied incubation temperature

Table 4.5 gives the weekly growth pattern of Japanese quail chicks hatched at 36^{o}C, 37^{o}C, 38^{o}C, 39^{o}C and 40^{o}C. There were significant differences ($P<0.05$) in all the parameters measured across the treatments during the period except in week 1, when the weight per chick value did not vary statistically ($P>0.05$). In week 2, absolute growth rate varied from 14.52g in T_2 to 24.84g in T_5, week 3 (12.94 to 26.25g), week 4 (5.26 to 30.61g) and in week 5, it was highest (16.61g) in T_1 and lowest (5.26g) in T_3.

Table 4.5: Weekly growth pattern of Japanese quail chicks hatched at 36 – 40°C

		Treatments					
		T_1	T_2	T_3	T_4	T_5	
Week	**Parameters**	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**SEM**
1	No. of chicks	44	69	72	67	61	-
	Weight/group (g)	290.69^{c}	377.35^{b}	509.14^{a}	441.67^{b}	368.90^{b}	0.54
	Weight/chick (g)	6.60	6.42	6.84	6.38	5.88	0.58
	Absolute growth rate	0.00	0.00	0.00	0.00	0.00	0.00
	No. of chicks	3	18	26	34	44	-
	Weight/group (g)	71.14^{e}	372.64^{d}	839.99^{c}	924.53^{b}	1249.90^{a}	0.53
	Relative growth rate	23.21^{b}	20.94^{b}	31.10^{a}	29.09^{a}	30.72^{a}	3.61
2	Absolute growth rate (g)	16.61^{c}	14.52^{c}	24.26^{a}	22.71^{b}	24.84^{a}	2.04
	No. of chicks	2	16	26	33	44	-
	Weight/group (g)	109.98^{e}	637.14^{d}	1490.69^{c}	1691.63^{b}	2207.46^{a}	15.28
	Relative growth rate	36.15^{c}	34.77^{c}	53.62^{a}	50.78^{b}	49.72^{b}	4.17
3	Absolute growth rate (g)	12.94^{c}	13.83^{c}	22.52^{b}	26.25^{a}	24.82^{b}	2.34
	No. of chicks	2	16	26	33	44	-
	Weight/group (g)	107.98^{e}	653.14^{d}	1466.19^{c}	1665.31^{b}	2164.46^{a}	14.71
	Relative growth rate	53.56^{b}	53.92^{b}	55.93^{ab}	55.34^{ab}	55.54^{ab}	6.99
4	Absolute growth rate (g)	30.61^{a}	19.52^{b}	5.26^{d}	6.71^{cd}	7.84^{c}	4.89
	No. of chicks	2	16	26	33	44	-
	Weight/group (g)	134.24^{e}	1016.95^{d}	1269.94^{c}	1305.31^{b}	1803.721^{a}	14.71
	Relative growth rate	66.56^{a}	63.92^{a}	60.93^{b}	61.78^{b}	62.72^{b}	6.99
5	Absolute growth rate (g)	16.61^{a}	9.52^{b}	5.26^{c}	6.71^{c}	6.84^{c}	11.89

abcde: Mean values on the same row with different superscript differ statistically at 5% probability test; SEM: Standard error of means; Group: Represent total number of birds per treatments.

4.1.4.4:Blood profiles of Japanese quail chicks hatched at varied incubation temperature

Table 4.6 shows the blood profiles of Japanese quail chicks hatched at 36^{o}C, 37^{o}C, 38^{o}C, 39^{o}C and 40^{o}C. There were no significant differences (P>0.05) in all the parameters evaluated except in packed cell volume, red blood cells and neutrophil. Packed cell volume was superior (P<0.05) in T_4 (45.21%), slightly followed by T_5 (44.79%), T_3 (40.48%), T_2 (40.00%)and T_1 (35.04%). While a similar trend was observed in red blood cells, T_2 value (28.52%) was highest in neutrophil, slightly followed by 28.00% in T_1 with the least value (17.51%) recorded in T_4. White blood cells were more (9.33 x 10^9/L) in T_4, slightly followed by T_5 (9.03 x 10^9/L), T_2 (8.36 x 10^9/L), T_1 (5.44 x 10^9/L) and T_3 (4.95 x 10^9/L). Even when monocytes were not detected in birds in T_1 (absolutely 0.00%), it was as high as 2.50% in T_4, lymphocytes value ranged between 68.67% and 81.02% and eosin was only detected in T_2 (0.63%) and T_4 (0.67%).

Table 4.6: Blood profiles of Japanese quail chicks hatched at 36 – 40^{o}C

	Treatments					Statistics			
	T_1	T_2	T_3	T_4	T_5			Overall	
Parameters	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**Min**	**Max**	**mean**	**SEM**
No. of birds	1	8	8	6	8	1	8	-	-
PCV (%)	35.04^{b}	40.00^{ab}	40.48^{ab}	45.21^{a}	44.79^{a}	28	50	41.42	0.86
RBC (x10^{12}/L)	0.82^{c}	1.43^{bc}	1.84^{abc}	2.77^{a}	2.09^{ab}	0.8	4.0	1.92	0.15
WBC (x10^{9}/L)	5.44	8.36	4.95	9.33	9.03	2.3	15.6	7.37	0.53
HB (g/dL)	8.44	9.75	9.57	9.43	8.78	6.0	13.8	9.34	0.28
Neut (%)	28.00^{a}	28.52^{a}	21.12^{ab}	17.51^{b}	22.02^{ab}	4.0	43.0	21.22	1.38
Lym (%)	72.00	68.67	76.55	81.02	76.89	49	96	76.6	1.81
Mono (%)	0.00	2.13	2.38	2.50	1.13	0.0	19.0	2.08	0.69
Eosin (%)	0.00	0.63	0.00	0.67	0.00	0.0	4.0	0.31	0.13

abc: Mean values on the same row with different superscript differ statistically at 5% probability test; Min: Minimum value; Max: Maximum value; SEM: Standard error of means; PVC: Packed cell volume; RBC: Red blood cells; WBC: White blood cells; HB: Haemoglobin; Neut:Neutrophils; Lym: Lymphocytes; Mono: Monocytes; Eosin: Eosinophil.

4.1.4.5: Serum biochemistry of Japanese quail chicks hatched at varied incubation temperature

Serum biochemistry of Japanese quail chicks hatched at 36^{o}C, 37^{o}C, 38^{o}C, 39^{o}C and 40^{o}C is provided in Table 4.7. There were statistical variations ($P<0.05$) in all the parameters determined except, in albumin whose value ranged from 13.89g/L (T_4) to 17.53g/L (T_1). Alanine aminotransferase value varied from 6.00U/L (T_1) to 26.56U/L (T_5), total protein (26.67g/L to 53.42g/L), creatinine (39.30Umol/L to 46.57Umol/L) and cholesterol value was lowest (162.42mg/dL) in T_5 with the highest value (200.12mg/dL) recorded in T_1.

Table 4.7: Serum biochemistry of Japanese quail chicks hatched at 36 – 40°C

	Treatments					Statistics			
	T_1	T_2	T_3	T_4	T_5				
Parameters	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**Min**	**Max**	**Mean**	**SEM**
No. of birds	1	8	8	6	8	-	-	-	-
ALT (U/L)	6.00^{b}	12.56^{ab}	15.14^{ab}	14.00^{ab}	26.56^{a}	4.0	52.0	15.67	1.36
TP (g/L)	53.42^{a}	30.10^{b}	32.78^{b}	26.67^{b}	31.02^{b}	20.4	53.4	31.33	1.36
ALB (g/L)	17.53	16.58	14.10	13.89	15.58	9.9	30.5	15.59	0.77
CREAT (Umol/L)	39.30^{b}	45.11^{ab}	42.89^{ab}	45.44^{ab}	46.57^{ab}	32.2	84.4	46.78	1.61
CHOL (mg/dL)	200.12^{a}	183.89^{ab}	168.78^{ab}	177.54^{ab}	162.42^{ab}	99.90	219.90	169.73	4.85

ab: Mean values on the same row with different superscript differ statistically at 5% probability test; Min: Minimum value; Max: Maximum value; SEM: Standard error of means; ALT: Alanine aminotransferase; TP: Total protein; ALB: Albumin; CREAT: Creatinine; CHOL: Cholesterol.

4.1.4.6: Sexual maturity, sex reversal, carcass quality and organs weight of Japanese quail chicks hatched at varied incubation temperature

Sexual maturity, sex reversal, carcass quality and organs weight of Japanese quail chicks hatched at 36^{o}C, 37^{o}C, 38^{o}C, 39^{o}C and 40^{o}C are presented in table 4.8. There were significant differences($P<0.05$) in all the parameters measured.While the hens in T_4 and T_5started laying eggs at the age of 5 weeks,those in T_1, $T_2$and T_3started laying eggs at 6 weeks of age. Though two cases of reversed sex from female to male were observed only in T_4, the sex ratio values were 0:1, 8:9; 12:13, 1:1 and 7:16 in T_1, T_2, T_3, T_4 and T_5 in that arrangement. While the live weight ranged from 99.89g (T_2) to 134.18g (T_1), carcass weight value (97.44g) was significantly best ($P<0.05$) in T_1compared to 68.12g recorded in T_2. The dressing percentage value varied between 66.78% (T_5) and 72.52% (T_1), gizzard weight (3.23 to 4.24g), liver weight (1.88 to 3.12g) and heart weight varied between 0.90g(T_2) and 1.30g (T_4). The right testis (1.84g) and left testis (1.81g) weight values were superior in T_4, compared to 1.22g (right) and 1.43g (left) recorded in T_3.

Table 4.8: Sexual maturity, sex reversal, carcass quality and organs weight of Japanese quail chicks hatched at 36 – 40°C

		Treatments					
		T_1	T_2	T_3	T_4	T_5	
Parameters		**36**°C	**37**°C	**38**°C	**39**°C	**40**°C	**SEM**
Sexual maturity							
Age at point of lay (wks)		6	6	6	5	5	-
Yolk		6.00^{a}	2.00^{b}	3.67^{b}	0.00^{c}	2.00^{b}	0.48
Formed eggs		0.00^{ab}	0.00^{b}	1.00^{a}	0.00^{b}	0.00^{b}	0.00
Sex ratio (M : F)		0:1	8:9	12:13	1.1	7:16	-
Reversed	M → F	0	0	0	0	0	-
sex	F → M	0	0	0	2	0	-
Carcass quality (g)							
No. of birds		1	8	8	6	8	-
Live weight		134.18^{a}	99.89^{c}	117.12^{ab}	110.89^{ab}	115.78^{bc}	6.79
Bled weight		128.67^{a}	95.67^{c}	111.67^{bc}	105.13^{bc}	110.50^{bc}	2.86
De-feathered weight		113.20^{a}	82.20^{c}	97.67^{ab}	90.78^{bc}	95.62^{bc}	2.65
Carcass weight		97.44^{a}	68.12^{c}	80.00^{bc}	74.28^{bc}	77.12^{bc}	1.97
Dressing percentage (%)		72.52^{a}	68.11^{b}	68.42^{b}	68.89^{b}	66.78^{b}	0.56
Organs weight (g)							
Gizzard weight		4.24^{a}	3.28^{b}	3.23^{b}	3.33^{b}	3.64^{ab}	0.12
Liver weight		3.12^{ab}	2.55^{ab}	2.09^{ab}	1.88^{b}	2.61^{ab}	0.17
Heart		1.13^{ab}	0.90^{b}	1.05^{ab}	1.30^{a}	1.14^{ab}	0.03
Testes	Right	0.00^{b}	1.51^{a}	1.22^{a}	1.84^{a}	1.56^{a}	0.13
weight	Left	0.00^{b}	1.47^{a}	1.43^{a}	1.81^{a}	1.58^{a}	0.11

abc: Mean values on the same row with different superscript differ statistically at 5% probability test;

SEM: Standard error of means.

4.1.4.7:Mortality rate of Japanese quail chicks hatched at varied incubation temperature

Mortality of Japanese quail chicks during the brooding and grower phases is given in table 4.9. There were significant differences ($P<0.05$) across the treatment mean values during the periods.Except at 4 – 5 weeks of age, when low mortality (0.67) was recorded in $T_2$with absolutely zero mortality in T_1, T_3, T_4 and T_5. Meanwhile, during the brooding phase (weeks 1 – 3), least mortality (1.89) was recorded in T_5, unlike in T_1 where as high as 18.00 chicks were lost, slightly followed by 13.73 (T_2),13.36 (T_3)and 6.85 (T_4). On the other hand, more mortality (3.00) was observed in T_4 and T_5 at sexual maturity (5 – 6 weeks of age), compared to 0.67 recorded in T_1.

Table 4.9: Mortality of Japanese quail chicks hatched at 36 – 40^{o}C

	Treatments					Statistics		
	T_1	T_2	T_3	T_4	T_5			
Age (weeks)	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**Min**	**Max**	**SEM**
1 - 3	18.00^{a}	13.73^{b}	13.36^{b}	6.85^{c}	1.89^{d}	0.00	18.00	0.41
3 - 4	0.00^{c}	1.00^{b}	2.50^{a}	1.17^{b}	1.00^{b}	0.00	4.00	0.09
4 - 5	0.00	0.67	0.00	0.00	0.00	0.00	2.00	0.16
5 - 6	0.67^{bc}	1.25^{b}	1.50^{b}	3.00^{a}	3.00^{a}	1.00	3.00	0.18

abc: Mean values on the same row with different superscript differ statistically at 5% probability test; Min: Minimum value; Max: Maximum value; SEM: Standard error of means.

4.1.4.8: Survival rate of Japanese quail chicks hatched at varied incubation temperature

Presented in table 4.10 is the survival rate of Japanese quail chicks hatched at 36^{o}C, 37^{o}C, 38^{o}C, 39^{o}C and 40^{o}C. Statistical variations ($P<0.05$) were observed in all the parameters evaluated except in hatched chicks,whose valueranged from 20.27 in T_5 to 24.00 in T_3.Whereas, the mortality rate varied from 33.50(T_5) to 85.47% (T_1), the survival rate value was statistically superior ($P<0.05$) in T_5 (66.50%), followed by 46.10% (T_4), 27.40% (T_2), 21.50% (T_3) and 14.53% (T_1) in that order.

Table 4.10: Survival rate of Japanese quail chicks hatched at 36 – 40°C

	Treatments					Statistics		
	T_1	T_2	T_3	T_4	T_5			
Parameters	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**Min**	**Max**	**SEM**
Hatched chicks	22.00	23.00	24.00	22.28	20.27	2.00	38.00	3.05
Chick mortality	18.50^{b}	16.67^{c}	18.80^{a}	11.67^{d}	6.67^{e}	1.00	38.00	2.73
Mortality rate (%)	85.47^{a}	72.60^{c}	78.50^{b}	53.90^{d}	33.50^{e}	16.70	100.00	6.57
Survival rate (%)	14.53^{e}	27.40^{c}	21.50^{d}	46.10^{b}	66.50^{a}	16.70	83.30	6.25

abcde: Mean values on the same row with different superscript differ statistically at 5% probability test; Min: Minimum value; Max: Maximum value; SEM: Standard error of means.

4.1.5 Discussion

4.1.6Optimum incubation temperature in Japanese quails production

Results showed that the incubation temperature values were similar to what was reported in imported incubators. However, some variations withina range of 0.2 – 0.4^{o}C that Poultry Hub (2018) reported not only to be safe for poultry eggs incubation but the effective incubation temperature for high hatchability rate. Also, it was within variations of up to 0.2 – 1.2°C that French (2000) found within an incubator and stated that reducing the air temperature variation required a uniform air flow throughout the incubator. On the other hand, it was lower than 0.4 – 3.0^{o}C fluctuation that was reported to be dependent on the incubator design (Mauldin and Buhr, 1995; French, 1997). It seemingly appeared that achieving the perfect precision value of 100% of calibrated incubation temperature in poultry eggs incubators may not be feasible. This is possible because, when the thermostat automatically trips off the heat source at the regulated temperature value, there will still be heat production for a while, which the thermometer responds to. Meanwhile, the recorded treatments incubation temperature values were similar to 33 – 40^{o}C reported to be the lower limit of optimal embryo development in avian species (Göth and Booth, 2005; Mani*et al.,* 2008; ECQ, 2015; Pam, 2015). Meanwhile, a range of 37 – 38^{o}C have been reported as the optimum operating temperatures (Archer and Cartwright, 2018; Sartell, 2018) for avian species and it was stated that deviations from this optimum value, could have a major impact on hatching success (Wilson, 1991). Also, it was reported that temperature outside the optimum range could affect post-hatch performance in avian species (Gladys *et al*., 2000; Hulet *et al*., 2000; Lourens and Middelkoop, 2000). French (2009) suggested that it would be more convenient to describe optimum incubation temperature in a range of values, rather than using a single value as an optimum incubation temperature. This could be largely due to fluctuations that are bound to occur in incubators, no matter the design and effectiveness. Nonetheless, in the present study, the hatchability output which is the essence of artificial incubation was recorded thus, the fabricated incubators were probably suitable for use in Japanese quail eggs incubation.

The incubation relative humidity recorded in each of the treatments was lower than a range of 55 – 65% observed in Japanese quails incubation (Pedroso *et al*. 2006), 45 – 55% in waterfowl, 40 – 50% in poultry, 35 – 45% in parrots and more than 65% in all avian

species (Cutler and Abbott, 1991). However, the recorded values were similar to 36% observed in quail eggs incubation with the highest hatchability rate (Romao *et al*., 2009a). Thus, low incubation relative humidity may not play a vital role in Japanese quail eggs incubation. The ambient temperature values recorded were within 20 – 34^{o}C, but the ambient relative humidity was less than 60 – 80% reported to be the ideal values in rearing poultry species (Poultry CRC, 2016). These values were both close to 20 – 27^{o}C and 50 – 90% predicted to prevail during the period (December/January) in Jos, Plateau State (EUMETSAT, 2017). Therefore, the microclimatic conditions required for the study that informed the conduct of the experiment in NVRI, Jos Plateau State was observed.

The average weight of the incubated eggs across the treatments was within 7.69 – 10.80g reportedin Japanese quails (Indreswari *et al.*, 2019; Ajide 2011) but somewhat less than 10 – 12g speculated to be the normal size of Japanese quail eggs (FAO, 2003). Therefore, the eggs used in this study were perhaps normal. Meanwhile, the observed incubation period was within the range of 16 – 18 days recorded, when Japanese quail eggs were subjected to microclimatic conditions (Sellier *et al*., 2006). The shortest incubation period (16 days) recorded in T4 (treatment incubator regulated at 39^{o}C), probably implied economic benefit as the total hatchery cost could be cut down. This observation agreed with the report of Romao *et al*. (2009b) that higher incubation temperature at 40^{o}C resulted in early hatching (16 days) in Japanese quails, compared to those incubated at 35^{o}C that hatched on day 23. At hatch, the chicks weighed close to a range of 7.0 – 7.96g reported by Farghly *et al*. (2015), when Japanese quails of similar age were subjected to photostimulated conditions during egg incubation. This probably reflected that the treated birds were seemingly healthy with little or no adverse effects on their health. When the unhatched eggs were windowed after embryonic day 23, it was observed that some of the incubated eggs were "caked", "infertile" and "not clear whether fertile or not". The caked eggs were probably due to cracks that led to the loss of water, leaving the egg yolk and albumen in dried form. While the "infertile" eggs were probably due to the improper sex ratio of the flock, mating or fertilization failure, while the "not clear whether fertile or not" eggs were perhaps due to failure of the zygote to cleave and differentiate. This probably resulted in the regression or death of the zygote that left a stain (brown, black or blood) on the yolk, making it difficult to ascertain whether the egg was fertile or not. This observation was similar to a

condition of the tiny spot, occasionally found on egg yolk described as blood spots or egg spots, which do not however signify fertility in poultry eggs (AEB, 2018). It was stated that this condition was caused by rupture of blood vessels on the yolk surface or accidental oviductal wall rupture during egg formation.

In some other unhatched eggs windowed, it was observed that the embryos were at different physiological statuses of growth and development, which were either dead or could not emerge as hatchlings after embryonic day 23. Such developmental stages were described in this study as "early dead embryos" [embryonic days 1 – 7], "fully developed but dead embryos" [embryonic days 8 – 15], "pipped and dead embryos" [embryonic days 16 – 17] as well as "pipped and alive embryos but not able to emerge" [embryonic days 17 – 24]. However, these stages were similar to the conditions described earlier (Romao *et al*., 2009b) as "infertile", "early embryo death", "intermediate embryo death", "late embryo death" and "pipped egg with dead embryo". Meanwhile, Yilmaz *et al*. (2011) simply described similar stages as "early", "middle" and "late embryonic death". Although the causes of hatching failure are not clear according to Ramteke *et al.* (2013), the causes of the observed different physiological statuses of the embryos that could not emerge as hatchlings, could purely be unfavourable microclimatic conditions that perhaps resulted in metabolic disorder. Also, it could be partly due to microbial contamination, resulting in infection of the developing embryos and possibly due to the poor nutritional plane of the hens, resulting in low quality yolk and albumen that were unsuitable for the developing embryos. However, Woodard *et al*. (1973) reported that most embryonic deaths occur during the first 3 days of incubation and/or just prior to hatching. Also, it was stated that most eggs removed at the first candling (8 days of incubation) were infertile and early embryo deaths. It was further stressed that fatal mortality was largely due to the inability of the developing embryos to form vital organs or malfunctioning of the organs during development. Critical functions in such conditions include a change in position of the embryo prior to pipping, utilization of the remaining albumen, absorption of the yolk sack and change from allantoic to pulmonary respiration. It was added that there was an indication of a slight mid-incubation peak of mortality, suggestive of chickens formally associated with dietary deficits.

It was observed that the hatchability was poor and less than a range of 78.67 – 85.56% reported in Japanese quails (El-Kholy *et al*., 2019), probably due to fairly low egg fertility of 49 – 61% recorded. In any case, the observation was similar to 2.9 – 75.0% reported when Japanese quail eggs were incubated at different temperatures (Romao *et al*., 2009b) and when Japanese quail eggs were stored for 20 days before incubating at 39^{o}C (Mani *et al*., 2008). The observed 41 – 67% chick yield was less than 72% recordedwhen Akpinar *et al*. (2019) evaluated growth traits in Japanese quails and 67 – 68% described as optimum chick yield in poultry species (Aviagen, 2015). However, chick yield in T_3 (38^{o}C) and T_4 (39^{o}C) were similar to 67% that Poultry Site (2014a) reported as an ideal target for best chick quality. Although chicks hatched in all the treatments incubators set at 36^{o}C (Very low), 37^{o}C (Low), 38^{o}C (Medium), 39^{o}C (High) and 40^{o}C (Very high) incubation temperatures, there was reduced incubation period at 39^{o}C, followed by 38^{o}C, but chick yield was best at 38^{o}C, followed by 39^{o}C whereas, hatchability was best at 37^{o}C, followed by 39^{o}C, 38^{o}C and 40^{o}C in that order. Consequently, a range of 37 – 40^{o}C may be recommended as the optimum incubation temperature in Japanese quails.

The brooding temperature was similar to 21 – 36^{o}C recommended by (Hyaline, 2018; Penn State Extension, 2016; The Poultry Site, 2014b) in poultry chicks. The ambient temperature was similar to 23 – 30^{o}C reported by EUMETSAT (2017) to prevail during that time of the year (December/January) in Jos, Plateau State. Yet, the brooding pen and ambient relative humidity were maintained at similar range during the brooding period. This probably resulted in the feed intake pattern observed, that remained increasing with chick age, indicating that the birds were comfortable with the environmental conditions. The observed feed intake was somewhat similar to 8g (at 0 – 2 weeks old), 19.19g (at 2 – 4 weeks old) but less than 27.37g (at 4 – 6 weeks old) reported in Japanese quails that were administered water-soluble vitamins *in ovo* (El-Kholy *et al*., 2019). The differences may be largely due to the strain of Japanese quails used in the studies and probably environmental or treatment effects on the birds. Therefore, feed intake of Japanese quails subjected to low and high incubation temperature may not be compromised. The growth rate pattern was also increasing with the chick age, but seemingly dropped on the 3rd and 4th week of age, probably implying growth peak that Hossner (2005) described as “growth spurt” in farm animals. This scenario was similar to the observation of El-Kholy *et al*.

(2019) who equally recorded decline in growth at 4 – 6 weeks in Japanese quails. This possibly signified that the chicks gained more weight up to week 3 or 4 and declined, when a seemingly stable growth rate up to week 5 or 6 was established, suggesting that the chicks have probably attained their full growth potentials. Therefore, at this growth phase, crude protein in the diet could be reduced to maximize profit, while the energy level may be maintained or increased in order to meet the energy requirement.

According to Samour (2006), avian blood analysis often expresses the health status of the birds. The observed value of packed cell volume was somewhat higher than a range of 29.42 – 37% reported to be normal in avian species (Anggraeni *et al*., 2016; Sturkie and Griminger, 1976) but close to 40.9 – 55.00% reported by El-Kholy *et al*. (2019) in Japanese quails at 6 weeks of age. The disparities could be due to the age of the birds, environmental conditions of where the birds were raised and probably due to the laboratory protocol adopted (Givens *et al*., 2000). The red blood cells were similar to a normal range of 2.30 – 3.86 x $10^6/mm^3$ reported in healthy birds of similar age (Anggraeni *et al*., 2016; El-Kholy *et al*., 2019). Also, white blood cells, haemoglobin, neutrophils, lymphocytes, monocytes and eosinophil were within the normal ranges reported (Sturkie and Griminger, 1976; Anggraeni *et al*., 2016). This probably indicated that the treated chicks were not stressed, as evidently provided by Tamzil *et al*. (2014) that there was no depression in the haematological values, when chickens were subjected to acute heat stress. Meanwhile, the consistent least values recorded in T_1 (36^{o}C), in all the parameters evaluated except in neutrophils, could be largely due to late hatching of the chicks and partly due to incubation temperature (36^{o}C) of the eggs that probably led to facultative hypothermia. Essentially, avian neutrophils have been reported to be normally higher than white blood cells unlike in mammals. More so, it was speculated that avian neutrophils were equivalent to heterophil in mammals (Samour, 2006).

The heart and liver weights were close to 0.95g and 2.29g but the gizzard was greater than 1.63g reported by El-Kholy *et al*. (2019) in Japanese quails. The organs function tests as revealed by serum alanine aminotransferase, total protein, albumin, creatinine and cholesterol values compared favourably well with 6.5 – 9.6U/L, 31.6 – 36.5g/L, 13.3 – 15.3g/L, 4.0 – 4.5µmol/L and 235 – 259mmol/L respectively reported by Scholtz *et al*. (2009) as reference values in adult Japanese quails. Both values were similar to those

given more recently, as normal range in avian species (Mnisi and Mlambo, 2017). Nevertheless, there were little discrepancies in some of the parameters, probably as influenced by the age, strains, environmental conditions, unit of measurements and the trial the birds were subjected to in the respective studies. This probably showed that the birds' physiological systems were not compromised by the low or high incubation temperatures. Consequently, Japanese quails could build thermotolerant traits during embryogenesis in order to resist, survive and thrive well in high ambient temperature at post-hatch growth phases. This observation corroborated the report of Piestun *et al*. (2008) that avian species could acquire thermotolerant traits during embryogenesis. To buttress this, the hens in T_4 (39^{o}C) and T_5 (40^{o}C), started laying eggs at the same age of 5 weeks, signifying the attainment of sexual maturity. This probably implied that the birds developed coping strategy, in microclimate temperature close to its body temperature, in order to thrive well at high ambient temperature (Tzschentke and Basta, 2002; Moraes *et al*., 2003; Yahav *et al*., 2004). Although hens in T_1 (36^{o}C), T_2 (37^{o}C) and T_3 (38^{o}C) were yet to start laying eggs at 5 weeks of age, yolk and formed eggs were found *in utero* indicating sexual maturity. Even though two cases of reversed sex both from female to male was recorded only in T_4 (39^{o}C), the sex ratio was uniformly 1male: 1female except in T_1 (36^{o}C) where there was no male at all and T_5 (40^{o}C) where there was approximately 1 male: 2females.The observed sex reversal was probably a mere occurrence, which Jacob and Mather (2000) speculated to be normally observed in a flock once in a while. Therefore, incubation temperature ranging from 36 to 40^{o}C may not play a vital role in sex reversal in Japanese quails.

Interestingly, birds in T_1 (36^{o}C) had the best carcass quality in all the parameters evaluated, probably indicating a catch-up growth that Chin *et al*. (2013) reported in Japanese quail and compensatory growth (Metcalfe and Monaghan, 2001; Radder *et al*., 2007; Zhan *et al*.,2007) that was reported to be possibly gained, when there is distortion in the physiological processes in animals. This superior growth rate recorded, seemingly buttressed the possibility of using incubation temperature to improve breast meat, myo-fibre aggregation and growth rate in broilers (Joseph *et al*., 2006; Piestun *et al*., 2011; Piestun *et al*., 2013b). Meanwhile, the values of live weight, carcass weight and dressing percentage compared favourably well with those reported in healthy poultry species

(Yannakopoulos and Tserveni-Gousi, 1986; Bonos *et al*., 2010; ;Raji *et al*., 2015; Muhammad *et al*., 2017; Nasr *et al*., 2017).

It was observed that mortality was highest during the first few days of life that gradually reduced during the grower phase and rose again at 5 – 6 weeks of age. This may be probably due to the nutritional plane of the birds, endemic diseases in the environment or just a mere coincidence due to unknown factors. This observation lent more credence to the reports of Musa *et al.*, (2013),as well asTaskin and Karadavut (2014) that Japanese quails mortality could be high during few days of life but will decline after the brooding phase. Though, the cause of high mortality at this stage of growth in Japanese quails was not provided, it could be due to physiological distortion during embryogenesis as influenced by parental genetic makeup, the integrity of the incubated eggs and mechanisms of the incubators. The relatively high survival rate (66.50%) of birds recorded in T_5 probably indicated efficiency of the fabricated incubators. Therefore, the fabricated incubators were probably effective during the study period. Since mortality was recorded in all the treated birds, Japanese quail eggs could be incubated at 36 – 40^{o}C, with little or no deleterious effects on the physiological processes. However, incubation period and hatchability were best at 37 – 40^{o}C, chick yield was best at 38 – 39^{o}C, growth rate and dressing percentage were best at 36^{o}C whereas, survivability was best at 38 – 40^{o}C. Therefore, a wide range of 36 – 40^{o}C may be adopted as the optimum incubation temperature in Japanese quails production.

4.2 Results

4.2.1 Growth and developmental stages of Japanese quail embryos in eggs incubated at varying temperature

4.2.2: Incubation temperature of Japanese quail eggs opened on embryonicdays 1 – 20

Presented in Table 4.11 is the daily incubation temperature during Japanese quail embryogenesis. There were significant differences ($P<0.05$) among the values in control(38°C), T39°Cand T40°Ctreatments with minimum values ranging from 33.00^{o}C to 36.30^{o}C, compared to maximum values of between 37.20^{o}Cand 41.00^{o}C across thetreatments.

Table 4.11: Daily incubation temperature during Japanese quail embryogenesis

	Treatments					
Embryonic day	Control (38°C)	T36°C	T37°C	T38°C	T39°C	T40°C
0	38.00[a]	36.10	37.77	38.37	40.13[a]	41.10[a]
1	36.20[ab]	36.60	37.63	38.77	39.57[ab]	40.63[ab]
2	38.20[a]	36.17	37.50	38.20	39.60[ab]	40.70[ab]
3	37.73[a]	36.70	37.60	38.53	38.93[ab]	40.30[b]
4	38.23[a]	36.00	37.50	38.20	38.37[b]	40.50[ab]
5	37.80[a]	36.43	37.47	38.63	39.27[ab]	40.47[ab]
6	38.20[a]	36.40	37.90	38.33	39.80[ab]	40.67[ab]
7	37.97[a]	36.83	36.70	38.50	39.43[ab]	40.00[b]
8	38.17[a]	36.97	37.10	38.57	39.30[ab]	40.30[ab]
9	38.20[a]	36.80	37.50	38.43	39.43[ab]	40.27[ab]
10	33.43[b]	36.30	37.67	38.13	39.00[ab]	40.73[ab]
11	37.00[ab]	36.73	37.53	38.50	39.13[ab]	40.90[ab]
12	37.87[a]	36.60	37.50	38.37	39.63[ab]	40.67[b]
13	38.03[a]	36.87	37.57	38.60	39.77[ab]	40.50[ab]
14	38.20[a]	36.97	36.57	38.23	39.83[ab]	40.20[ab]
15	37.47[ab]	36.53	37.33	38.43	39.10[ab]	40.50[ab]
16	38.00[a]	36.50	37.50	38.03	39.10[ab]	40.27[ab]
17	36.07[ab]	36.17	37.40	38.43	39.77[ab]	40.60[ab]
18	38.13[a]	36.93	37.00	38.47	39.40[ab]	40.40[ab]
19	38.17[a]	36.30	37.70	38.33	39.73[ab]	40.40[ab]
20	38.13[a]	36.20	37.70	38.37	39.60[ab]	40.43[ab]
Minimum	34.40	33.00	34.50	35.10	35.70	36.30
Maximum	38.50	37.20	37.95	38.90	40.30	41.00
SEM	0.25	0.07	0.08	0.24	0.09	0.06

ab: Mean values on the same column with different superscript differ statistically at 5% probability test;SEM: Standard error of means.

4.2.3: Ambient temperature of incubated Japanese quail eggs

Table 4.12 presents the daily ambient temperature during Japanese quail embryogenesis. There were significant differences ($P<0.05$) in all the treatments with minimum values of 20.00^{o}C, 18.80^{o}C, 18.50^{o}C, 20.10^{o}C, 20.00^{o}C, 19.80^{o}C and maximum values of 32.50^{o}C, 31.50^{o}C, 31.50^{o}C, 38.20^{o}C, 32.50^{o}C, 32.20^{o}C in control(38^{o}C), T36^{o}C, T37^{o}C, T38^{o}C, T39^{o}C and T40^{o}Ctreatments,accordingly.

Table 4.12: Daily ambient temperature during Japanese quail embryogenesis

	Treatments					
Embryonic day	Control (38°C)	T36°C	T37°C	T38°C	T39°C	T40°C
0	32.13^{a}	30.83^{a}	30.90^{a}	31.57^{a}	32.13^{a}	31.53^{a}
1	29.37^{ab}	27.77^{ab}	28.40^{b}	31.80^{a}	29.37^{ab}	28.73^{ab}
2	29.50^{ab}	27.43^{ab}	28.87^{b}	28.90^{ab}	29.50^{ab}	28.53^{ab}
3	29.13^{ab}	28.33^{ab}	27.97^{b}	28.77^{ab}	29.13^{ab}	28.40^{ab}
4	29.23^{ab}	28.73^{ab}	28.50^{b}	32.30^{a}	29.23^{ab}	28.67^{ab}
5	30.43^{ab}	29.33^{ab}	29.47^{b}	26.77^{c}	30.43^{ab}	29.73^{ab}
6	30.13^{ab}	29.00^{ab}	28.87^{b}	29.57^{ab}	30.13^{ab}	29.37^{ab}
7	30.23^{ab}	27.90^{ab}	29.03^{b}	29.60^{ab}	30.23^{ab}	29.67^{ab}
8	28.30^{ab}	26.77^{ab}	27.20^{c}	27.83^{b}	28.30^{ab}	27.57^{ab}
9	29.27^{ab}	28.57^{ab}	28.23^{b}	28.73^{ab}	29.27^{ab}	28.83^{ab}
10	28.47^{ab}	27.20^{ab}	27.07^{c}	27.83^{b}	28.47^{ab}	27.70^{ab}
11	28.47^{ab}	27.53±^{ab}	27.30^{c}	28.00^{b}	28.47^{ab}	27.63^{ab}
12	27.90^{ab}	26.33^{ab}	26.47^{cd}	30.77^{ab}	27.90^{ab}	27.00^{ab}
13	27.53^{ab}	26.30^{ab}	25.77^{d}	27.10^{b}	27.53^{ab}	26.73^{ab}
14	28.63^{ab}	27.63^{ab}	25.93^{d}	28.03^{b}	28.63^{ab}	27.43^{ab}
15	26.63^{b}	25.40^{b}	25.43^{d}	26.37^{c}	26.63^{b}	26.30^{b}
16	27.77^{ab}	26.83^{ab}	26.23^{cd}	27.77^{b}	27.77^{ab}	27.27^{ab}
17	29.50^{ab}	27.90^{ab}	27.73^{b}	28.73^{ab}	29.50^{ab}	28.77^{ab}
18	28.60^{ab}	27.20^{ab}	26.23^{cd}	27.90^{b}	28.60^{ab}	27.73^{ab}
19	28.43^{ab}	26.93^{ab}	26.30^{cd}	27.83^{b}	28.43^{ab}	27.57^{ab}
20	29.10^{ab}	27.97^{ab}	27.07^{c}	28.60^{ab}	29.10^{ab}	28.37^{ab}
Minimum	20.00	18.80	18.50	20.10	20.00	19.80
Maximum	32.50	31.50	31.50	38.20	32.50	32.20
SEM	0.28	0.29	0.33	0.36	0.29	0.28

ab: Mean values on the same column with different superscript differ statistically at 5% probability test; SEM: Standard error of means.

4.2.4: Incubation relative humidity of incubated Japanese quail eggs

Expressed in Table 4.13 is the daily incubation relative humidity during Japanese quail embryogenesis. There were statistical differences ($P<0.05$) in all the mean values across the treatments, with minimum values that varied from 17.00^{o}C (T37^{o}C, T38^{o}C, T39^{o}C, T40^{o}C) to 22.00^{o}C (T36^{o}Cand maximum values between 24.00^{o}C (T36^{o}C) and 38.00^{o}C.in T37^{o}C, T38^{o}C, T39^{o}C and T40^{o}C.

Table 4.13: Daily incubation relative humidity during Japanese quail embryogenesis

	Treatments(%)					
Embryonic day	Control (38°C)	T36°C	T37°C	T38°C	T39°C	T40°C
0	20.33^{b}	26.00^{fgh}	34.00^{ab}	25.67^{ghij}	28.33^{efgh}	19.00^{m}
1	20.33^{b}	26.33^{efgh}	36.0^{a}	27.33^{efghi}	26.00^{ghij}	25.67^{ghij}
2	20.33^{b}	30.00^{cde}	20.67^{lm}	25.33^{hij}	26.33^{fghij}	27.33^{efghi}
3	22.67^{ab}	29.33^{cdef}	30.67^{bcde}	23.67^{ijkl}	30.00^{cdef}	25.33^{hij}
4	22.67^{ab}	24.33^{ghi}	32.33^{abcd}	24.33^{ijkl}	29.33^{defg}	23.00^{jkl}
5	23.67^{ab}	27.33^{defg}	35.67^{a}	21.00^{klm}	24.67^{hijk}	24.33^{ijkl}
6	22.67^{ab}	25.33^{fgh}	32.33^{bcd}	20.67^{lm}	32.33^{abcd}	21.00^{klm}
7	21.67^{ab}	33.00^{abc}	33.33^{abc}	30.00^{cdef}	33.33^{abc}	34.00^{ab}
8	21.67^{ab}	28.33^{defg}	28.33^{efgh}	28.33^{efgh}	20.67^{lm}	36.00^{a}
9	22.00^{ab}	32.33^{abc}	26.00^{ghij}	26.00^{ghij}	19.003^{m}	20.67^{lm}
10	24.33^{a}	35.67^{a}	26.33^{fghij}	26.33^{fghij}	25.67^{ghij}	30.67^{bcde}
11	21.00^{ab}	32.33^{abc}	30.00^{cdef}	34.00^{ab}	27.33^{efghi}	32.33^{abcd}
12	20.67^{b}	20.67^{ij}	29.33^{defg}	29.33^{defg}	25.33^{hij}	23.67^{ijkl}
13	22.00^{ab}	24.67^{ghi}	24.67^{hijk}	24.67^{hijk}	23.67^{ijkl}	24.67^{hijk}
14	21.33^{ab}	23.00^{hij}	23.00^{jkl}	23.00^{jkl}	23.00^{jkl}	26.00^{ghij}
15	21.67^{ab}	25.67^{fgh}	24.33^{ijkl}	35.67^{a}	24.33^{ijkl}	26.33^{fghij}
16	21.33^{ab}	22.33^{hij}	21.00^{klm}	32.33^{abcd}	21.00^{klm}	30.00^{cdef}
17	21.00^{ab}	23.00^{hij}	20.67^{lm}	33.33^{abc}	34.00^{ab}	20.67^{lm}
18	22.33^{ab}	19.33^{j}	19.00^{m}	19.00^{m}	20.67^{lm}	35.67^{a}
19	21.67^{ab}	23.00^{hij}	25.67^{ghij}	36.00^{a}	20.67^{lm}	32.33^{abcd}
20	21.00^{ab}	34.00^{ab}	27.33^{efghi}	20.67^{lm}	30.67^{bcde}	28.33±^{efgh}
Minimum	18.00	22.00	17.00	17.00	17.00	17.00
Maximum	30.00	24.00	38.00	38.00	38.00	38.00
SEM	0.21	0.58	0.63	0.63	0.63	0.63

abcdefghijklm: Mean values on the same column with different superscript differ statistically at 5% probability test; SEM: Standard error of means.

4.2.5: Incubation relative humidity of incubated Japanese quail eggs

The daily ambient relative humidity during Japanese quail embryogenesis is given in Table 4.14. There were significant differences ($P<0.05$) in the mean values across the treatments. While the minimum values ranged from 10.00^{o}C to 20.00^{o}C, the maximum values varied between 12.00^{o}C and 22.0^{o}C.

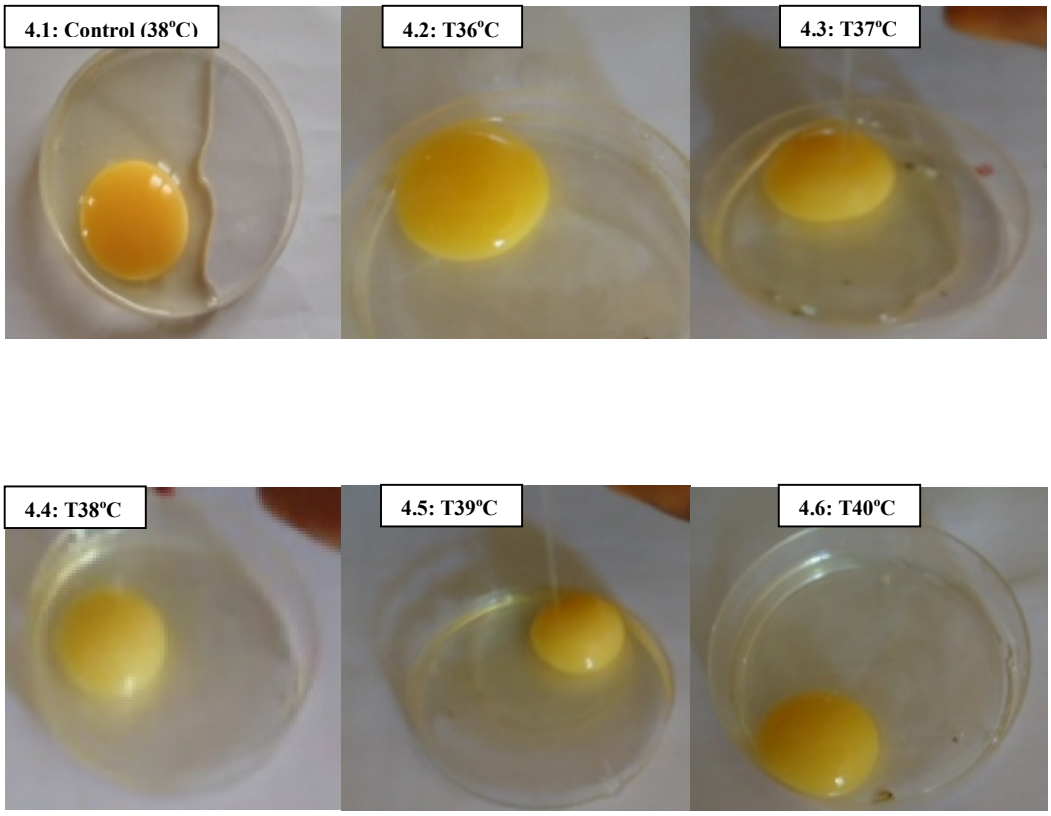
Table 4.14: Daily ambient relative humidity during Japanese quail embryogenesis

	Treatments(%)					
Embryonic day	Control (T38°C)	T36°C	T37°C	T38°C	T39°C	T40°C
0	14.33^{bcde}	10.00^{b}	11.33^{ab}	20.00^{b}	15.67^{abc}	20.00^{b}
1	12.33^{cde}	12.67^{bc}	13.00^{ab}	20.00^{b}	16.00^{ab}	20.00^{b}
2	12.33^{cde}	14.00^{bc}	12.67^{ab}	20.33^{ab}	17.33^{a}	20.33^{ab}
3	13.00^{bcde}	10.00^{b}	14.00^{a}	20.33^{ab}	16.00^{ab}	20.67^{a}
4	10.67^{de}	10.00^{b}	12.33^{ab}	20.67^{a}	17.00^{ab}	20.33^{ab}
5	10.00^{e}	13.33^{bc}	12.67^{ab}	20.33^{ab}	17.67^{a}	20.33^{ab}
6	10.00 ^{e}	17.00^{ab}	13.00^{ab}	20.00^{b}	15.00^{abcd}	20.00^{b}
7	15.67^{bc}	10.00^{b}	10.33^{b}	20.33^{ab}	14.00^{bcde}	20.00^{b}
8	17.33^{ab}	16.67^{ab}	10.00^{b}	20.33^{ab}	10.67^{ef}	20.00^{b}
9	16.33^{abc}	20.00^{a}	10.00^{b}	20.67^{a}	11.33^{ef}	20.00^{b}
10	17.00^{ab}	13.33^{bc}	10.00^{b}	20.33^{ab}	10.67^{ef}	20.33^{ab}
11	17.00^{ab}	10.00^{b}	10.00^{b}	20.00^{b}	12.33^{def}	20.33^{ab}
12	16.00^{abc}	10.00^{b}	10.00^{b}	20.33^{ab}	10.00^{f}	20.67^{a}
13	14.67^{bcd}	10.00^{b}	10.00^{b}	20.33^{ab}	10.00^{f}	20.33^{ab}
14	13.33^{bcde}	10.00^{b}	10.00^{b}	20.67^{a}	10.00^{f}	20.00^{b}
15	10.67^{de}	10.00^{b}	10.00^{b}	20.33^{ab}	15.00^{abcd}	20.00^{b}
16	14.00^{bcde}	11.33^{b}	10.00^{b}	20.00^{b}	11.33^{ef}	20.00^{b}
17	20.00^{a}	13.67^{bc}	10.00^{b}	20.33^{ab}	10.67^{ef}	20.00^{b}
18	20.00^{a}	10.00^{b}	10.00^{b}	20.33^{ab}	10.67^{ef}	20.33^{ab}
19	20.00^{a}	10.00^{b}	10.33^{b}	20.67^{a}	12.67^{cdef}	20.67^{a}
20	17.33^{ab}	10.00^{b}	11.00^{b}	20.33^{ab}	12.33^{def}	20.33^{ab}
Minimum	10.00	10.00	10.00	20.00	10.00	20.00
Maximum	12.00	21.00	16.00	22.00	20.00	21.00
SEM	0.45	0.42	0.22	0.07	0.37	0.05

abcdef: Mean values on the same column with different superscript differ statistically at 5% probability test; SEM: Standard error of means.

Plates 4.1 – 4.6: Freshly laid Japanese quailegg yolkon embryonicday 0

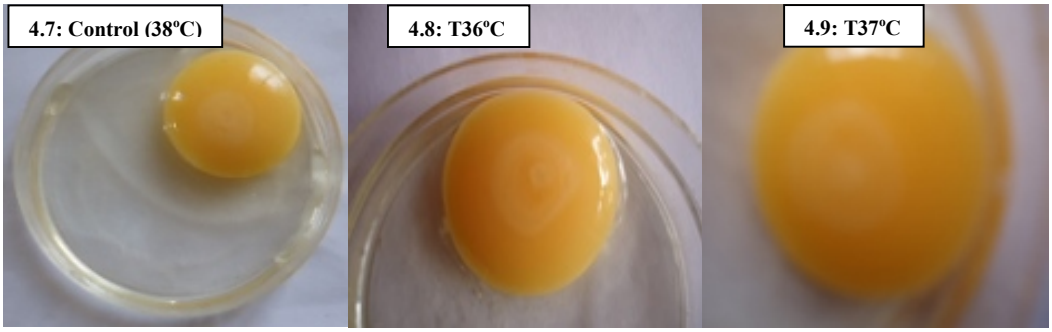
Plates 4.1 – 4.6 present freshly laid Japanese quail egg yolk. It was observed that all the egg yolk was intact in the inner membrane with yellowish colouration and completely submerged in the albumen that was clear, transparent and colourless.

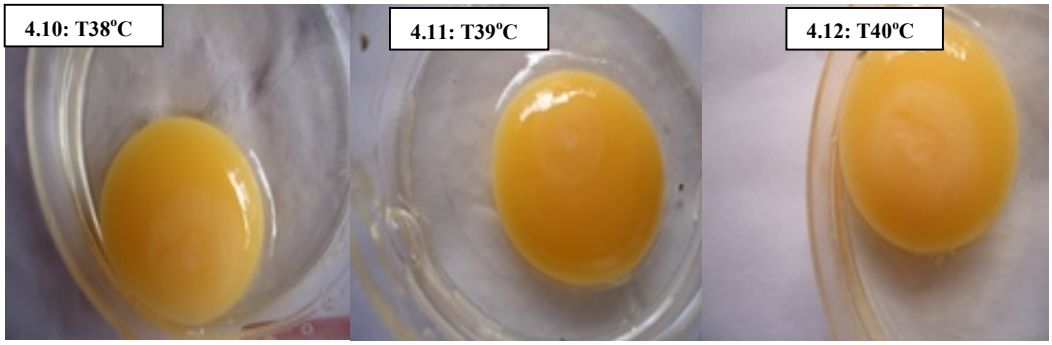


Plates 4.1 – 4.6: Freshly laid Japanese quail egg yolk on embryonic day 0

Plates 4.7 – 4.12: Developing blastoderm in Japanese quail egg on embryonic day 1

Plates 4.7 – 4.12show developing blastoderm on the yolk of Japanese quail eggs on day 1 of incubation. In all the treatments, there was growth on the surface of the egg yolk that spread like “microbial growth” in two concentric layers. The yolk and albumen were still intact but the albumen became less viscous.

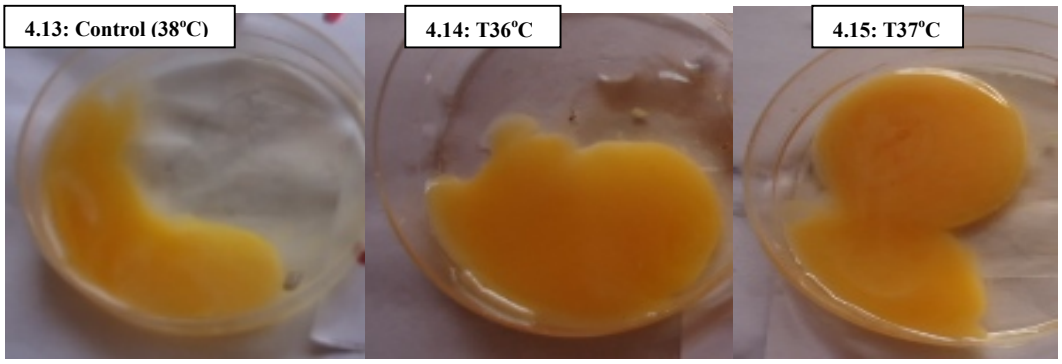


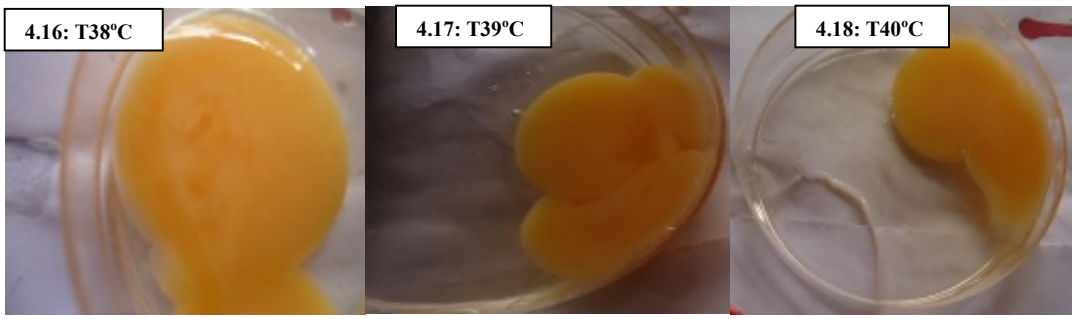


Plates 4.7 – 4.12:Developing blastoderm in the yolk of Japanese quail eggs on incubation day 1

Plates 4.13 – 4.18: Growth of blastoderm in Japanese quail egg on embryonic day 2

Given in Plates 4.13 – 4.18 are growth of blastoderm on the yolk of Japanese quail egg on day 2 of incubation. In all the treatments, the two concentric layers growth pattern observed on day 1 disappeared with disintegrated yolk and albumen. The growth covered the entire yolk surface towards the airspace in the egg. There were gel-like frames suspected to be the endochondral ossification of the embryo structure. Although red stream-like structure suspected to be blood vessels were observed faintly in T38^{o}Conly, blood-clot-like lump suspected to be the heart was observed in all the treatments.

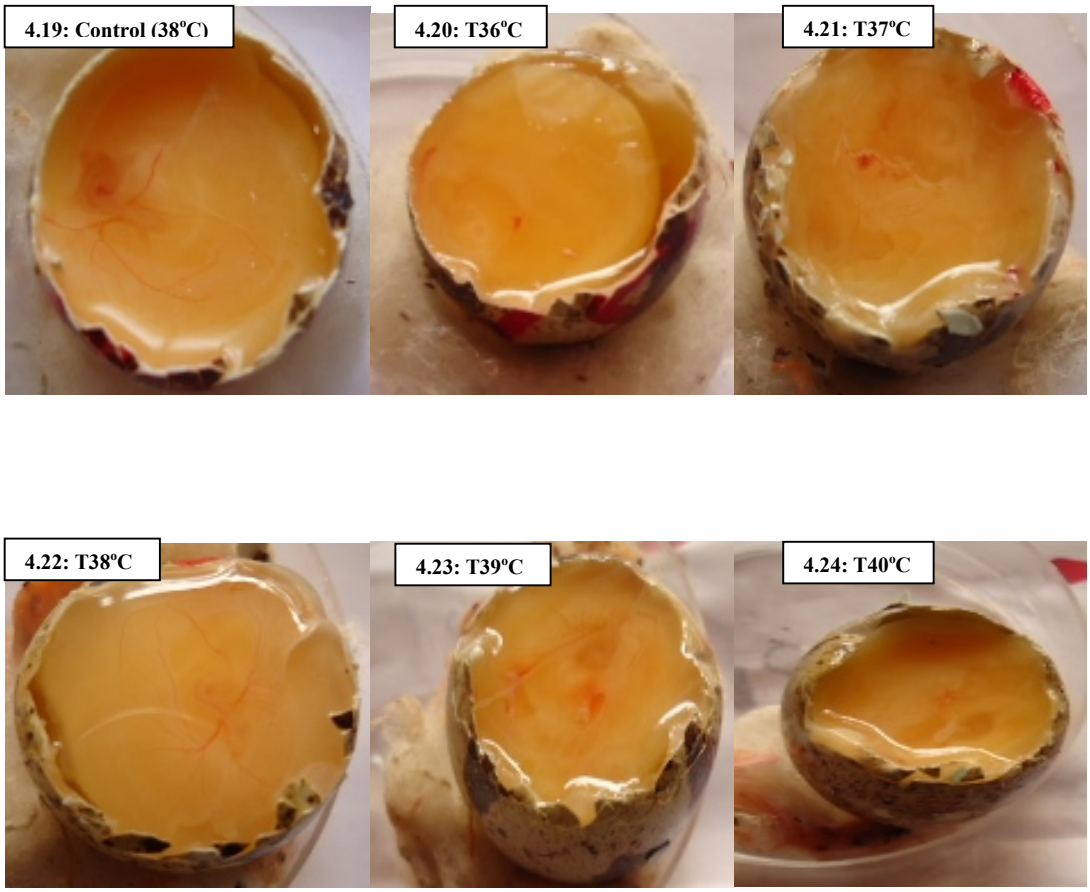




Plates 4.13 – 4.18: Blastoderm growth on Japanese quailyolk on incubation day 2

Plates 4.19 – 4.24: Developing embryos in Japanese quail eggs on embryonic day 3

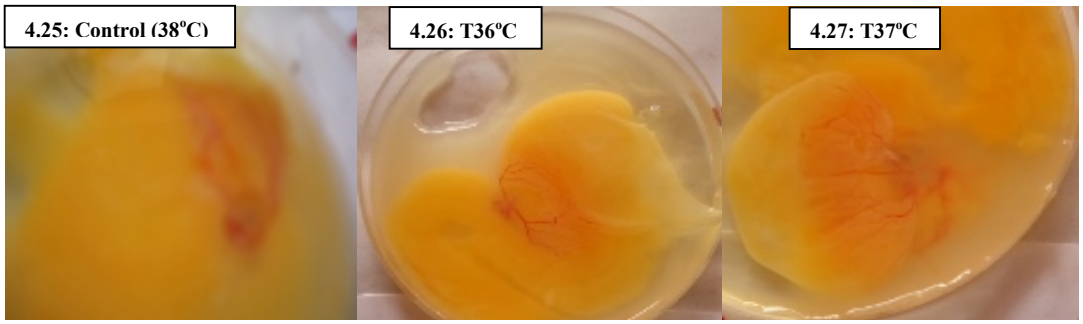
Plates 4.19 – 4.24 describe developing Japanese quail embryos on incubation day 3. The yolk and albumen became inconsistent and the growth covered the entire yolk surface yet the yellowish colouration was retained. The endochondral ossification of the embryos structure became more obvious. Also, the blood vessels became more conspicuous in all the treatments but the heart beat was observed only in T36^{o}C, T37^{o}C and T39^{o}C.

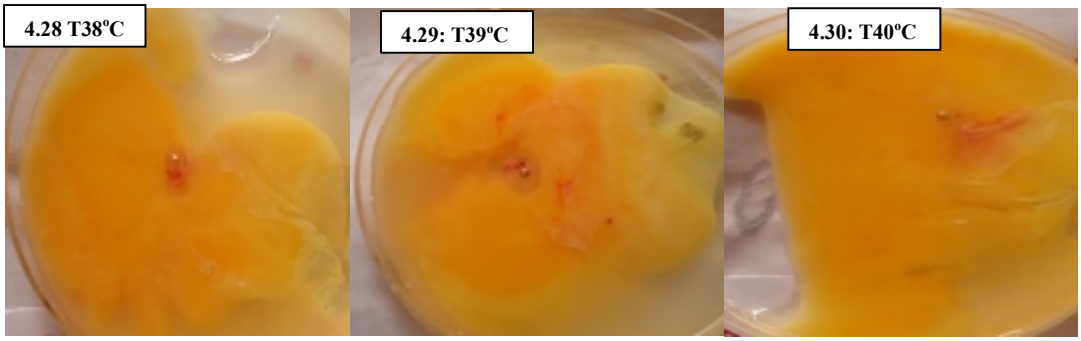


Plates 4.19 – 4.24:Developing Japanese quail embryos on incubation day 3

Plates 4.25– 4.30: Developing embryos in Japanese quail eggs on embryonic day 4

Developing Japanese quail embryos on day 4 of incubation is expressed in Plates 4.25 – 4.30. The endochondral ossification increased with the size of the embryos, indicative of deposition of calcium salts in a matrix of osteoid tissue (true bone formation) and muscle accretion. Black refractive ball-like structure suspected to be the eyeball was observed in all the treatments. Blood vessels became more conspicuous in all the treatments. Obvious heartbeat was observed in all the treatments.

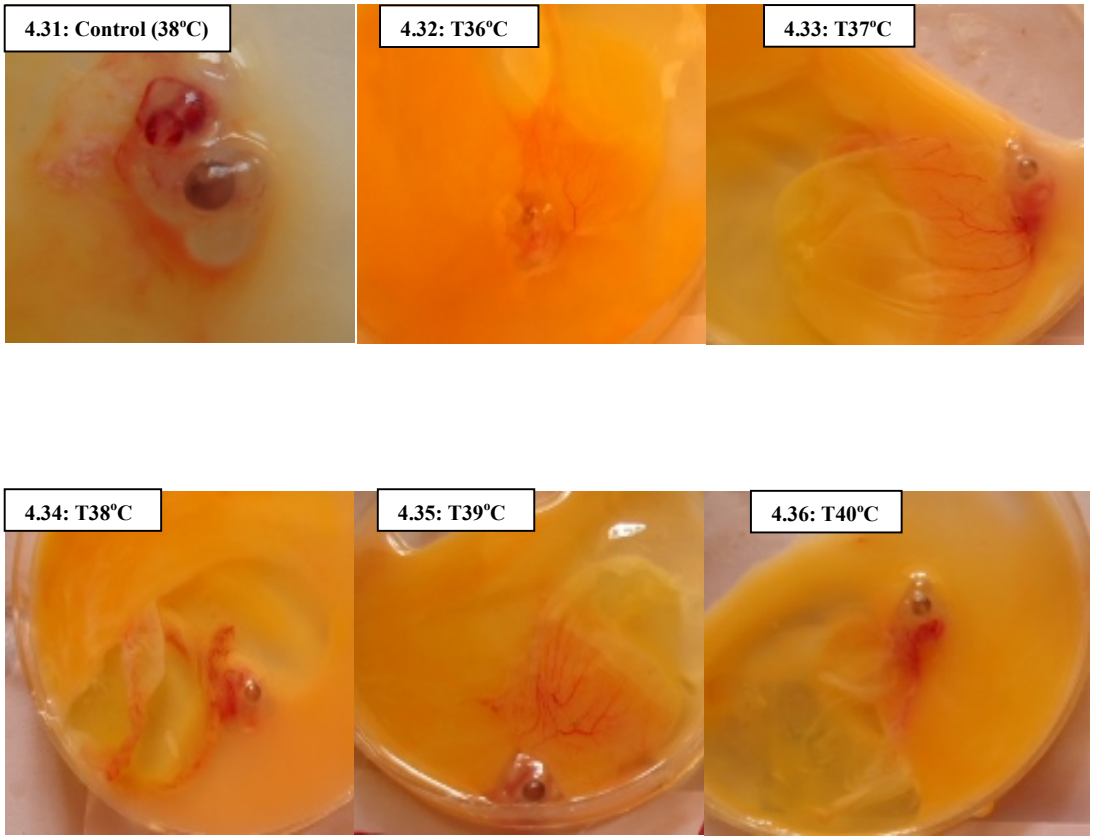




Plates 4.25 – 4.30:Developing Japanese quail embryos on day 4 of incubation

Plates 4.31 – 4.36: Developing embryos in Japanese quail eggs on embryonic day 5

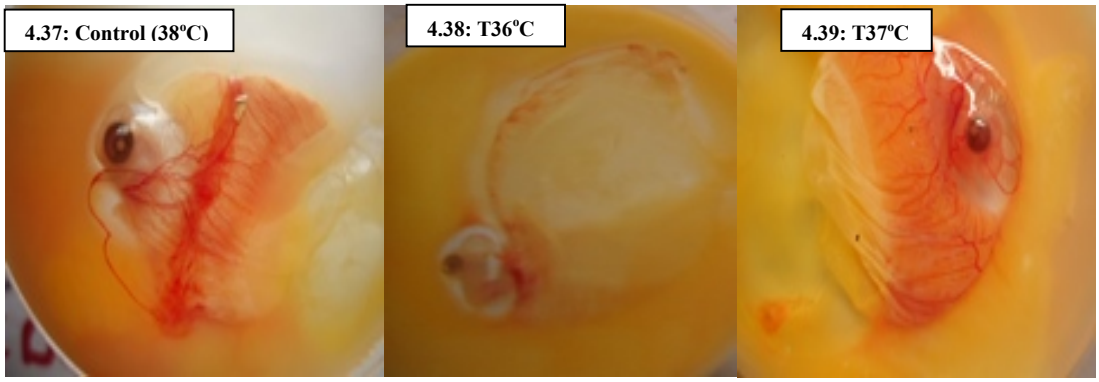
Shown in Plates 4.31 – 4.36 are developing embryosin Japanese quail eggs on day 5 of incubation. Plexus of blood vessels with adequate invaginations were observed in all the treatments. The eyeball became more conspicuous across the treatments. The yolk and albumen became more inconsistent yet separated. While the yolk retained its yellow colour, the albumen was seemingly stained and became yellowish.

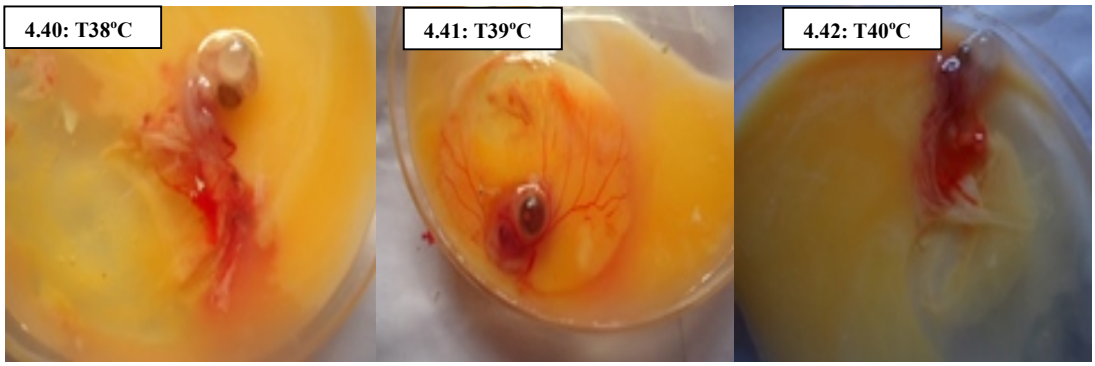


Plates 4.31 – 4.36: Developing embryosin Japanese quail eggs on day 5 of incubation

Plates 4.37 – 4.42: Developing embryos in Japanese quail eggs on embryonic day 6

Developing embryos in Japanese quail eggs on incubation day 6 is presented in Plates 4.37 – 4.42. The yolk became more inconsistent yet colour was retained and albumen surrounding the developing embryo was well reduced and very thick. The developing embryos increased in size with a well-formed network of blood vessels,in a more complex plexus suspected to be the allantois in all the treatments. The eyeballs became more conspicuous in all the treatments. Embryos in T36^{o}C andT37^{o}C seemed to be less developed compared to those in control (T38^{o}C), T38^{o}C, T39^{o}C and T40^{o}C.

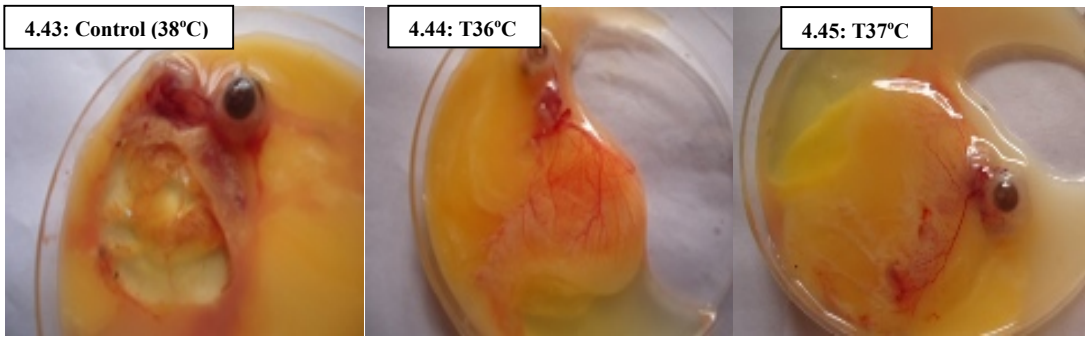


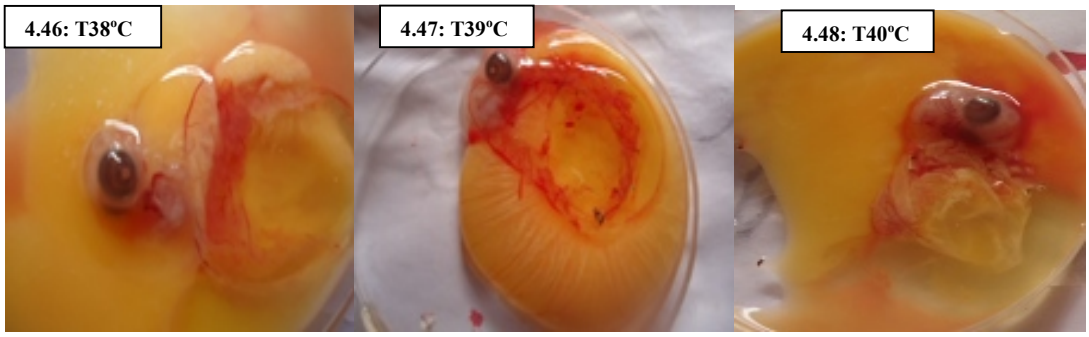


Plates 4.37 – 4.42: Developing embryos in Japanese quail eggs on incubation day 6

Plates 4.43 – 4.48: Developing embryos in Japanese quail eggs on embryonic day 7

Plates 4.43 – 4.48 show developing embryos in Japanese quail eggs on incubation day 7. The eyeballs became more conspicuous in a structure suspected to be the head. The blood vessels became more complex with invaginations all over a structure suspected to be the allantois and limbs projections were observed in all the treatments. However, embryos in T36^{o}C and T37^{o}C seemed to be less developed when compared with those in control T38^{o}C,T38^{o}C, T39^{o}C and T40^{o}C.

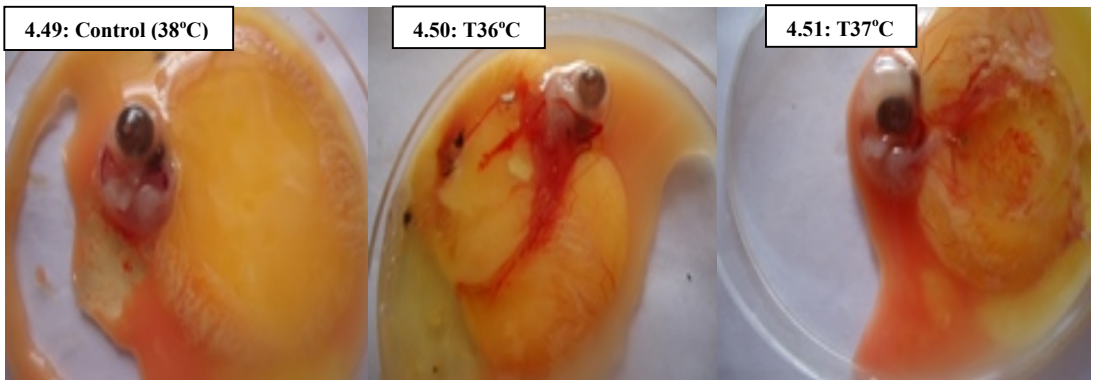


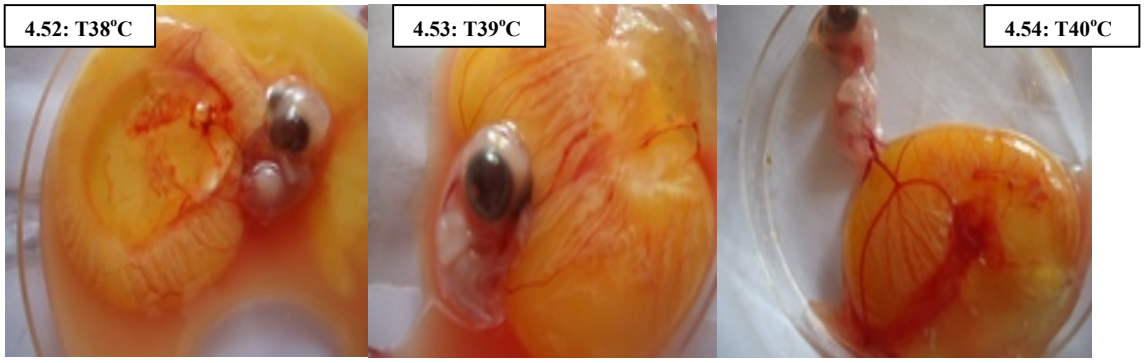


Plates 4.43 – 4.48: Developing embryos in Japanese quail eggs on incubation day 7

Plates 4.49 – 4.54: Developing embryos in Japanese quail eggs on embryonic day 8

Presented in plates 4.49 – 4.54 are developing embryos in Japanese quail eggs on incubation day 8. Movement of the developing embryos was observed across the treatments and they all appeared to be at the same rate of growth. The head became more conspicuous, yolk and albumen formed a gel-like mass with plexus of blood vessels connected by allantois to the developing embryo, which was transparent showing some internal organs being formed. Projections suspected to be the beak and wings as well as legs were observed across the treatments.

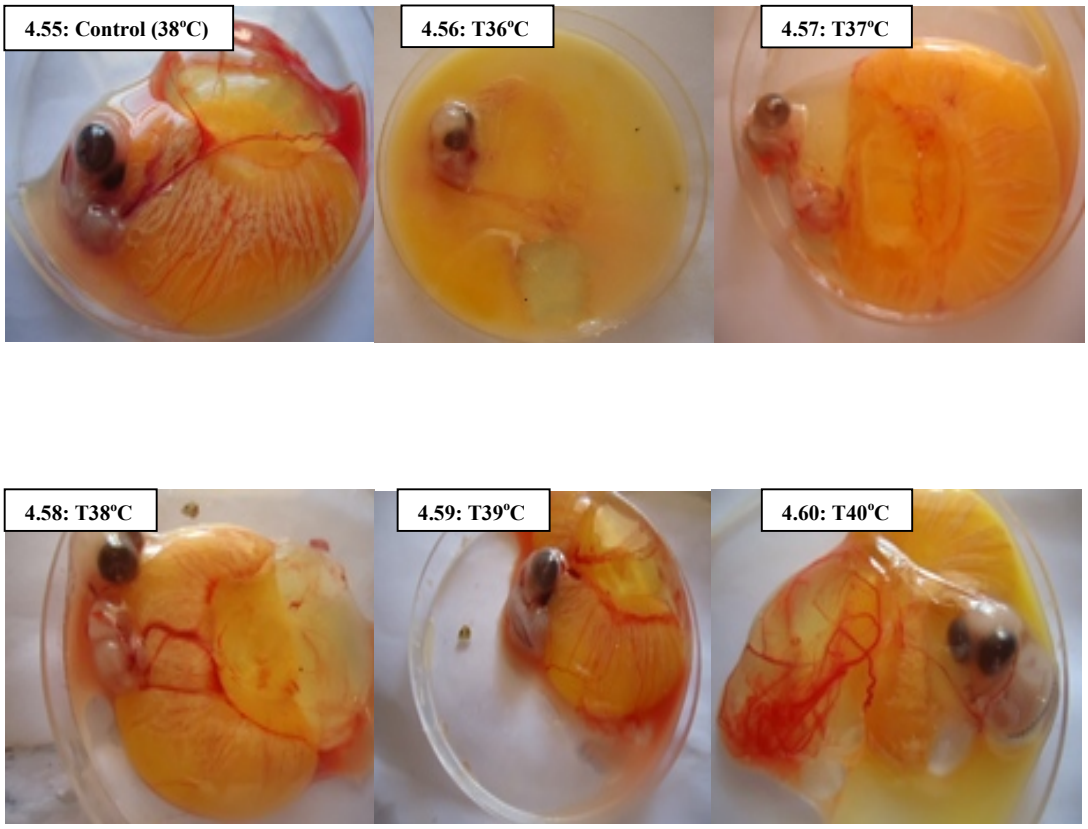




Plates 4.49 – 4.54: Developing embryos in Japanese quail eggs on incubation day 8

Plates 4.55 – 4.60: Developingembryos in Japanese quail eggs on embryonic day 9

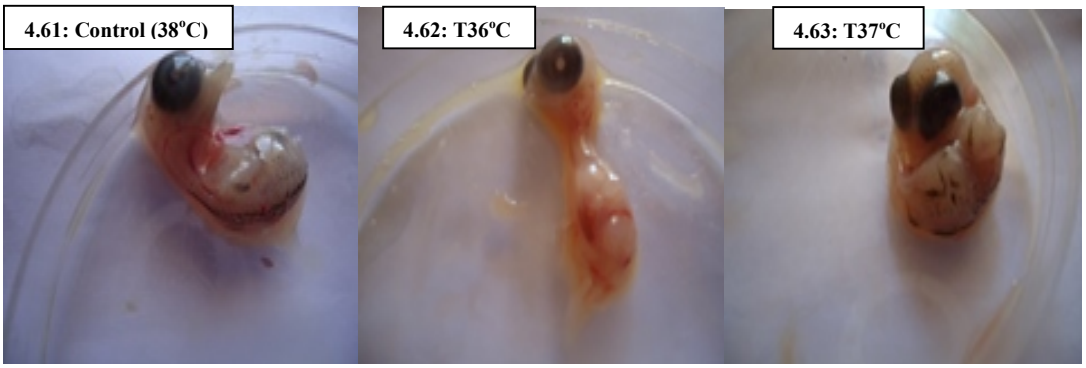
Expressed in Plates 4.55 – 4.60is Japanese quailembryos developmental stages on incubation day 9. Embryos in T39^{o}C and T40^{o}C developed black and white strips suspected to be the plumage pattern but this was not observed in control(38^{o}C), T36^{o}C, T37^{o}C and T39^{o}C. Embryos were in a coiled posture with the head bent backwards either through the left orright. The beaks, wings and legs became more conspicuous. Yolk and albumen formed a mass that was with plexus of invaginations attached by allantois to the developing embryo.

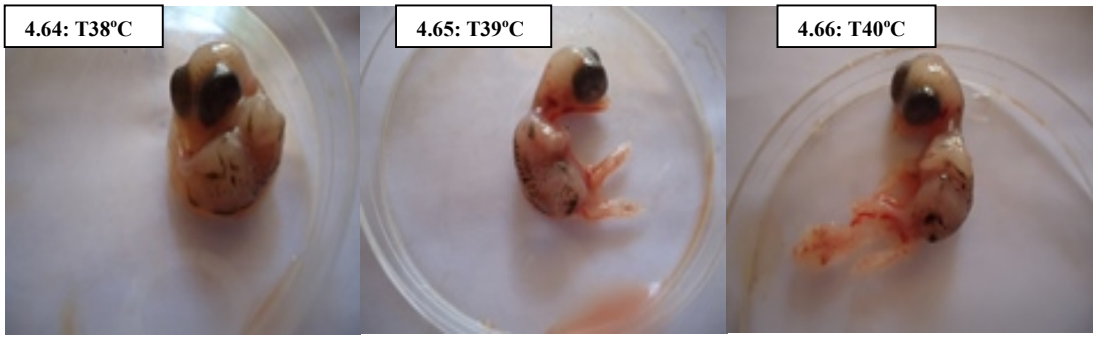


Plates 4.55 – 4.60: Japanese quail embryos developmental stages on incubation day 9

Plates 4.61 – 4.66: Developing embryos in Japanese quail eggs on embryonic day 10

Plates 4.61 – 4.66give the stages of Japanese quail embryos growth on incubation day 10. The embryos in T36^{o}C and T37^{o}C still did not develop black strips suspected to be the plumage, unlike in control(38^{o}C), T38^{o}C, T39^{o}C and T40^{o}C where the embryos grew feathers on their thighs, wings, back and the head. All the embryos were in a coiled posture with the head bent backwards either through the left or right. The yolk and albumen appeared separately and became smaller in size, forming more viscous mass with plexus of invaginations connected by allantois believed to be conducting materials in and out of the developing embryos.

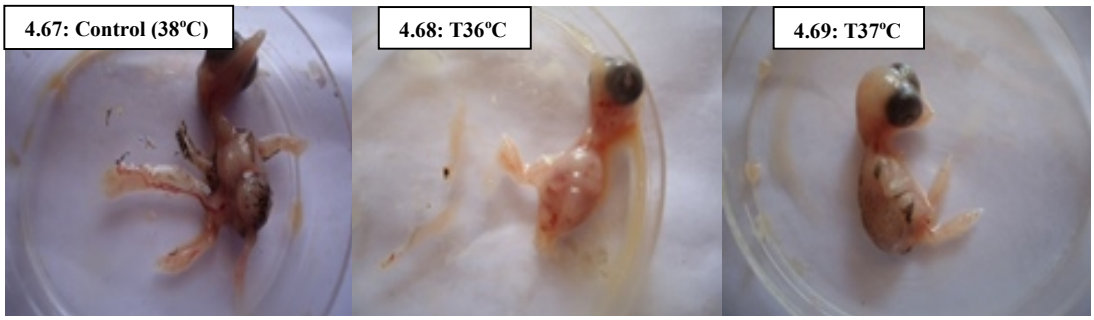


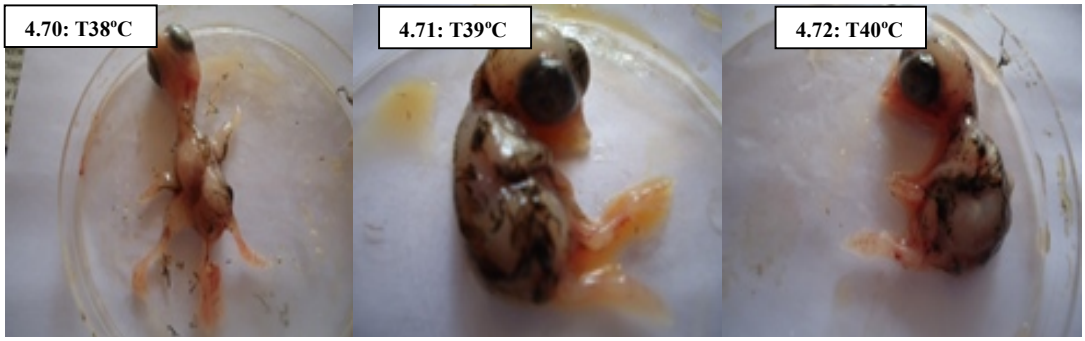


Plates 4.61 – 4.66: Stages of Japanese quail embryos growth on incubation day 10

Plates 4.67 – 4.72: Developing embryos in Japanese quail eggs on embryonic day 11

Given in plates 4.67 – 4.72are Japanese quail embryo stages of development on incubation day 11. The embryos in T36^{o}C and T37^{o}C developed black strips suspected to be the plumage, whereas those in control(38^{o}C), T38^{o}C, T39^{o}C and T40^{o}C grew feathers on their thighs, wings, back and the head. Embryos in a coiled posture with the head bent backwards either through the left or right. The yolk and albumen were seemingly separated and gradually reducing in size with more viscous gel-like mass with plexus of invaginations of blood vessels observed only in embryos in control(38^{o}C), T38^{o}C, T39^{o}C and T40^{o}C.

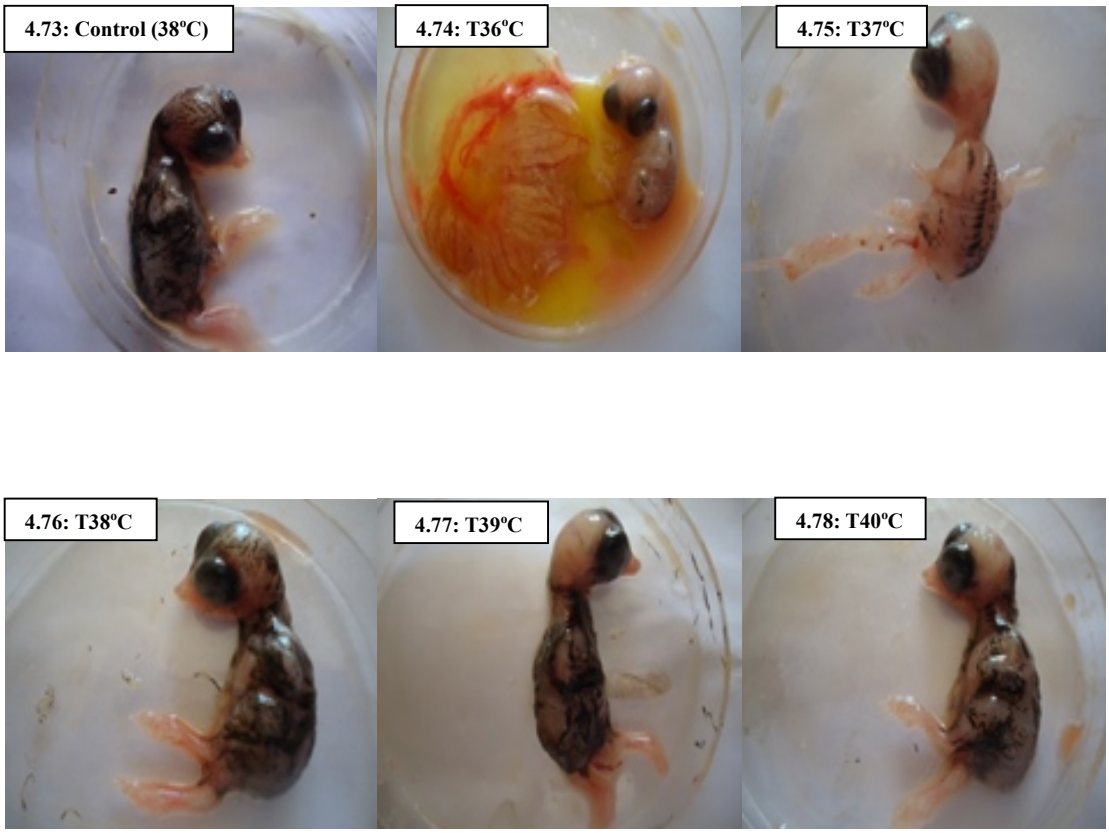




Plates 4.67 – 4.72:Japanese quail embryo developmentalstages on incubation day 11

Plates 4.73 – 4.78: Developing embryos in Japanese quail eggs on embryonic day 12

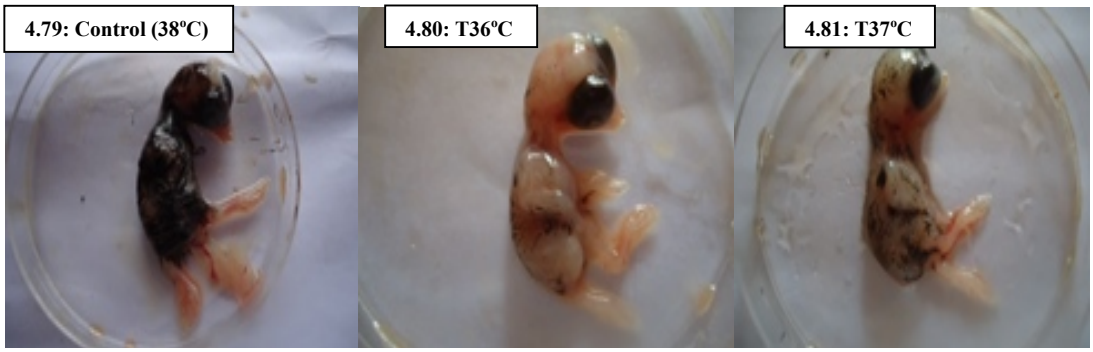
Plates 4.73 – 4.78show the developing embryos in Japanese quail eggs on incubation day 12. Embryos in T36^{o}C and T37^{o}C appeared to be less developed compared to those in control38^{o}C, T38^{o}C, T39^{o}C and T40^{o}C. While feathers grew all over the body of the embryos in control38^{o}C, T38^{o}C, T39^{o}C and T40^{o}C, it was only observed on the thighs, wings and back of the embryos in T36^{o}C and T37^{o}C. The yolk and albumen further reduced in size becoming more viscous and formed a mass that was with plexus of invagination of blood vessels attached by allantois to the developing embryos in all the treatments.

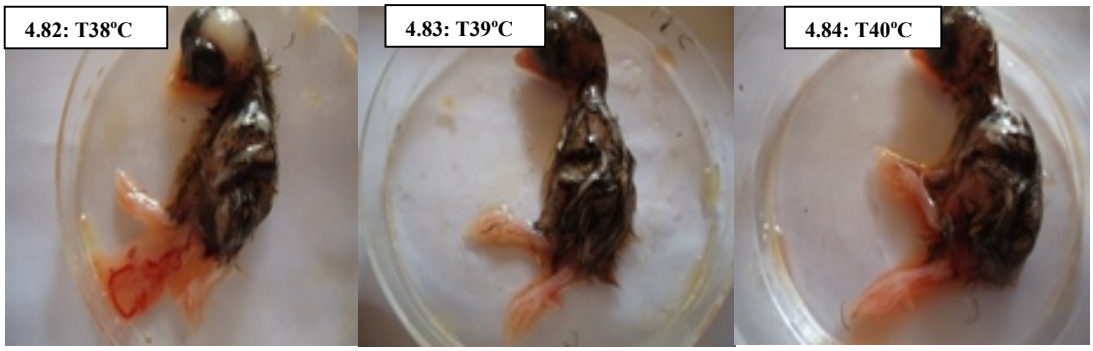


Plates 4.73 – 4.78: Developing embryos in Japanese quail eggs on incubation day 12

Plates 4.79 – 4.84: Developing embryos in Japanese quail eggs on embryonic day 13

Developing embryos in Japanese quail eggs on incubation day 13 is provided in Plates 4.79 – 4.84. The embryos in T36^{o}C and T37^{o}C grew feathers but were fewer than what was observed in control(38^{o}C), T38^{o}C, T39^{o}C and T40^{o}C. All the embryos were in a coiled posture with the head bent backwards. Yolk and albumen formed a mass that was with plexus of invagination attached by allantois to the developing embryos. But the yolk and albumen gel-like mass in embryoin T39^{o}Chad green and yellow colours.

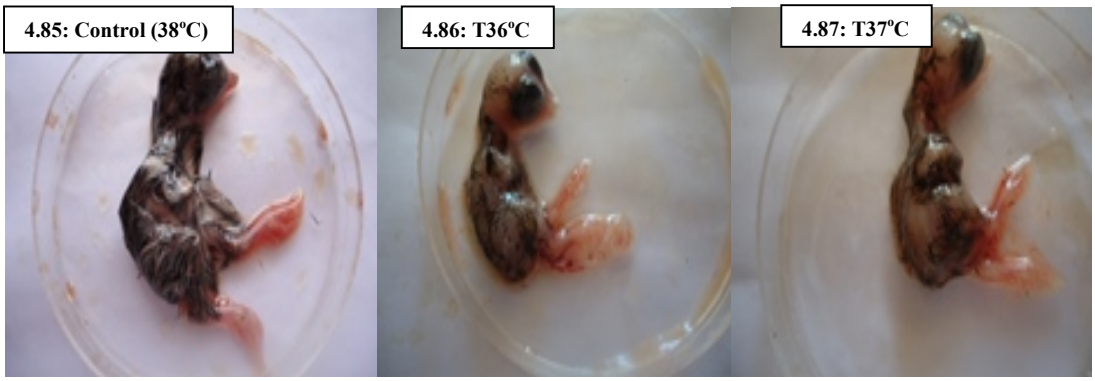


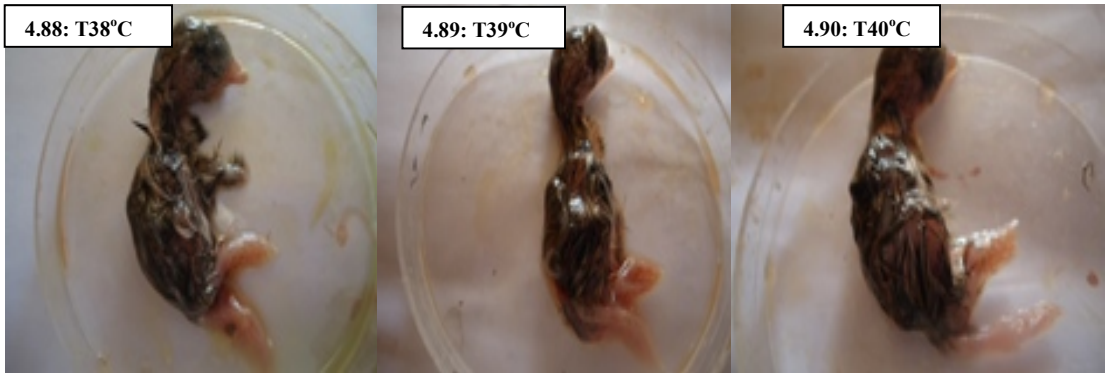


Plates 4.79 – 4.84: Developing embryos in Japanese quail eggs on incubation day 13

Plates 4.85 – 4.90: Developing embryos in Japanese quail eggs on embryonic day 14

Developing embryos in Japanese quail eggs on incubation day 14 are shown in Plates 4.85 – 4.90. The developing embryos in T36^{o}C and T37^{o}C grew feathers yet appeared less developed compared to those in control(38^{o}C), T38^{o}C, T39^{o}C and T40^{o}C that appeared fully grown with well reduced yolk mass. The yolk and albumen gel-like mass (unabsorbed yolk) in embryos in T38^{o}C and T39^{o}C were greenish with faint yellow patches.

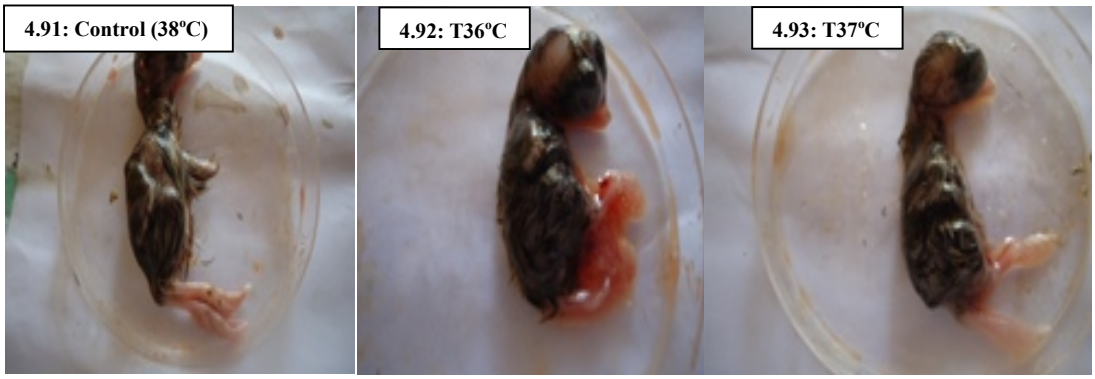


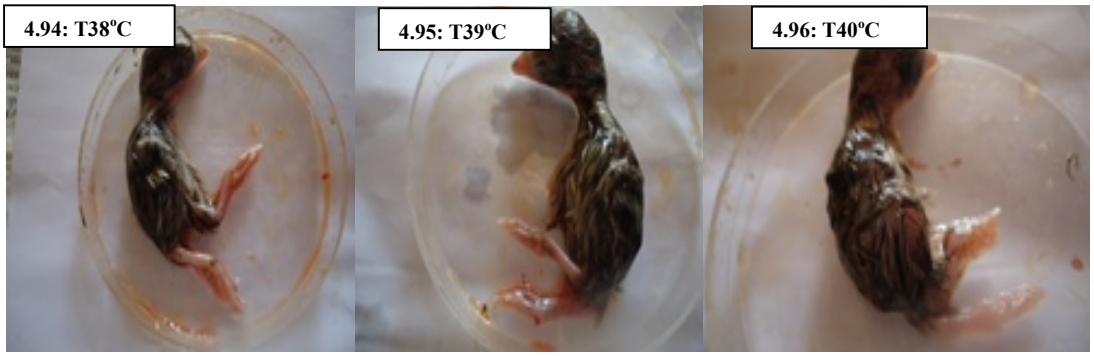


Plates 4.85 – 4.90: Developing embryos in Japanese quail eggs on incubation day 14

Plates 4.91– 4.96: Developing embryos in Japanese quail eggs on embryonic day 15

Embryo developmental stages in Japanese quail eggs on incubation day 15 are presented in Plates 4.91– 4.96. Embryos in all the treatments seemingly developed at equal rate, unlike the previous incubation days, when embryos in T36^{o}C and T37^{o}Cobviously appeared less developed than those in control(38^{o}C), T38^{o}C, T39^{o}C and T40^{o}C. However, the yolk and albumen gel-like mass (unabsorbed yolk) was well reduced in control(38^{o}C), T38^{o}C, T39^{o}C and T40^{o}C compared to those in T36^{o}C and T37^{o}C, where the yolk and albumen gel-like mass were larger in size.

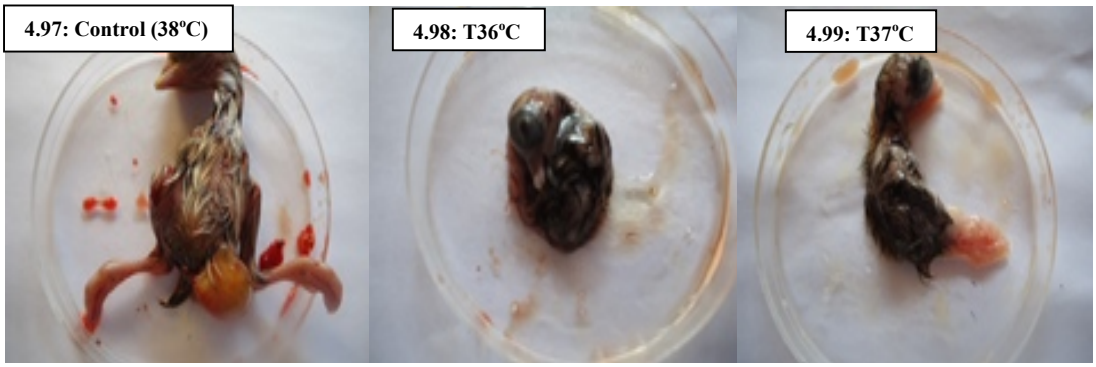


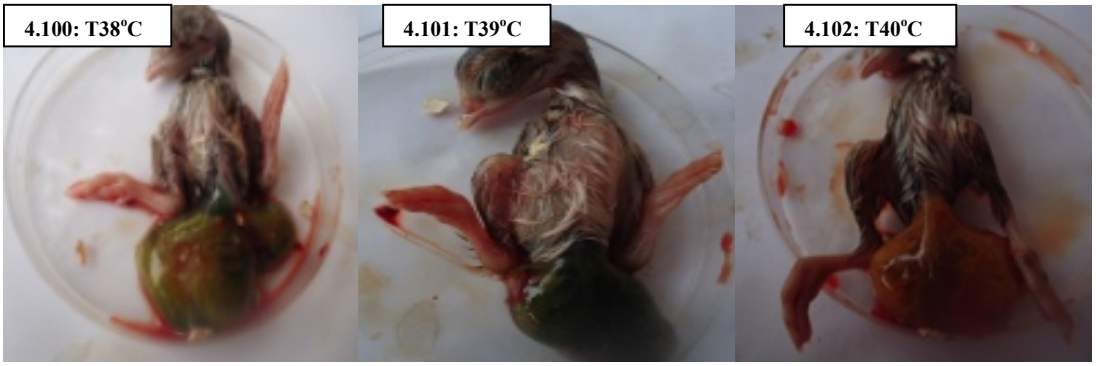


Plates 4.91 – 4.96: Japanese quail embryo developmental stages on incubation day 15

Plates 4.97 – 4.102: Developing embryos in Japanese quail eggs on embryonic day 16

Japanese quail embryos developmental stages on incubation day 16 are provided in Plates 4.97 – 4.102. The seemingly equal development across the treatments observed on the previous incubation day 15, was maintained with the yolk and albumen mass in all the embryos appearing to be well reduced. Meanwhile, yolk and albumen mass of embryos in T36^{o}C and T37^{o}C were still large. The yolk and albumen in T36^{o}C, T38^{o}C and T39^{o}C were greenish yet the network of blood vessels which formed plexus of invaginations attached by allantois to the developing embryos was still visible.

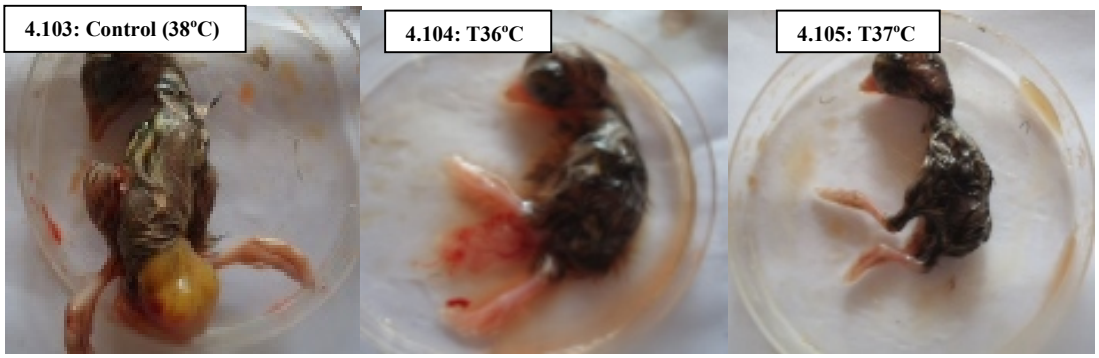


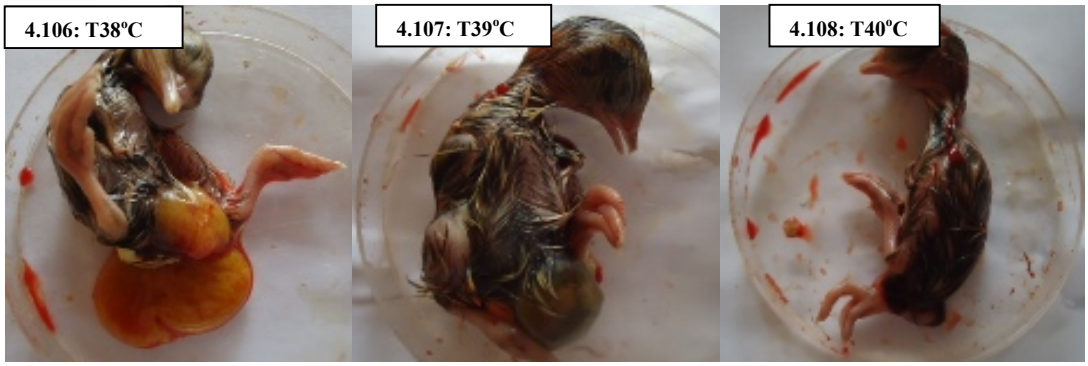


Plates 4.97 – 4.102: Japanese quail embryos developmental stages on incubation day 16

Plates4.103 – 4.108: Japanese quail developing embryos on embryonic day 17

Plates 4.103 – 4.108indicate the level of Japanese quail embryos growth on incubation day 17. The embryo in T39^{o}C made a very audible sound (cried) like that of a hatchling and was returned to the incubator after data collection. The yolk and albumen gel-like mass of embryos in control(38^{o}C), T38^{o}C, T39^{o}C and T40^{o}C became smaller, without fluid and appeared to be at the point of final withdrawal particularly in control(38^{o}C) and T39^{o}C. Meanwhile, the yolk and albumen mass of embryos in T37^{o}C, T39^{o}C and T40^{o}C were greenish. Nonetheless, the network of blood vessels that formed plexus of invaginations attached by allantois to the developing embryos was still conspicuous.



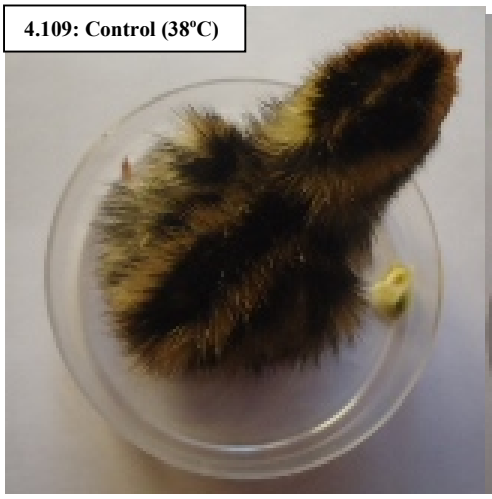


Plates 4.103 – 4.108: Japanese quail embryos growth on incubation day 17

Plates 4.109– 4.112: Pipping and hatching ofJapanese quail chicks in control (38^{o}C), T38^{o}C, T39^{o}C and T40^{o}C

Plates 4.109 – 4.112show pipping and hatching stages of Japanese quail chick on incubation day 18. The embryos in control(38^{o}C), T38^{o}C, T39^{o}C and T40^{o}C emerged as chicks. Interestingly, the embryo in T39^{o}C that made anaudible sound and was returned to the incubator on the previous incubation day (17) survived and weighed 5.59g on day 18. While, all the hatched chicks in control(38^{o}C), T38^{o}C and T39^{o}C were fluffy dried, some chicks in T40^{o}C were piping and those in T36^{o}C and T37^{o}C did not show any sign of pipping or hatching. It was observed that the embryo head was bent dorsally either through the left or right and at pipping, the chick used the egg tooth to window the eggshell while reversing the head ventrally.

4.109: Control (38ºC)



4.110: T38ºC

4.111: T39ºC

4.112: T40ºC

Plates 4.109 – 4.112: Pipping and hatching stages of Japanese quail chick on incubation day 18

Plate4.113: Hatching of Japanese quail chicks in T37^{o}Con incubation day 19

Plate 4.113 presents hatched chick of Japanese quail on incubation day 19. Some chicks were observed to be emerging in T37^{o}Con incubation day 19and those in T36^{o}C were yet to present any sign of pipping or hatching which probably signified delayed hatching due to comparatively low incubation temperature.

Plate 4.113:HatchedJapanese quail chick in T37^{o}C on incubation day 19

Plates 4.114: Hatching of Japanese quail chicks in T36^{o}C on day 20 of Incubation

Plate 4.114 provides Japanese quail chick hatched on incubation day 20. Some chicks in T36^{o}Cfinally emerged on incubation day 20,displaying delayed hatching probably as influenced by the comparatively low incubation temperature.

Plate 4.114: Hatched Japanese quail chick on incubation day 20

4.2.6 Discussion

4.2.7. Growth and developmental stages of Japanese quail embryos

The incubation temperature monitored on daily basis for a period of 20 embryonic days indicated that the calibrated values were achieved in each of the treatment incubators. However, a fluctuation of 0.03 – 0.97^{o}C higher than 0.03 – 0.05^{o}C reported by Poultry Hub (2018) to be normal was observed, suggesting that it might not be possible to achieve perfect precision of calibrated incubation temperature value. Meanwhile, the temperature provided in each of the treatment incubators, were within the range of 36 – 40.5^{o}C given to be ideal levels of incubation temperature and assumed to be the same in all avian species (French, 1997; Pedroso *et al.* 2006; Romao *et al*. 2009b). Though, optimum incubation temperature for poultry species has been given to be between 37.0 and 38.0^{o}C (Woodard *et al*., 1973; Ferguson 1994; Scott *et al*., 2015; ISA, 2016), French (1997) stated that hatchability was also possible at between 35.0 – 40.5^{o}C. Notably, it was stated that embryos were more sensitive to high incubation temperature, than when it was low and the effect of a suboptimal temperature, will depend on the degree of deviation from optimum temperature and the length of time applied. More so, it was stated that embryos appeared to be more sensitive to suboptimal temperature at the start of incubation than at the completion of incubation. More significantly, it was speculated that optimum incubation temperature depended on the poultry species or size of the eggs (Christensen *et al*., 1994; Decuypere, 1994; French, 1994). Consequently, all the treatment incubators were probably suitable for Japanese quail eggs incubation. The ambient temperature revealed a wide range of daily fluctuation of up to 3.6^{o}C throughout the study period. This may be largely due to gradual rotation of the earth, resulting in gradual sunset bringing coolness and gradual sunrise which results in hot weather condition. This phenomenon perhaps influenced the incubators mechanisms resulting in seemingly uncontrollable fluctuation in the incubation temperature. Although, the incubation relative humidity was lower than 60 – 70% recommended in avian egg incubation (Romao *et al*., 2009a; Brinsea, 2018), it was higher than the ambient relative humidity that varied from 10 – 20%. This may be due to the prevailing weather conditions at that time of the year (February/March) in Jos, Plateau State that made it suitable for this study. Since the Japanese quails were observed to be metamorphosing through different physiological

stages, it probably showed that the water placed in the incubator was enough and the fabricated incubators were efficient during the study period.On embryonic day 0, the egg yolk and albumen integrity were observed to be seemingly intact with a germinal disc, on the egg yolk where the ovum or egg is found. This perhaps suggested that the eggs used in this study were probably normal. On day 1 of the incubation period, the suspected germinal disc developed into two concentric layers, resembling the primitive streak, notochord, head fold and somite described by Tyler (2006) and Ainsworth *et al*.(2010), but the growth looked more like microbial growth on the surface of a substrate. On the following day, the concentric layers spread over the entire surface of the yolk, with a lump of blood believed to be the heart found in the yolk around the developing embryo. At this stage, the somite became more evident, indicating a metameric process similar to what Ainsworth *et al*.(2010) described in Japanese quail embryo after 40 to 64 hours of incubation. On embryonic day 3, there were network of blood vessels emanating from the beating heart region, buttressing the report of Pas Reform (2010) that the heart of avian embryo starts to beat after 72 hours of incubation. On embryonic day 4, a glittering part of the embryo was found inside the yolk, at a little distance far away from the beating heart. This was suspected to be the eyeball though was not pigmented and this stage was similar to the distinct un-pigmented eye which Ainsworth *et al*.(2010) stated would develop on embryonic day 3. Meanwhile, the features that look more like limbs and beak buds as well as the maxillary process were observed to be protruding. Interestingly, on embryonic day 5, the eyeballs became more conspicuous, beak outgrowth and limb joints became distinctive with well-formed amniotic fluid, allantois and plexus of blood vessels. This conformed to the reports of Ainsworth *et al*.(2010) and Pas Reform (2010) that blood vessels invagination of the yolk and albumen, forming a mass attached to the developing embryo by allantois with a network of innervation became prominent on day 5 of incubation. The observed network of the blood vessels probably showed that the developing embryos were deriving materials required for sustenance from the yolk and albumen mass. Between embryonic days 6 and 9, all the embryos in each of the treatment incubators were fully formed with all the morphological features observed. In each case, the developing embryo was found on top of the yolk and albumen mass toward the blunt end of the egg, probably demonstrating that avian embryo normally develops close to the

air sac region of the egg. This perhaps showed that developing Japanese quail embryo draws in its required gases and loses its wasted substances for sustenance, through the air sac that was widening by the day. Thus, the embryo may no longer depend on the egg pores for sustenance rather, on the widen air sac because the created vacuum in the air sac provides better gaseous exchange medium. Hence, the need to always keep the eggs upright, even if egg turning inside the incubator is necessary, in order not to distort the normal physiological processes for a well-formed chick. Meanwhile, it was observed that the embryos in T36^{o}C and T37^{o}C were not as developed as the embryos in other treatments, probably due to relatively low incubation temperatures they were subjected to. This concurred with the report of TNAU (2015), that low temperature slows down the growth of embryo and higher temperature (more than optimum) speeds up the embryonic growth. It was further stressed that if irregular temperature settings persist over a long period, hatchability would be deleteriously affected. More so, embryonic mortality, prolonged incubation period as well as the emergence of weak and deformed chicks was adduced to low incubation temperature (Shubber *et al*., 2012). On embryonic day 10, feathers were found on the entire body of the developing embryos compared to just strips of feather follicles observed in T36^{o}C and T37^{o}C. The differences in growth rate continued until the 15th day of incubation, when all the embryos in all the treatment incubators, were apparently at the same growth pace. This probably demonstrated a compensatory growth, described by Hector and Nakagawa (2012) to be observed in living things, when there is distortion in physiological processes influenced by poor nutritional plane or undue microclimatic conditions. It was observed in this study that Japanese quail embryos may not yield readily to varying incubation temperatures from 36 to 40^{o}C. This is because, it was least expected that the embryos in T36^{o}C, 37^{o}C (relatively low incubation temperature) and T39^{o}C, T40^{o}C (relatively high incubation temperature) will progress up to fully developed embryos, talk more of pipping and emerging as chicks. Consequently, the present findings contradicted the report of Wilson (1991) who gave a range of 37 – 38^{o}C as optimum operating temperatures for poultry species and emphasised that any deviations from this will have a major impact on hatching success. However, the observed disparities could be attributed to differences in the climatic conditions of the study areas and position of the incubated eggs in the incubators. Also, it could be due to

effectiveness and performance of the incubators to provide the required microclimatic conditions necessary for embryo development and growth. On embryonic day 13, it was astonishing to observe green yolk and albumen gel-like mass that continued up to day 17 in some of the developing embryos. But respite ensued when green excreta was found on the chick crate after transportation of the hatchlings. This probably indicated embryo maturity stage, when the unabsorbed yolk would be rapidly and completely withdrawn into the body prior to pipping and hatching. This deviation from the normal yellow yolk and colourless albumen could be largely due to metabolic pathway disorder resulting in green excreta often passed out by some day-old hatchlings. Also, it could be a symptom of microbial infection that could lead to high chick mortality often recorded during the first few days of life.On embryonic day 15, it was observed that all the embryos apparently had the same growth rate, contrasting the records of previous incubation days, when embryos in T36^{o}C and T37^{o}C clearly seemed less developed than those in control (38^{o}C), T38^{o}C, T39^{o}C and T40^{o}C. This observation probably suggested that there was a physiological catch-up-growth between the embryos in T36^{o}C and T37^{o}C as well as those in control (38^{o}C), T38^{o}C, T39^{o}C and T40^{o}C that were at the verge of pipping. Even at that, the embryos in T36^{o}C and T37^{o}C that were comparatively less developed, eventually hatched much later than those in control (38^{o}C), T38^{o}C, T39^{o}C and T40^{o}C, undermining the observed seemingly catch-up growth. Therefore, incubation temperature may play a vital role in Japanese quail embryos development and growth. On incubation day 16, the seemingly equal development observed across the treatments on the previous incubation day 15, was maintained with the yolk and albumen mass in all the embryos appearing to be well reduced. This observation possibly showed that there was a gradual withdrawal of the unabsorbed yolk into the developing chicks' body. The growth rate was apparently synchronized, perhaps through compensatory growth resulting in close incubation periods that were seemingly within the range of 16 – 18 days reported to be suitable for chick survival (Archer and Cartwright, 2018; Sartell, 2018). Similar embryo growth and developmental pattern was observed across the treatments, suggesting that the incubators were efficient. While the chicks in Control(38^{o}C), T38^{o}C and T39^{o}C emerged on embryonic day 18, those in T40^{o}C were at the pipping phase and the chicks in T37^{o}C and T36^{o}C hatched on days 19 and 20, respectively. Therefore, the delayed chick emergence

in T36^{o}C and T37^{o}C may be purely attributed to the microclimatic conditions the embryos were subjected to. As a result, low and high incubation temperature, could influence embryo growth and development in Japanese quails hence, the need for optimal incubation temperature determination.

4.3 Results

4.3.1 Role of incubation temperature on Japanese quail sex reversal

4.3.2 Altered incubation temperature during early embryogenesis in Japanese quails

The incubation temperature altered on incubation days 3, 4 and 5 is given in table 4.15. There were statistical differences ($P<0.05$) in all the daily mean values across the treatments. However, the temperature alteration resulted in fall of the calibrated treatment values, to physiological zero temperature (26.34 – 28.43^{o}C) except in control that maintained a range of 37.60 – 38.60^{o}C. While the minimum values ranged from 26.30^{o}C to 37.60^{o}C, the maximum values were between 36.40^{o}C and 40.40^{o}C.

Table 4.15: Alteration of incubation temperature during early embryogenesis inJapanese quails

	Treatments					
Embryonic day	T_A Control (38^oC)	T_B 36^oC	T_C 37^oC	T_D 38^oC	T_E 39^oC	T_F 40^oC
0	38.30^{ab}	35.90^{b}	36.50^{ab}	38.03^{a}	39.03^{a}	40.23^{a}
1	38.03^{bc}	36.27^{a}	37.30^{b}	37.83^{a}	38.83^{ab}	39.50^{ab}
2	38.20^{bc}	35.96^{b}	36.70^{ab}	37.87^{a}	38.43^{b}	40.03^{a}
3	38.07^{bc}	26.90^{d}	26.34^{d}	27.20^{c}	26.62^{d}	27.03^{d}
4	38.33^{a}	28.43^{c}	26.53^{d}	27.52^{c}	26.47^{d}	27.20^{d}
5	38.17^{bc}	27.70^{cd}	28.23^{c}	27.10^{c}	27.60^{c}	28.30^{c}
6	38.20^{bc}	35.93^{b}	36.97^{ab}	38.30^{a}	39.40^{a}	40.20^{a}
7	38.13^{bc}	35.80^{b}	36.53^{ab}	38.77^{a}	38.50^{b}	40.43^{a}
8	38.17^{bc}	35.90^{b}	37.03^{b}	37.93^{a}	39.23^{a}	39.70^{ab}
9	38.17^{bc}	36.34^{a}	37.17^{ab}	38.23^{a}	39.00^{a}	40.03^{a}
10	38.03^{bc}	36.00^{a}	37.00^{b}	37.73^{ab}	38.90^{a}	39.60^{ab}
11	37.97^{bc}	35.97^{b}	36.97^{ab}	37.97^{a}	38.93^{a}	39.70^{ab}
12	37.97^{bc}	36.47^{a}	36.80^{ab}	38.07^{a}	39.07^{a}	40.20^{a}
13	37.97^{bc}	36.37^{a}	36.73^{ab}	38.77^{a}	39.40^{a}	40.30^{a}
14	38.07^{bc}	35.90^{b}	37.47^{a}	38.03^{a}	39.30^{a}	40.13^{a}
15	38.00^{bc}	36.30^{a}	37.57^{a}	37.93^{a}	39.13^{a}	40.43^{a}
16	37.93^{c}	36.33^{a}	36.90^{ab}	38.37^{a}	39.00^{a}	40.30^{a}
17	38.10^{bc}	35.70^{b}	37.27^{ab}	37.60^{ab}	39.20^{a}	39.60^{ab}
18	38.10^{bc}	35.74^{b}	37.43^{a}	37.60^{ab}	38.77^{b}	40.07^{a}
19	38.03^{bc}	36.03^{a}	37.20^{ab}	38.07^{a}	38.70^{b}	39.73^{ab}
20	38.03^{bc}	35.90^{b}	37.39^{a}	38.23^{a}	38.80^{b}	40.27^{a}
21	38.07^{bc}	36.30^{a}	37.03^{b}	37.70^{ab}	38.60^{b}	39.93^{ab}
22	37.93^{c}	36.37^{a}	37.27^{ab}	38.33^{a}	39.37^{a}	40.33^{a}
Minimum	37.60	26.90	26.30	27.2	26.40	27.00
Maximum	38.60	36.40	37.50	38.80	39.50	40.40
SEM	0.18	0.24	0.27	0.32	0.37	0.32

abcd: Mean values on the same column with different superscript differ statistically at 5% probability test; SEM: Standard error of means.

4.3.3: Ambient temperature during incubation temperature alteration at early embryogenesis in Japanese quails

Table 4.16 displays the ambient temperature during incubation temperature alteration at early embryogenesis in Japanese quails. There were significant variations ($P<0.05$) in all the daily mean values across the treatments. While the minimum value varied from 23.10^{o}C to 33.90^{o}C, the maximum value was between 33.90^{o}C and 39.10^{o}C.

Table 4.16: Ambient temperature during incubation temperature alteration at early embryogenesis in Japanese quails(°C)

	Treatments					
Embryonic day	T_A Control (38°C)	T_B 36°C	T_C 37°C	T_D 38°C	T_E 39°C	T_F 40°C
0	35.40^{a}	28.80	29.77^{ef}	29.20	32.63^{ab}	35.00^{ab}
1	35.13^{abc}	29.67	28.27^{f}	28.60	28.27^{d}	28.77^{d}
2	34.83^{abc}	29.87	31.20^{de}	29.80	29.93^{bcd}	29.90^{d}
3	35.27^{ab}	26.70	29.70^{ef}	29.13	28.57^{cd}	30.47^{cd}
4	34.43^{abcd}	32.40	32.70^{bcd}	32.33	31.33^{ab}	34.40^{ab}
5	33.20^{abcdef}	32.87	33.63^{bcd}	32.43	31.03^{abc}	35.00^{ab}
6	34.00^{abcd}	34.13	34.23^{abc}	33.10	31.53^{ab}	35.87^{ab}
7	32.43^{defg}	34.53	34.03^{abc}	33.30	31.70^{ab}	35.73^{ab}
8	33.10^{bcdef}	26.13	34.37^{abc}	33.00	32.63^{ab}	35.60^{ab}
9	33.30^{abcdef}	34.37	36.67^{a}	34.57	33.57^{a}	37.27^{a}
10	33.00^{cdefg}	34.50	34.77^{abc}	33.60	33.10^{a}	36.10^{ab}
11	34.57^{abcd}	32.60	35.60^{abc}	33.33	32.70^{ab}	36.77^{a}
12	33.60^{abcde}	33.80	34.50^{abc}	32.77	32.27^{ab}	36.40^{a}
13	32.90^{cdefg}	32.20	35.30^{abc}	32.57	31.93^{ab}	35.53^{ab}
14	33.00^{cdefg}	33.30	34.27^{abc}	31.13	31.07^{abc}	35.53^{ab}
15	32.90^{cdefg}	31.83	32.97^{bcd}	31.93	31.30^{abc}	35.00^{ab}
16	32.57^{defg}	34.30	34.13^{abc}	31.13	31.03^{abc}	34.03^{ab}
17	31.93^{efg}	35.03	33.07^{bcd}	31.40	31.07^{abc}	32.90^{bc}
18	31.13^{fg}	33.23	35.07^{abc}	32.97	33.00^{a}	35.63^{ab}
19	30.80^{g}	33.63	35.20^{abc}	31.73	32.10^{ab}	35.10^{ab}
20	28.90^{h}	34.13	34.47^{abc}	32.73	32.73^{ab}	35.13^{ab}
21	33.07^{bcdef}	33.53	35.33^{abc}	33.20	33.17^{a}	35.63^{ab}
22	31.07^{fg}	28.80	34.20^{abc}	32.57	31.67^{ab}	34.60^{ab}
Minimum	26.70	23.10	33.80	31.70	25.40	33.90
Maximum	36.70	39.10	34.50	33.90	34.40	35.00
SEM	1.82	0.52	0.28	0.36	0.21	0.30

abcdefg: Mean values on the same column with different superscript differ statistically at 5% probability test;SEM: Standard error of means.

4.3.4: Paused incubation temperature during early embryogenesis in Japanese quails

Showed in table 4.17 is the hourly incubation temperature pausing duration at the early phase of Japanese quail embryogenesis. There were significant differences ($P<0.05$) in the mean value of incubation temperature across the treatments whereas, the ambient mean temperature value did not differ ($P>0.05$) throughout the period. At the initial hour (0 hour), the treatments temperature values were seemingly close to the calibrated values but declined to as low as 32.07^{o}C (T_B) and as high as 35.90^{o}C in T6 after an hour later except the control. At the 5th hour, the incubation temperature dropped to as low as 26.83^{o}C (T_E) with the highest (27.80^{o}C) recorded in T_C.

Table 4.17: Hourly incubation temperature pausing duration at early embryogenesis in Japanesequails

Parameters		Treatments						
		T_A	T_B	T_C	T_D	T_E	T_F	
Pausing time (hr)	Temperature	Control (38^oC)	36^oC	37^oC	38^oC	39^oC	40^oC	SEM
0	Incubation	38.27^{ab}	35.97^{c}	36.53^{bc}	37.70^{abc}	38.63^{a}	39.77^{a}	0.32
	Ambient	27.07	27.97	28.27	27.70	29.23	29.33	0.44
1	Incubation	38.00^{a}	32.07^{c}	34.90^{abc}	34.50^{bc}	32.67^{c}	35.90^{ab}	0.59
	Ambient	27.87	27.87	27.90	27.97	28.63	27.23	0.27
2	Incubation	38.20^{a}	29.93^{b}	31.47^{b}	29.83^{b}	29.33^{b}	31.10^{b}	0.77
	Ambient	27.23	26.83	27.67	27.03	27.53	27.53	0.31
3	Incubation	38.20^{a}	28.37^{b}	28.53^{b}	28.13^{b}	27.47^{b}	28.57^{b}	0.93
	Ambient	27.63	27.40	27.83	27.47	27.90	27.73	0.33
4	Incubation	38.13^{a}	27.83^{b}	27.33^{b}	27.47^{b}	27.10^{b}	27.77^{b}	0.97
	Ambient	26.73	27.00	26.93	27.33	27.10	26.67	0.25
5	Incubation	38.17^{a}	27.67^{b}	27.80^{b}	26.93^{b}	26.83^{b}	27.43^{b}	0.99
	Ambient	29.43	29.43	29.33	27.37	28.70	28.83	0.27

abc: Mean values on the same row with different superscript differ statistically at 5% probability test; SEM: Standard error of means.

4.3.5: Incubation relative humidity during incubation temperature alteration at early embryogenesis in Japanese quails

Given in Table 4.18 is the daily incubation relative humidity when incubation temperature was paused during early embryogenesis in Japanese quails. All the mean values across the treatments differ statistically ($P<0.05$) with minimum values ranging from 20.00% (T_A, T_C, T_F) to 36.00% (T_D) and maximum values from 34.00% (T_A) to 61.00% (T_D).

Table 4.18: Incubation relative humidity during incubation temperature alteration at early embryogenesis in Japanese quails

	Treatments (%)					
Embryonic day	T_A Control (38°C)	T_B 36°C	T_C 37°C	T_D 38°C	T_E 39°C	T_F 40°C
0	21.33^{ghi}	23.00^{k}	35.33^{de}	45.00^{gh}	28.67^{ghi}	26.00^{k}
1	20.00^{i}	28.67^{j}	30.33^{fghi}	45.67^{gh}	27.67^{hij}	29.33^{hijk}
2	21.33^{ghi}	28.67^{j}	32.67^{efgh}	46.00^{ghi}	24.67^{ij}	30.00^{ghijk}
3	20.33^{hi}	29.00^{j}	28.00^{hi}	45.00^{gh}	22.00^{j}	28.00^{jk}
4	22.00^{fghi}	30.67^{ij}	27.00^{i}	46.33^{ghi}	24.67^{ij}	26.33^{k}
5	32.67^{a}	34.00^{hi}	29.67^{ghi}	44.00^{i}	31.00^{fghi}	30.33^{fghijk}
6	31.67^{a}	35.33^{ghi}	32.00^{efgh}	48.00^{fgh}	32.33^{efgh}	31.00^{fghijk}
7	32.00^{a}	36.00^{fgh}	30.00^{ghi}	46.67^{ghi}	30.67^{fghi}	29.00^{ijk}
8	31.00^{ab}	39.33^{efg}	30.67^{fghi}	51.33^{def}	35.00^{defg}	33.00^{cdefgh}
9	25.67^{cdef}	40.33^{efg}	38.33^{cd}	54.33^{bcd}	41.33^{abcd}	40.67^{ab}
10	25.33^{defg}	41.00^{cdef}	35.67^{de}	56.00^{bc}	40.00^{abcd}	42.67^{a}
11	25.67^{cdef}	40.67^{efg}	36.33^{de}	51.67^{de}	39.67^{abcd}	39.00^{abc}
12	25.67^{cdef}	42.00^{cde}	35.33^{de}	54.33^{bcd}	38.67^{bcde}	40.00^{ab}
13	24.00^{efghi}	41.33^{def}	33.67^{efg}	52.00^{cde}	35.67^{cdef}	35.00^{bcdefg}
14	26.67^{cde}	36.00^{fgh}	32.33^{efgh}	52.33^{cde}	32.67^{efgh}	31.67^{fghijk}
15	31.33^{ab}	40.33^{efg}	36.33^{de}	52.67^{cde}	35.67^{cdef}	36.67^{abcd}
16	29.00^{abcd}	39.67^{efg}	33.33^{efg}	54.00^{bcd}	29.33^{fghi}	35.67^{bcdef}
17	28.67^{abcd}	39.33^{efg}	34.67^{de}	49.67^{efg}	29.33^{fghi}	31.33^{fghijk}
18	24.33^{efgh}	46.33^{bc}	42.00^{bc}	53.33^{cde}	35.33^{cdef}	33.33^{efghij}
19	25.33^{defg}	46.00^{bcd}	41.67^{bc}	54.33^{bcd}	36.00^{def}	$34.33^{bcdefgh}$
20	29.67^{abc}	50.00^{ab}	45.67^{ab}	57.33^{a}	42.00^{abc}	39.00^{abc}
21	27.33^{bcde}	52.33^{a}	49.00^{a}	60.33^{a}	46.00^{a}	42.67^{a}
22	21.33^{ghi}	52.67^{a}	49.67^{a}	60.33^{a}	43.00^{ab}	36.33^{bcde}
Minimum	20.00	21.00	20.00	36.00	21.00	20.00
Maximum	34.00	54.00	50.00	61.00	47.00	45.00
SEM	0.52	0.95	0.77	0.61	0.83	0.68

abcdefghijk: Mean values on the same column with different superscript differ statistically at 5% probability test; SEM: Standard error of means.

4.3.6: Ambient relative humidity during incubation temperature alteration at early embryogenesis in Japanese quails

The daily ambient relative humidity when incubation temperature was paused during early embryogenesis in Japanese quails is presented in table 4.19. All the mean values across the treatments were statistically different ($P<0.05$). The minimum mean value was between 20.00 (T_A, T_B) and 27.00% (T_D, T_F) and the maximum was between 45.00% (T_B) and 61.00% (T_C, T_D) across the treatments.

Table 4.19: Ambient relative humidity during incubation temperature alteration at early embryogenesis in Japanese quails

	Treatments(%)					
Embryonic day	T_A Control (38°C)	T_B 36°C	T_C 37°C	T_D 38°C	T_E 39°C	T_F 40°C
0	26.33^{h}	30.33^{ghij}	48.00^{fgh}	35.00^{g}	33.33^{ef}	44.00^{ab}
1	30.33^{gh}	28.33^{ijk}	26.67^{k}	34.00^{g}	27.67^{ef}	48.33^{a}
2	31.00^{gh}	25.67^{k}	30.00^{j}	35.00^{g}	24.67^{f}	34.67^{bcd}
3	29.00^{gh}	28.00^{ijk}	45.00^{hi}	45.00^{ef}	45.00^{abc}	34.33^{bcd}
4	33.00^{gh}	26.33^{jk}	46.33^{ghi}	46.33^{def}	46.33^{ab}	45.67^{a}
5	40.67^{ef}	30.33^{ghij}	44.00^{i}	44.00^{f}	44.00^{abcd}	46.33^{a}
6	42.67^{def}	31.00^{fghi}	48.00^{fgh}	48.00^{cdef}	48.33^{a}	47.67^{a}
7	41.33^{def}	29.00^{hijk}	46.67^{ghi}	46.67^{def}	34.67^{de}	43.67^{ab}
8	44.00^{de}	33.00^{defg}	51.33^{def}	51.33^{abcdef}	34.33^{def}	35.00^{bcd}
9	48.00^{abcde}	40.67^{ab}	54.33^{bcd}	54.33^{abcd}	45.67^{ab}	29.67^{d}
10	47.67^{abcde}	42.67^{a}	56.00^{bc}	56.00^{abc}	46.33^{ab}	39.33^{abc}
11	52.67^{abc}	39.00^{abc}	51.67^{def}	51.67^{abcdef}	47.67^{a}	46.33^{a}
12	54.67^{a}	40.00^{ab}	54.33^{bcd}	54.33^{abcd}	43.67^{abcd}	47.67^{a}
13	54.00^{ab}	35.00^{cdef}	52.00^{cde}	52.00^{abcdef}	35.00^{de}	43.67^{ab}
14	36.00^{fg}	31.67^{efghi}	52.33^{de}	52.33^{abcdef}	31.67^{ef}	47.67^{a}
15	45.67^{cde}	36.67^{bcd}	52.67^{cde}	52.67^{abcdef}	36.67^{bcde}	46.33^{a}
16	46.33^{bcde}	35.67^{cde}	54.00^{bcd}	54.00^{abcd}	35.67^{cde}	46.33^{a}
17	47.67^{abcde}	31.33^{efghi}	49.67^{efg}	49.67^{bcdef}	31.33^{ef}	47.67^{a}
18	43.67^{def}	33.33^{defg}	53.33^{cd}	53.33^{abcde}	33.33^{ef}	43.67^{ab}
19	47.67^{abcde}	34.33^{defg}	54.33^{bcd}	54.33^{abcd}	53.00^{a}	35.00^{bcd}
20	49.33^{abcd}	39.00^{abc}	57.33^{ab}	57.33^{ab}	51.67^{a}	33.00^{cd}
21	53.33^{abc}	42.67^{a}	60.33^{a}	60.33^{a}	52.67^{a}	40.67^{abc}
22	55.33^{a}	43.00	60.33^{a}	60.33^{a}	33.33^{ef}	42.67^{ab}
Minimum	20.00	20.00	26.00	27.00	22.00	27.00
Maximum	57.00	45.00	61.00	61.00	56.00	51.00
SEM	1.12	0.68	0.98	0.99	1.11	0.84

abcdefghijk: Mean values on the same column with different superscript differ statistically at 5% probability test; SEM: Standard error of means.

4.3.7: Brooding temperature and relative humidity of Japanese quail chicks hatched at manipulated temperature in early embryogenesis

The brooding temperature and relative humidity of Japanese quail chicks are showed in table 4.20. The mean values were not significantly different (P>0.05) in all the parameters measured across the treatments. However, the brooding pen mean temperature ranged from 25.90^{o}C (weeks 1 and 3) to 34.80^{o}C (weeks 1, 2 and 3), ambient mean temperature (26.73to 26.87^{o}C), brooding pen and ambient relative humidity varied from 68.82 to 69.56% and 68.44 to 68.87%, respectively throughout the 3 weeks brooding period.

Table 4.20: Pen temperature and relative humidity during brooding of Japanese quail chicks hatched at manipulated temperature in early embryogenesis

	Brooding period											
	Week 1				Week 2				Week 3			
Parameters	**Mean**	**Min**	**Max**	**SEM**	**Mean**	**Min**	**Max**	**SEM**	**Mean**	**Min**	**Max**	**SEM**
Temperature (^{o}C)												
Brooding pen	34.72	25.90	34.80	0.11	34.73	26.90	34.80	0.13	33.83	25.90	34.80	0.12
Ambient	26.80	24.90	29.50	0.12	26.87	24.90	29.50	0.14	26.73	24.90	29.50	0.12
Relative humidity(%)												
Brooding pen	68.91	60.00	77.00	0.70	69.56	60.00	77.00	0.75	68.82	60.00	77.00	0.40
Ambient	68.87	62.00	79.00	0.56	68.64	62.00	79.00	0.49	68.44	62.00	79.00	0.30

SEM: Standard error of means; Min: Minimum value; Max: Maximum value.

4.3.8: Physiological status of Japanese quail eggs incubated at manipulated temperature during early embryogenesis

Table 4.21 gives the physiological status of Japanese quail eggs subjected to paused incubation temperature,hatchability and chick yield. There were statistical differences ($P<0.05$) in all the mean values of the parameters monitored except, total egg weight, average egg weightand not clear whether fertile eggs or not. The incubation period was 18 days in all the treatments except the eggs in T_B that hatched on incubation day 19. Though there was no occurrence of pipped and alive but not able to emerge embryos in any of the treatments, pipped and dead embryos (3.34) as well as fully developed but dead embryos (14.33) were highest in T_C.

Even when fertile eggs were more (46.33) in T_C, hatched chicks were more (37.00) in T_A and the least value (0.33) was recorded in T_D. While the average egg weight ranged from 9.45g (T_F) to 9.62g (T_C), average chick weight value varied from 5.46g (T_E) to 6.48g (T_B) with superior hatchability ($P<0.05$) of 81.01% recorded in T_A, compared to 1.52% recorded in T_D and absolutely zero in T_F. While fertility rate was lowest (6.67%) in $T_F$and highest (66.18%) in T_C, chick yield value was however lowest (57.90%) in T_E and was as high as 67.73% in T_B.

Table 4.21: Physiological status of Japanese quail eggs incubated at manipulated temperature,hatchability and chick yield

	Treatments						
	T_A	T_B	T_C	T_D	T_E	T_F	
Parameters	**Control (38°C)**	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**SEM**
Totaleggs set	210	210	210	210	210	210	-
TEW (g)	1993.80	2011.14	2021.34	2012.35	1994.12	1984.61	1.80
AEW (g)	9.49	9.57	9.62	9.57	9.49	9.45	0.03
IP (days)	18	19	18	18	18	-	-
Unfertile eggs: No evidence of embryo growth							
Infertile eggs	10.67^{b}	14.33^{b}	5.67^{c}	22.33^{a}	23.67^{a}	26.67^{a}	3.42
Caked eggs	6.33^{b}	7.00^{b}	10.00^{b}	20.00^{a}	17.33^{a}	24.67^{a}	1.87
NCWFN	7.33	7.67	8.00	6.00	7.67	9.32	0.83
Fertile eggs: Evidence of embryo growth							
EDE	1.33^{e}	7.67^{bc}	7.33^{bc}	11.34^{a}	6.33^{c}	4.67^{d}	1.38
FDDE	4.67^{b}	13.00^{a}	14.33^{a}	9.00^{ab}	9.33^{ab}	0.00^{c}	1.63
PDE	2.67^{a}	2.66^{a}	3.34^{a}	1.00^{ab}	3.00^{a}	0.00^{b}	0.45
PAE	0.00	0.00	0.00	0.00	0.00	0.00	0.0
Hatched chicks	37.00^{a}	17.67^{b}	21.33^{b}	0.33^{d}	2.67^{c}	0.00^{d}	4.26
Fertile eggs	45.67^{a}	41.00^{a}	46.33^{a}	21.67^{ab}	21.33^{ab}	4.67^{b}	4.97
Fertility (%)	65.24^{a}	58.57^{a}	66.18^{a}	30.95^{b}	30.47^{b}	6.67^{c}	7.10
Hatchability and chick yield							
Hatchability (%)	81.01^{a}	43.09^{b}	46.04^{b}	1.52^{c}	12.52^{bc}	0.00^{c}	7.57
TCW (g)	226.78^{a}	115.24^{b}	138.94^{b}	1.95^{d}	14.47^{c}	0.00^{d}	26.67
ACW(g)	5.95^{a}	6.48^{a}	6.32^{a}	5.84^{a}	5.46^{a}	0.00^{b}	0.70
Chick yield (%)	62.69^{a}	67.73^{a}	65.77^{a}	60.49^{a}	57.90^{a}	0.00^{b}	7.35

abc: Mean values on the same row with different superscript differ statistically at 5% probability test; SEM: Standard error of means; TEW: Total egg weight; AEW: Average egg weight; IP: Incubation period; NCWFN: Not clear whether fertile eggs or not; EDE: Early dead embryos; FDDE: Fully developed but dead embryos; PDE: Pipped and dead embryos; PAE: Pipped and alive but not able to emerge embryos; TCW: Total chick weight; ACW: Average chick weight.

4.3.9: Post-hatchperformance of Japanese quail chicks subjected to paused incubation temperature

4.3.9.1 Feed intake by Japanese quail chicks subjected to paused incubation temperature during early embryogenesis

Daily feed intake by Japanese quail chicks subjected to paused incubation temperature during early embryogenesis is shown in table 4.22. There were statistical variations ($P<0.05$) in all the mean values of the parameters measured across the treatments. It was observed that the population size was somewhat inconsistent throughout the 6 weeks study period, yet feed intake value was increasing with age among all the birds.

In week 2 however, feed intake per group of chicks was highest (973.72g) in T_A and lowest (222.04g) in T_B, but feed intake per chick was best (14.20g) in T_C and poorest (10.27g) in T_B. Similarly, at 3 weeks of age, the feed intake per group was highest (1005.35g) in $T_A$and feed intake per chick was best (19.59g) in T_C. In week 4, both feed intake per group and chick werebest in T_A but were observed to be somewhat diminishing in weeks 5 and 6, respectively.

Table 4.22: Daily feed intake by Japanese quail chicks subjected to paused incubation temperature during early embryogenesis

		Treatments						
		T_A	T_B	T_C	T_D	T_E	T_F	
		Control						
Week	Parameters	38°C	36°C	37°C	38°C	39°C	40°C	SEM
1	No. of chicks	111	56	69	1	8	0	-
	Feed intake/group (g)	ND	ND	ND	ND	ND	ND	ND
	Feed intake/chick (g)	ND	ND	ND	ND	ND	ND	ND
2	No. of chicks	85	21	26	0	0	0	-
	Feed intake/group (g)	973.72^{a}	222.04^{c}	382.86^{b}	0.00^{d}	0.00^{d}	0.00^{d}	12.77
	Feed intake/chick (g)	11.36^{a}	10.27^{a}	14.20^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.45
3	No. of chicks	79	20	26	0	0	0	-
	Feed intake/group (g)	1005.35^{a}	315.70^{c}	503.42^{b}	0.00^{d}	0.00^{d}	0.00^{d}	23.20
	Feed intake/chick (g)	12.26^{b}	15.32^{ab}	19.59^{a}	0.00^{c}	0.00^{c}	0.00^{c}	0.33
4	No. of chicks	78	20	26	0	0	0	-
	Feed intake/group (g)	1296.50^{a}	282.93^{c}	405.43^{b}	0.00^{d}	0.00^{d}	0.00^{d}	25.49
	Feed intake/chick (g)	16.34^{a}	14.16^{a}	15.93^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.34
5	No. of chicks	77	19	26	0	0	0	-
	Feed intake/group (g)	1336.49^{a}	337.73^{b}	429.56^{b}	0.00^{c}	0.00^{c}	0.00^{c}	28.29
	Feed intake/chick (g)	17.36^{a}	17.82^{a}	16.82^{a}	0.00^{b}	0.00^{b}	0.00^{b}	
6	No. of chicks	77	19	21	0	0	0	-
	Feed intake/group (g)	1330.07^{a}	291.57^{b}	347.00^{b}	0.00^{c}	0.00^{c}	0.00^{c}	24.70
	Feed intake/chick (g)	17.30^{a}	15.12^{a}	16.75^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.5

abcd: Mean values on the same row with different superscript differ statistically at 5% probability test; SEM: Standard error of means; ND: Not determined due to irregular incubation periods and 100% mortality in T_D and T_E; Group: Represent total number of birds per treatments.

4.3.9.2: Growth rate of Japanese quail chicks subjected to paused incubation temperature at early embryogenesis

Table 4.23 presents the weekly growth pattern of Japanese quail chicks subjected to paused incubation temperature at early embryogenesis. The mean values of all the parameters measured across the treatments differed significantly ($P<0.05$). The weight values were increasing with age, signifying that the chicks were gaining weight except in week 4, when the values dropped tremendously. In week 5 however, the weight values increased again across the treatments indicative of not attaining full growth yet. In week 2, absolute growth rate varied from 20.20g in T_B to 23.68g in T_A, week 3 (24.00 to 26.02g), week 4 (19.68 to 21.04g) and in week 5, it was highest (26.78g) in T_C and lowest (22.75g) in T_A.

Table 4.23: Growth pattern of Japanese quail chicks subjected to paused incubation temperature at early embryogenesis

		Treatments						
		T_A Control	T_B	T_C	T_D	T_E	T_F	
Week	**Parameters**	**38°C**	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**SEM**
1	No. of chicks	111	56	69	1	8	0	-
	Weight/group (g)	680.89[a]	382.20[c]	446.07[b]	5.84[e]	43.42[d]	0.00[e]	21.02
	Weight/chick (g)	6.13[a]	6.82[a]	6.46a	5.84[a]	5.38[a]	0.00[b]	0.58
	Absolute growth rate	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	No. of chicks	85	21	26	0	0	0	-
	Weight/group (g)	2641.62[a]	572.77[b]	777.39[b]	0.00[c]	0.00[c]	0.00[c]	76.82
	Relative growth rate	30.92a	27.03[a]	29.94[a]	0.00[b]	0.00[b]	0.00[b]	2.59
	Absolute growth rate(g)	23.68[a]	20.20[a]	22.52[a]	0.00[b]	0.00[b]	0.00[b]	1.66
3	No. of chicks	79	20	26	0	0	0	-
	Weight/group (g)	4372.35[a]	1027.24[b]	1478.60[b]	0.00[c]	0.00[c]	0.00[c]	128.88
	Relative growth rate	55.01[a]	51.47[a]	56.31[a]	0.00[b]	0.00[b]	0.00[b]	3.99
	Absolute growth rate (g)	24.02[a]	24.00[a]	26.02[a]	0.00[b]	0.00[b]	0.00[b]	0.96
4	No. of chicks	78	20	26	0	0	0	-
	Weight/group (g)	5888.37[a]	1432.29[b]	2013.45[b]	0.00[c]	0.00[c]	0.00[c]	172.49
	Relative growth rate	75.44[c]	71.58[b]	77.49[a]	0.00[d]	0.00[d]	0.00[d]	5.12
	Absolute growth rate (g)	19.68[a]	19.83[a]	21.04[a]	0.00[b]	0.00[b]	0.00[b]	2.06
5	No. of chicks	77	19	26	0	0	0	-
	Weight/group (g)	7616.18[a]	1851.99[b]	2732.53[b]	0.00[c]	0.00[c]	0.00[c]	224.06
	Relative growth rate	98.58[a]	97.34[a]	104.85[a]	0.00[b]	0.00[b]	0.00[b]	7.03
	Absolute growth rate (g)	22.75[a]	25.95[a]	26.78[a]	0.00[b]	0.00[b]	0.00[b]	1.66

abcde: Mean values on the same row with different superscript differ statistically at 5% probability test; SEM: Standard error of means; Group: Represent total number of birds per treatments.

4.3.9.3: Blood profiles of Japanese quail chicks subjected to paused incubation temperature at early embryogenesis

The blood profiles of Japanese quail chicks subjected to paused incubation temperature at early embryogenesis is shown in table 4.24. There were no significant differences ($P>0.05$) in all the parameters evaluated in T_A, T_B and T_C except, in lymphocytes where birds in T_C (57.83%) were statistically inferior ($P<0.05$) compared to those in T_A(84.00%). Packed cell volume was statisticallysuperior ($P<0.05$) in T_B (40.83%), slightly followed by T_C (38.67%) and T_B (38.33%). While a similar trend was observed in red blood cells, white blood cells were more ($5.03x\ 10^9/L$) in T_A and least ($3.30x10^9/L$) in T_B, haemoglobin varied from 8.07 to 9.47g/dL, neutrophil (15.83 to 40.67%) and monocytes ranged from 0.17% in T_A to 0.67%) in T_C.

Table 4.24: Blood profiles of Japanese quail chicks subjected to paused incubation temperature at early embryogenesis

	Treatments						Statistics			
Parameters	T_A Control (38°C)	T_B 36°C	T_C 37°C	T_D 38°C	T_E 39°C	T_F 40°C	Min	Max	Overall mean	SEM
No. of birds	6	6	6	0	0	0				-
PCV (%)	38.33^{a}	40.83^{a}	38.67^{a}	0.00^{b}	0.00^{b}	0.00^{b}	27.00	46.00	39.27	1.34
RBC (x10^{12}/L)	1.08^{a}	1.33^{a}	1.30^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.70	2.00	1.24	0.11
WBC (x10^{9}/L)	5.03^{a}	3.30^{a}	4.72^{a}	0.00^{b}	0.00^{b}	0.00^{b}	1.60	10.40	4.35	0.49
HB(g/dl)	9.43^{a}	9.47^{a}	8.07^{a}	0.00^{b}	0.00^{b}	0.00^{b}	6.20	12.00	8.99	0.79
Neut (%)	15.83^{b}	25.17^{ab}	40.67^{a}	0.00^{c}	0.00^{c}	0.00^{c}	4.00	75.00	27.22	3.09
Lym (%)	84.00^{a}	74.50^{ab}	57.83^{b}	0.00^{c}	0.00^{c}	0.00^{c}	22.00	96.00	72.11	6.45
Mono (%)	0.17^{a}	0.33^{a}	0.67^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00	2.00	0.39	0.08

abc: Mean values on the same row with different superscript differ statistically at 5% probability test; Min: Minimum value; Max: Maximum value; SEM: Standard error of means; PVC: Packed cell volume; RBC: Red blood cells; WBC: White blood cells; HB: Haemoglobin; Neut:Neutrophils; Lym: Lymphocytes; Mono: Monocytes.

4.3.9.4: Serum biochemistry of Japanese quail chicks subjected to paused incubation temperature at early embryogenesis

Serum biochemistry of Japanese quail chicks subjected to paused incubation temperature at early embryogenesis is provided in table 4.25. There were no statistical variations ($P<0.05$) in all the parameters determined in T_A, T_B and T_C except, in creatinine (51.29Umol/L) and cholesterol (211.68mg/dL) that were both superior ($P<0.05$) in T_A. Alanine aminotransferase value varied from 59.00U/L (T_C) to 61.67U/L (T_B), total protein (30.46g/L to 33.22g/L) and albumin ranged from 15.67g/L in T_B to 18.16g/L in T_C.

Table 4.25: Serum biochemistry of Japanese quail chicks subjected to paused incubation temperature at early embryogenesis

	Treatments						Statistics			
	T_A Control	T_B	T_C	T_D	T_E	T_F				
Parameters	**38**^{o}C	**36**^{o}C	**37**^{o}C	**38**^{o}C	**39**^{o}C	**40**^{o}C	**Min**	**Max**	**Mean**	**SEM**
No. of birds	6	6	6	0	0	0	-	-	-	-
ALT(U/L)	60.17^{a}	61.67^{a}	59.00^{a}	0.00^{b}	0.00^{b}	0.00^{b}	53.00	67.00	60.28	0.89
TP (g/L)	30.46^{a}	31.25^{a}	33.22^{a}	0.00^{b}	0.00^{b}	0.00^{b}	19.74	45.32	31.65	1.74
ALB (g/L)	16.56^{a}	15.67^{a}	18.16^{a}	0.00^{b}	0.00^{b}	0.00^{b}	10.76	27.19	16.79	1.19
CREAT (Umol/L)	51.29^{a}	40.46^{b}	45.72^{ab}	0.00^{c}	0.00^{c}	0.00^{c}	29.14	62.52	45.82	2.47
CHOL (Mg/dL)	211.68^{a}	193.48^{ab}	189.08^{b}	0.00^{c}	0.00^{c}	0.00^{c}	168.50	243.00	198.08	6.02

abc: Mean values on the same row with different superscript differ statistically at 5% probability test; Min: Minimum value; Max: Maximum value; SEM: Standard error of means; ALT: Alanine aminotransferase; TP: Total protein; ALB: Albumin; CREAT: Creatinine; CHOL: Cholesterol.

4.3.9.5:Sexual maturity, sex reversal, carcass quality and organs weight of Japanese quail chicks subjected to paused incubation temperature at early embryogenesis

Table 4.26 presents the sexual maturity, sex reversal,carcass quality and organs weight of Japanese quail chicks subjected to paused incubation temperature at early embryogenesis. There were no significant differences ($P>0.05$) in all the parameters measured in T_A, T_B and T_C. The hens in all the treatments started laying eggs at the age of 5 weeks and developing yolk as well as fully formed eggs that were purely white (without pigmentation) were found in the uteri of the sacrificed hens.

There was a case of reversed sex from male to female in T_A and the sex ratio (male: female) was 40:37, 10:9, 10:11 in T_A, T_B and T_C in that order. While the live weight ranged from 120.23g in T_B to 127.58g in T_A, the carcass weight was highest (89.74g) in T_A, followed by 85.05g (T_B) and 83.61g in T_C. The dressing percentage value varied between 68.56 and 70.56%, gizzard weight (3.29 to 3.53g), liver weight (2.19 to 2.86g) and heart weight varied between 1.21 and 1.43g. The right testis mean weight ranged from 0.77 to 0.87g while the left testis weight was between 0.79 and 0.98g.

Table 4.26: Sexual maturity, sex reversal, carcass quality and organs weight of Japanese quail chicks subjected to paused incubation temperature at early embryogenesis

	Treatments						
	T_A	T_B	T_C	T_D	T_E	T_F	
Parameters	**Control (38°C)**	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**SEM**
Sexual maturity							
Age at lay (week)	5	5	5	0	0	0	-
Yolk	1.67[a]	1.75[a]	1.508[a]	0.00[b]	0.00[b]	0.00[b]	0.32
Formed eggs	0.42[a]	0.17[a]	0.25[a]	0.00[b]	0.00[b]	0.00[b]	0.07
Sex ratio (M : F)	40:37	10:9	10:11	0	0	0	-
Sex M → F	1	0	0	0	0	0	-
Reversal F → M	0	0	0	0	0	0	-
Carcass quality (g)							
No. of birds	12	12	12	0	0	0	-
Live weight	127.58[a]	120.23[a]	121.49[a]	0.00[b]	0.00[b]	0.00[b]	2.29
Bled weight	122.18[a]	115.06[a]	115.55[a]	0.00[b]	0.00[b]	0.00[b]	2.09
Def. weight	105.74[a]	98.97[a]	100.41[a]	0.00[b]	0.00[b]	0.00[b]	2.13
Carcass weight	89.74	85.05	83.61	0.00[b]	0.00[b]	0.00[b]	2.04
D. percentage (%)	70.19[a]	70.56[a]	68.56[a]	0.00[b]	0.00[b]	0.00[b]	0.48
Organs weight (g)							
Gizzard weight	3.35[a]	3.29[a]	3.53[a]	0.00[b]	0.00[b]	0.00[b]	0.12
Liver weight	2.62[a]	2.19[a]	2.86[a]	0.00[b]	0.00[b]	0.00[b]	0.22
Heart	1.21[a]	1.22[a]	1.43[a]	0.00[b]	0.00[b]	0.00[b]	0.07
Testes Right	0.85[a]	0.87[a]	0.77[a]	0.00[b]	0.00[b]	0.00[b]	0.15
weight Left	0.98[a]	0.94[a]	0.79[a]	0.00[b]	0.00[b]	0.00[b]	0.16

ab: Mean values on the same row with different superscript differ statistically at 5% probability test; SEM: Standard error of means; D. percentage: Dressing percentage; Def. weight: Defeathered weight.

4.3.9.6: Mortality of Japanese quail chicks subjected to paused incubation temperature at early embryogenesis

Expressed in table 4.27 is the mortality of Japanese quail chicks that were subjected to paused incubation temperature at early embryogenesis. There were no significant differences (P>0.05) across the treatments mean values, except during the brooding period (week 1 – 3) whenhighest mortality (2.07) was recorded in T_A, followed by 1.58 (T_C), 1.54 (T_B), 1.14 (T_E) and 0.33 (T_D), respectively. It was observed that all the chicks in T_D and T_E were lost before the 4^{th} week. At 3 – 4 weeks of age, 0.33 chick mortality was recorded in T_A and T_B with absolutely zero in T_C. At 4 – 5 weeks of age chick mortality (0.33) was recorded only in T_A, whereas at 5 – 6 weeks, there was no chick mortality in $T_A$nevertheless, 0.67 and 0.50 were recorded in $T_B$andT_C, accordingly.

Table 4.27: Mortality of Japanese quail chicks subjected to paused incubation temperature at early embryogenesis

	Treatments						Statistics		
	T_A Control	T_B	T_C	T_D	T_E	T_F			
Age (week)	(38°C)	36°C	37°C	38°C	39°C	40°C	Min	Max	SEM
1 - 3	2.07^{b}	1.54^{bc}	1.58^{bc}	0.33^{bc}	1.14^{a}	0.00^{c}	0.00	6.00	0.14
3 - 4	0.33	0.33	0.00	0.00	0.00	0.00	0.00	1.00	0.09
4 - 5	0.33	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.07
5 - 6	0.00	0.67	0.50	0.00	0.00	0.00	0.00	2.00	0.15

abc: Mean values on the same row with different superscript differ statistically at 5% probability test; Min: Minimum value; Max: Maximum value; SEM: Standard error of means.

4.3.9.7 Survival rate of Japanese quail chicks subjected to paused incubation temperature at early embryogenesis

Presented in table 4.28 is the survival rate of Japanese quail chicks subjected to paused incubation temperature at early embryogenesis. There were significant differences ($P<0.05$) in all the parameters monitored. More hatchlings (37.00) was recorded in T_A, followed by 21.33 in T_C, 17.67 (T_B), 0.33 (T_D) and there was no hatching (absolutely zero) in T_F. More chick mortality (13.33) was recorded in T_B, slightly followed by 13.00 (T_C), 9.67 (T_A), 2.67 (T_E) and 0.33 in T_D. The mortality rate was least (50.77%) in T_A, followed by 61.13% in T_C and 77.80% in $T_B$compared to absolutely 100% recorded in $T_D$and T_E.The survival rate was best (49.23%) in T_A, slightly followed by 38.87% and 22.20% in T_C and T_B, respectively.

Table 4.28: Survival rate of Japanese quail chicks subjected to paused incubation temperature at early embryogenesis

	Treatments						Statistics		
	T_A Control	T_B	T_C	T_D	T_E	T_F			
Parameters	**38°C**	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**Min**	**Max**	**SEM**
Hatched chicks	37.00^{a}	17.67^{ab}	21.33^{ab}	0.33^{b}	2.67^{b}	0.00^{b}	0.00	60.00	4.25
Chick mortality	9.67^{ab}	13.33^{a}	13.00^{a}	0.33^{c}	2.67^{bc}	0.00^{c}	0.00	19.00	1.67
Mortality rate (%)	50.77^{ab}	77.80^{a}	61.13^{ab}	100^{a}	100^{a}	0.00^{c}	0.00	100.00	9.61
Survival rate (%)	49.23^{a}	22.20^{ab}	38.87^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00	76.00	6.06

abc: Mean values on the same row with different superscript differ statistically at 5% probability test; Min: Minimum value; Max: Maximum value; SEM: Standard error of means.

4.3.10 Discussion

4.3.11 Role of pausing incubation temperature during early embryogenesis on Japanese quails on sex reversal

There were fluctuations on the daily incubation temperature ranging from 0.03 to 0.77^{o}C in all the treatment incubators including control, throughout the study period probably as influenced by heat production, even after shut down by the thermostat and partly due to ambient weather conditions. The calibrated treatment incubators temperatures (except control) were altered to physiological zero temperature (26 – 36^{o}C) on embryonic days 3, 4 and 5, which Boerjan (2016) described as "state of no embryo growth and development". Meanwhile, Conway and Thomas (2000) described this condition as "lower limit of optimal embryo growth and development" in avian species. It was stated that though embryo growth and development may be slow, it will not be impaired but prolonged exposure, could cause developmental abnormalities. Meanwhile, it was reported that this developmental abnormality could be reversed if the normal incubation temperature is restored (Webb, 1987; Conway and Thomas, 2000; Boerjan, 2016). However, it is possible that the gonadogenesis may not reverse rather there may be swift development of the rudimentary sex characters resulting in sex reversal. During the incubation temperature pausing that lasted for 5 hours on embryonic days 3, 4 and 5, it was observed that the temperature gradually declined swiftly over time until both incubation and ambient temperatures were at equilibrium. The recorded daily ambient temperature value was higher than 20 – 25^{o}C yet, relative humidity was lower than 70 – 87% respectively predicted to prevail that season (April/May) of the year in Jos, Plateau State (EUMETSAT, 2018). The differences could be mainly due to the emission of heat from the perforations in all the incubators and partly due to shutting of the door and windows in the pen. In any case, the hourly incubation temperature monitored during embryonic days 3, 4 and 5, when the incubation temperature was paused for 5 hours, was within 26 – 36^{o}C, described as physiological zero temperature in avian embryogenesis (Webb, 1987; Conway and Thomas, 2000; Fasenko, 2007; Boerjan, 2016).

The relative humidity in each treatment incubator including the control as well as the ambient relative humidity was not seemingly affected by the incubation temperaturepausing. Incubation temperaturepausing could be described as the halting of

heat supply to the incubator, in order to reduce theincubation temperaturebelow the recommended optimum range of 37 to 38^{o}C. This temperature reduction probably helped in maintaining relative humidity range of 21 – 61% across all the incubators throughout the incubation period. These values were however, less than 60 – 70% recommended for successful hatching and survival of the hatchlings (Tullett, 1990; Romao *et al*., 2009a). The observed fluctuations in the ambient relative humidity probably resulted in the low values observed in the treatment incubators. Also, it could be possibly due to insufficient air circulation in the treatment incubators and the pens, since the door and the windows were shut. The incubation period was close to the normal range of 16 – 18 days reported by Sellier *et al*. (2006) and the chick weight as well as chick yield was somewhat within 5 – 7g and 60 – 70%, respectively, purported to be a normal range in Japanese quails (Romao *et al*., 2009b; Alkan *et al*., 2011). However, the recorded chick weight was less than 8.20g reported in Japanese quails when *in ovo* Rutin injection was administered (Genc *et al*., 2019). The chick yield value compared favourably well with 66.0% reported by Akpınar and Günenç (2019) in Japanese quails. Consequently, altered incubation temperature during early embryogenesis may not affect Japanese quail hatchling weight. The hatchability value was less than a range of 52.4 to 69.30% reported in Japanese quail eggs stored and transported prior to incubation (Akpınar and Günenç 2019). The observed hatching failure in T_F and low hatchability in T_D and T_E, were probably due to the impact of temperature alteration, on the embryos growth and developmental processes. Also, it could be partly due to the low egg fertility recorded in T_D, T_E and T_F, that resulted in the poor hatchability and hatching failure. This seemingly contradicted the observations of Elmehdawi (2013), who observed no adverse effects of incubation temperature alteration on hatchability, when broiler breeder eggs were subjected to low and high incubation temperatures. The disparities could be possibly due to the incubation temperature fluctuations range and probably due to the avian species used in the studies. Nonetheless, the observation apparently buttressed the reports of Romao *et al*. (2009b), who recorded poor hatchability in Japanese quail eggs incubated at different temperatures and Mani *et al*. (2008), when Japanese quail eggs were stored for 20 days before incubating at high incubation temperature. It was revealed that some of the unhatched eggs opened were “caked”, “infertile” and “not clear whether fertile or not”. Meanwhile, some of the eggs

were observed to have developing embryos at different stages of growth and development described either as “early dead embryos”, “fully developed but dead embryos”, “pipped and dead embryos” or “pipped and alive but not able to emerge embryos” in this study. All these physiological statuses were observed in all the treatments, except “pipped and alive but not able to emerge embryos” that was not recorded in all the treatments and in T_F, where there was seemingly no fertile eggs at all, which probably explained the absolute hatching failure recorded. In any case, the described physiological statuses of either “early dead embryos”, “fully developed but dead embryos”, “pipped and dead embryos” or “pipped and alive but not able to emerge embryos” were before now described as early, middle and late embryonic death (Yilmaz *et al*., 2011) and infertile, early embryo death, intermediate embryo death, late embryo death and pipped egg with dead embryo (Romao *et al*., 2009b). Meanwhile, Woodard *et al*. (1973) reported that most embryonic deaths occur during the first 3 days of incubation and at about pipping. This was evidently shown in that study when most of the eggs removed at first 8 days of incubation during candling were infertile and early death embryos. The observed fatal mortality was however ascribed to the failure of the embryo to develop vital organs or a malfunction of its development. Recently, Genc *et al*. (2019) described embryo death in three distinct stages as early (1 to 6 days), intermediate (7 to 14 days) and late (15 to 18 days). The weekly brooding temperature was similar to 30 – 34^{o}C recommended for avian chicks(Poultry Site, 2014b; Penn State Extension, 2016; Hyaline, 2018), even when the prevailing ambient temperature was as low as 24.9 – 29.5^{o}C. This perhaps, indicated that the heat emission from the electric bulbs used was able to raise the brooding temperature which was probably suitable for the survival of the chicks at that age. During this period, the relative humidity was close to 70 – 80% predicted by EUMETSAT (2018) to prevail in Jos, Plateau State. The daily feed intake by the birds in all the treatments was seemingly lower than 23.3 – 30g, 38 – 57g, 56.7 – 92g, 85 – 127.3g and 87 – 166.3g recorded in weeks 1, 2, 3, 4 and 5, respectively, when Japanese quails were subjected to high incubation temperature (Rashid *et al*., 2016). The disparities could be largely due to feed intake result presentation per group or individual, which was however not stated in that study. Meanwhile, the values were relatively higher than 10.56g, 10.39g, 8.93g and 8.87g in weeks 3, 4, 5 and 6 in that order, reported when Japanese quails were fed varying levels

of sundried edible frog meal (Adesina, 2013). These observations could be largely due to the experimental treatments differences, environmental weather conditions, feed quality and the age or sex of the treated birds. According to Ozbey and Ozcelik (2004), high temperature has negative effects on feed consumption, feed efficiency, live weight, growth rate as well as declined productivity and egg quality.

It was speculated that cold weather could make poultry birds eat more, in order to cope with the necessary metabolic processes(Butler and Woakes, 2001; Cooper and Gessaman, 2005). The growth rate in all the birds across the treatments was similar to the values reported in Japanese quails of similar age (Rabie *et al*., 2015). This could be purely due to the feed intake pattern indicative of healthy birds. Meanwhile, the highest growth rate was attained at 3 weeks of age but declined gradually at 4 and 5 weeks of age. This probably showed that the Japanese quails attained "growth spurt" at about 4 weeks of age. Therefore, inclusion level of protein which is often the most expensive component of feed formulation could be reduced, in order to cut down the total cost of production but the energy level may be maintained or increased to meet the energy requirement at this growth phase. Blood profiles of the birds in control, T_B and T_C that were seemingly uniform, were within 29.0 – 58% (packed cell volume), 1.6 – 4.9 $x10^{12}$/L (red blood cells), 3.5 – 52.0$x10^9$/L (white blood cells), 7.8 – 18.5g/dL (haemoglobin), 13 – 72% (neutrophil), 27 – 83% (lymphocytes) and 0.0 – 7.0% (monocytes) given to be normal in healthy avian species (Fudge, 2000; Samour, 2006; Bickford, 2007). Similarly, serum alanine aminotransferase (37 – 50U/L), total protein (3.5 – 5.5mg), albumin (18 – 24g/dL), creatinine (12 – 19Umol/L) and cholesterol (100 – 300mg) were close to the values reported in healthy Japanese quails (Sakas, 2002; Mnisi and Mlambo, 2017). These observations probably indicated that the birds' physiological systems were not compromised.Thus, agreed with the report of Sgavioli *et al*. (2016), that incubation temperature may not be detrimental to poultry species and Piestun *et al.* (2008), who stated that avian species could acquire thermotolerant traits during embryogenesis. The hens in control, T_B and T_C started laying eggs at the age of 5 weeks and yolk as well as formed eggs was found in all the treatments, suggesting that their reproductive potentials were not possibly hampered. The sex ratio was approximately 1 male: 1 female and there was sex reversal from male to female only in control.Therefore, incubation temperature

pausing for 5 hours during early embryogenesis in Japanese quails may not reverse sex. Meanwhile, the live weightwas less than a range of 168.8 to 185.1g reported in Japanese quails when probiotic was administered (Mahrose *et al*., 2019) but the carcass weight and dressing percentage were similar to the values reported in normal healthy Japanese quails of similar age (Lonita *et al*., 2008; Boni *et al*., 2010; Ribarski and Genchev, 2013; Muhammad *et al*., 2017). The liver and heart weight values were similar to 3g and 1g, respectively, reported in Japanese quails (Mahrose *et al*., 2019). Generally, the organs weight were similar to normal values reported in healthy birds thus, all the birds across the treatments were probably not affected by the manipulated incubation temperature during early embryogenesis in Japanese quails. The total loss of all the hatchlings in T_D and $T_E$may be a mere coincidence and never as a result of the incubation temperature treatments. More so, it could be purely due to very few hatchlings recorded in T_D and T_E and possibly due to mortality rate that Nanda *et al*. (2015) stated was expected to be highest before the age of 3 weeks in poultry species. Although there was mortality across the treatments, majority of the birds in T_B and T_C were lost. This could be purely due to the late hatching and low chick yield that Mallik *et al*. (2001) and Aviagen (2015) reported could account for the high mortality rate in Japanese quails. However, the survival rate was lower than 70 – 90% reported when Japanese quails at different ages were subjected to different weather conditions (Nanda *et al*., 2015), but was higher than 5 – 30% annual survival rate in both hunted and unhunted quails population in Florida (Giuliano and Selph, 2015). Meanwhile, the observation compared favourably well with 30 – 60%, speculated to be typical in avian species (MacArthur, 1972; Ricklefs, 1973; Skutch 1985; Karr *et al*., 1990).

Since the number of survived birds in all the treatments was similar, it probably impliedthat pausing incubation temperature for 5 hours during early embryogenesis may not influence post-hatch performance in Japanese quails. However, the loss of all the hatchlings in T_D and T_E as well as the absolute hatching failure recorded in T_F probably showed that pausing incubation temperature during early embryogenesis for 5 hours may not be suitable for sex reversalin Japanese quails.

4.4 Results

4.4.1 Role of incubation temperature manipulation during late embryogenesis in Japanese quail sex reversal

4.4.2Incubation temperature alteration during late embryogenesis in Japanese quails

The daily incubation temperature that was paused during late embryogenesis in Japanese quails is shown in table 4.29. There were no significant differences ($P>0.05$) in all the treatments except on temperature pausing days (11, 12 and 13), that statistically differed ($P<0.05$) from other incubation days which were maintained throughout the incubation period. Minimum value ranged from 26.22 to 37.70^{o}Canda maximum of between 37.20^{o}C and 41.12^{o}C was recorded.

Table 4.29: Alteration of incubation temperature during late embryogenesis in

Japanese quails

	Treatments					
Embryonic day	T_I Control (38°C)	T_{II} 36°C	T_{III} 37°C	T_{IV} 38°C	T_V 39°C	T_{VI} 40°C
0	38.33	35.97[a]	36.98[a]	38.13[a]	38.98[a]	40.10[a]
1	38.03	36.10[a]	37.27[a]	37.73[a]	38.97[a]	40.33[a]
2	38.17	35.87[a]	36.89[a]	37.87[a]	38.87[a]	39.77[a]
3	38.17	36.17[a]	37.23[a]	38.37[a]	39.37[a]	40.70[a]
4	38.23	36.13[a]	37.11[a]	38.53[a]	39.47[a]	39.93[a]
5	38.27	36.20[a]	37.20[a]	37.88[a]	39.30[a]	40.27[a]
6	38.30	36.03[a]	36.90[a]	37.97[a]	39.22[a]	40.30[a]
7	38.27	36.17[a]	37.17[a]	38.30[a]	39.07[a]	40.23[a]
8	38.23	36.03[a]	37.10[a]	38.23[a]	39.62[a]	40.03[a]
9	38.20	36.43[a]	37.37[a]	38.37[a]	38.97[a]	40.03[a]
10	38.10	36.17[a]	37.17[a]	38.27[a]	39.17[a]	40.03[a]
11	38.20	26.77[b]	27.03[b]	26.73[b]	27.23[b]	26.83[b]
12	38.23	27.00[b]	26.77[b]	26.90[b]	27.07[b]	27.10[b]
13	38.10	26.90[b]	26.70[b]	27.53[b]	26.83[b]	26.90[b]
14	38.10	36.40[a]	36.97[a]	38.20[a]	39.03[a]	40.00[a]
15	38.07	36.47[a]	37.27[a]	38.03[a]	39.20[a]	40.03[a]
16	38.10	35.97[a]	36.93[a]	38.37[a]	39.27[a]	40.20[a]
17	38.13	36.23[a]	37.07[a]	38.13[a]	39.30[a]	39.87[a]
18	38.10	36.30[a]	37.30[a]	38.80[a]	39.13[a]	39.90[a]
19	37.87	36.37[a]	37.13[a]	38.53[a]	39.07[a]	40.17[a]
20	38.00	36.50[a]	37.27[a]	38.73[a]	39.33[a]	39.93[a]
21	37.97	36.47[a]	37.07[a]	38.33[a]	39.30[a]	40.30[a]
22	38.20	36.43[a]	36.87[a]	38.43[a]	39.07[a]	40.07[a]
Minimum	37.70	26.70	26.30	26.22	26.40	26.70
Maximum	38.38	37.20	38.09	38.90	40.12	41.10
SEM	0.23	0.24	0.27	0.32	0.37	0.32

ab: Mean values on the same column with different superscript differ statistically at 5% probability test; SEM: Standard error of means.

4.4.3 Ambient temperature during incubation temperature alteration at late embryogenesis in Japanese quails

Given in table 4.30 is the daily ambient temperature when incubation temperature was paused during late embryogenesis in Japanese quails. There were significant variations ($P<0.05$) in all the treatments with minimum value of 28.40^{o}C – 29.90^{o}C and maximum value of 35.50^{o}C – 37.10^{o}C recorded throughout the incubation period.

Table 4.30: Ambient temperature during incubation temperature alteration at late embryogenesis in Japanese quails

	Treatments					
	T_I Control	T_{II}	T_{III}	T_{IV}	T_V	T_{VI}
Embryonic day	(38°C)	36°C	37°C	38°C	39°C	40°C
0	35.57[a]	34.67[a]	33.23[a]	31.15[b]	35.40[a]	34.87[a]
1	32.70[b]	35.57[a]	34.27[a]	32.43[b]	35.80[a]	35.50[a]
2	32.07[b]	32.70[b]	33.03[a]	32.07[b]	32.37[b]	35.43[a]
3	33.80[a]	32.07[b]	33.63[a]	30.27[b]	31.63[b]	35.73[a]
4	33.23[a]	33.80[a]	33.67[a]	34.23[a]	33.80[a]	35.53[a]
5	33.77[a]	33.23[a]	33.93[a]	32.40[b]	32.53[b]	35.77[a]
6	32.13[b]	36.77[a]	34.10[a]	33.77[a]	33.43[a]	36.43[a]
7	33.57[a]	32.13[b]	32.87[b]	31.57[b]	31.93[b]	34.67[a]
8	33.90[a]	33.57[a]	34.07[a]	32.63[b]	33.00[a]	35.80[a]
9	31.47[b]	33.90[a]	33.93[a]	32.67[b]	33.40[a]	35.73[a]
10	31.53[b]	31.47[b]	31.33[b]	30.77[b]	31.00[b]	32.23[b]
11	29.87[c]	31.20[b]	32.00[b]	30.37[b]	30.40[b]	32.80[b]
12	31.23[b]	29.87[c]	29.40[c]	29.57[b]	28.80[b]	30.07[b]
13	33.30[a]	31.23[b]	31.60[b]	29.10[b]	29.97[b]	32.17[b]
14	32.83[b]	33.30[a]	33.17[a]	29.27[b]	30.77[b]	33.73[b]
15	33.43[a]	32.83[b]	33.07[a]	30.10[b]	31.10[b]	33.47[b]
16	34.40[a]	33.43[a]	33.07[a]	31.17[b]	32.30[b]	34.60[a]
17	32.70[b]	34.4 [a]	33.23[a]	31.20[b]	32.37[b]	34.50[a]
18	31.60[b]	32.70[b]	33.33[a]	30.70[b]	31.90[b]	34.57[a]
19	32.97[b]	31.60[b]	32.93[b]	29.03[b]	31.20[b]	34.23[a]
20	33.60[a]	32.97[b]	32.97[b]	29.97[b]	31.77[b]	34.83[a]
21	33.23[a]	33.60[a]	33.40[a]	31.17[b]	32.40[b]	34.90[a]
22	35.57[a]	33.23[a]	33.17[a]	31.23[b]	32.03[b]	34.57[a]
Minimum	28.70	28.94	28.80	28.70	28.40	29.90
Maximum	36.70	37.10	35.50	35.91	36.40	37.00
SEM	0.12	0.71	0.81	0.88	0.95	0.39

ab: Mean values on the same column with different superscript differ statistically at 5% probability test; SEM: Standard error of means.

4.4.4Paused incubation temperature duration at late embryogenesis in Japanesequails

Table 4.31 shows the hourly incubation temperature pausing duration in late stages of Japanese quails embryogenesis. There were significant differences ($P<0.05$) in all the mean values of all the parameters measured across the treatments except at 0, 4th and 5th hour, when the ambient temperature was seemingly constant. At 0 hour, the mean values were close to the treatments' calibrated values but gradually dropped from the 1st hour to the 5th hour, when as low as between 27.83(T_{II}) and 29.23^{o}C (T_{VI}) that were similar to the ambient temperature whichvaried from 28.57%(T_{V}) to 29.43^{o}C(T_{II}) were recorded.

Table 4.31: Incubation temperature pausing duration at late embryogenesis in Japanese quails

Parameters		Treatments						
		T_I	T_{II}	T_{III}	T_{IV}	T_V	T_{VI}	
Pausing time (hr)	Temperature	Control (38^oC)	36^oC	37^oC	38^oC	39^oC	40^oC	SEM
0	Incubation	38.20^{abc}	35.87^{d}	36.97^{cd}	37.83^{bc}	38.77^{ab}	39.50^{a}	0.32
	Ambient	29.17	29.63	28.83	28.83	28.70	29.77	0.21
1	Incubation	38.17^{a}	35.00^{c}	36.03^{bc}	36.13^{bc}	37.07^{ab}	38.37^{a}	0.35
	Ambient	29.90^{b}	30.67^{ab}	30.27^{b}	30.80^{ab}	29.97^{b}	32.00^{a}	0.23
2	Incubation	38.20^{a}	32.67^{b}	33.40^{b}	33.77^{b}	33.17^{b}	34.43^{b}	0.56
	Ambient	31.10^{b}	32.42^{a}	31.07^{b}	31.43^{b}	31.80^{ab}	33.73^{a}	0.32
3	Incubation	38.17^{a}	31.00^{b}	31.17^{b}	31.33^{b}	30.57^{b}	31.30^{b}	0.74
	Ambient	32.53^{ab}	32.67^{ab}	31.43^{b}	31.43^{b}	32.90^{ab}	34.73^{a}	0.39
4	Incubation	38.13^{a}	29.80^{b}	29.70^{b}	29.57^{b}	29.40^{b}	29.47^{b}	0.83
	Ambient	30.83	29.67	29.93	30.00	29.90	31.00	0.44
5	Incubation	38.10^{a}	27.83^{b}	28.97^{b}	29.03^{b}	28.97^{b}	29.23^{b}	0.94
	Ambient	29.10	29.43	28.87	28.87	28.57	29.23	0.33

abcd: Mean values on the same row with different superscript differ statistically at 5% probability test; SEM: Standard error of means.

4.4.5 Incubation relative humidity during incubation temperature alteration at late embryogenesis in Japanese quails

Table 4.32 provides the daily incubation relative humidity during late embryogenesis in Japanese quails. There were statistical differences ($P<0.05$) in all the mean values across the treatments with minimum values varying from 17.00% (T_V) to 41.00% (T_{IV}) while the maximum values varied between 45.00 (T_V, T_{VI}) and 61.00% (T_{IV}).

Table 4.32:Incubation relative humidity during incubation temperature alterationat late embryogenesis in Japanese quails

	Treatments (%)					
	T_I Control	T_{II}	T_{III}	T_{IV}	T_V	T_{VI}
Embryonic day	(38°C)	36°C	37°C	38°C	39°C	40°C
0	32.00^{d}	39.00^{b}	39.00^{b}	51.67^{a}	35.33^{b}	44.33^{a}
1	31.00^{d}	39.67^{b}	39.00^{b}	53.67^{a}	35.67^{b}	42.67^{a}
2	31.00^{d}	41.00^{b}	39.00^{b}	49.33^{b}	35.67^{b}	38.67^{a}
3	25.00^{e}	41.67^{b}	38.33^{b}	49.33^{b}	38.67^{b}	32.67^{b}
4	28.67^{d}	39.00^{b}	33.67^{c}	48.33^{b}	31.67^{c}	31.00^{b}
5	29.00^{d}	41.67^{b}	36.00^{b}	49.33^{b}	34.00^{b}	32.33^{b}
6	29.67^{d}	44.00^{a}	43.33^{a}	50.67^{a}	40.67^{a}	36.00^{b}
7	32.33^{d}	44.00^{a}	40.00^{b}	52.00^{a}	38.67^{b}	37.00^{b}
8	27.67^{d}	44.67^{a}	40.67^{b}	54.33^{a}	39.33^{b}	36.67^{b}
9	32.00^{d}	45.67^{a}	45.00^{a}	54.00^{a}	43.67^{a}	41.00^{a}
10	31.00^{d}	47.00^{a}	43.33^{a}	53.67^{a}	42.00^{a}	41.33^{a}
11	37.00^{c}	46.33^{a}	46.33^{a}	52.33^{a}	42.33^{a}	39.33^{a}
12	38.33^{b}	48.00^{a}	45.00^{a}	55.00^{a}	44.00^{a}	45.00^{a}
13	44.33^{b}	44.33^{a}	42.67^{a}	55.00^{a}	39.33^{b}	38.00^{a}
14	44.33^{b}	40.00^{b}	33.00^{c}	49.00^{b}	29.33^{d}	29.33^{c}
15	41.33^{b}	41.67^{b}	38.33^{b}	45.67^{b}	35.67^{b}	32.33^{b}
16	36.67^{c}	41.00^{b}	35.33^{c}	49.00^{b}	31.67^{c}	31.67^{b}
17	29.00^{d}	43.67^{a}	40.33^{b}	51.00^{a}	35.33^{b}	33.33^{b}
18	29.33^{d}	41.00^{b}	34.00^{c}	49.33^{b}	20.67^{e}	30.67^{b}
19	48.33^{a}	33.33^{c}	28.67^{d}	43.33^{b}	17.33^{e}	26.00^{c}
20	36.00^{c}	38.33^{b}	32.33^{c}	45.00^{b}	28.33^{d}	29.67^{c}
21	29.33^{d}	39.00^{b}	34.00^{c}	46.33^{b}	29.67^{d}	30.00^{b}
22	24.33^{e}	38.33^{b}	45.00^{a}	45.00^{b}	29.67^{d}	27.00^{c}
Minimum	23.00	30.00	28.00	41.00	17.00	27.00
Maximum	50.00	50.00	47.00	61.00	45.00	45.00
SEM	0.49	0.54	0.62	0.77	0.73	0.18

abcde: Mean values on the same column with different superscript differ statistically at 5% probability test; SEM: Standard error of means.

4.4.6 Ambient relative humidity during incubation temperature alteration at late embryogenesis in Japanese quails

The daily ambient relative humidity during late embryogenesis in Japanese quails is given in Table 4.33. There were significant differences ($P<0.05$) in all the mean values across the treatments. Meanwhile, the minimum values ranged from 27.00 (T_{II}) to 47.00% (T_{IV}) and the maximum values were between 45.00 (T_{II}) and 56.00% (T_{III},T_{IV}, T_{V}, T_{VI}) throughout the incubation period.

Table 4.33: Ambient relative humidity during incubation temperature alterationat late embryogenesis in Japanese quails

	Treatments(%)					
Embryonic day	T_I Control (38°C)	T_{II} 36°C	T_{III} 37°C	T_{IV} 38°C	T_V 39°C	T_{VI} 40°C
0	44.33[a]	44.33[a]	51.67[a]	47.67[b]	51.67[a]	50.33[a]
1	42.67[a]	42.67[a]	53.67[a]	48.67[b]	53.67[a]	50.67[a]
2	38.67[b]	38.67[b]	49.33[a]	50.67[a]	49.33[b]	52.00[a]
3	38.67[b]	32.67[c]	49.33[a]	51.33[a]	49.33[b]	54.33[a]
4	32.67[c]	31.00[c]	48.33[a]	53.67[a]	48.33[b]	54.00[a]
5	31.00[c]	32.33[c]	49.33[a]	54.33[a]	49.33[b]	53.67[a]
6	32.33[c]	36.00[b]	50.67[a]	52.67[a]	50.67[a]	52.33[a]
7	36.00[b]	37.00[b]	52.00[a]	53.00[a]	52.00[a]	55.00[a]
8	37.00[b]	36.67[b]	54.33[a]	54.67[a]	54.33[a]	55.00[a]
9	36.67[b]	41.00[a]	54.00[a]	55.00[a]	54.00[a]	51.67[a]
10	41.00[a]	41.33[a]	53.67[a]	52.67[a]	53.67[a]	53.33[a]
11	41.33[a]	39.33[b]	52.33[a]	51.67[a]	52.33[a]	52.00[a]
12	39.33[b]	45.00[a]	55.00[a]	53.67[a]	55.00[a]	45.67[b]
13	45.00[a]	38.00[b]	55.00[a]	49.33[b]	55.00[a]	49.00[b]
14	38.00[b]	29.33[d]	49.00[a]	51.00[a]	49.00[b]	51.00[a]
15	29.33[d]	32.33[c]	45.67[b]	50.67[a]	45.67[b]	49.33[b]
16	32.33[c]	31.67[c]	49.00[a]	51.33[a]	49.00[b]	43.33[b]
17	31.67[c]	33.33[c]	51.00[a]	53.67[a]	51.00[a]	45.00[b]
18	33.33[c]	30.67[c]	49.33[a]	54.33[a]	49.33[b]	46.33[b]
19	30.67[c]	29.33[d]	43.33[b]	52.67[a]	43.33[b]	43.00[b]
20	29.33[d]	29.67[d]	45.00[b]	53.00[a]	45.00[b]	42.67[b]
21	29.67[c]	30.00[c]	46.33[b]	54.67[a]	46.33[b]	35.33[c]
22	30.00[c]	27.00[d]	45.00[b]	52.67[a]	45.00[b]	32.67[c]
Minimum	29.00	27.00	43.00	47.00	43.00	32.00
Maximum	46.00	45.00	56.00	56.00	56.00	56.00
SEM	1.37	0.57	1.03	0.88	1.43	0.92

abcd: Mean values on the same column with different superscript differ statistically at 5% probability test;

SEM: Standard error of means.

4.4.7 Brooding temperature and relative humidity of Japanese quail chicks hatched at manipulated incubation temperature in late embryogenesis

The brooding temperature and relative humidity of Japanese quail chicks hatched at manipulated incubation temperature in late embryogenesis are shown in table 4.34. The mean values were not significantly different (P>0.05) in all the parameters measured across the treatments. However, the brooding penmean temperature ranged from 33.95 (week 3) to 34.84^{o}C (week 1), while the ambient temperature varied from 28.38^{o}C (week 1) to 28.54^{o}C. Itwas observed that brooding pen and ambient relative humidity values varied from 70.16 to 71.67% and 71.93 to 72.71% respectively.

Table 4.34: Brooding temperature and relative humidity during brooding of Japanesequail chicks hatched at manipulated incubation temperaturein late embryogenesis

	Brooding period											
	Week 1				Week 2				Week 3			
Parameters	**Mean**	**Min**	**Max**	**SEM**	**Mean**	**Min**	**Max**	**SEM**	**Mean**	**Min**	**Max**	**SEM**
Temperature (^{o}C)												
Brooding pen	34.84	26.20	35.20	0.18	34.66	26.20	35.20	0.21	33.95	26.20	34.20	0.18
Ambient	28.38	27.60	30.10	0.14	28.54	27.60	30.10	0.12	28.52	27.60	30.10	0.13
Relative humidity (%)												
Brooding pen	70.16	64.00	82.00	0.86	71.09	64.00	82.00	0.88	71.67	64.00	82.00	0.87
Ambient	72.44	64.00	81.00	0.79	71.93	64.00	81.00	0.78	72.71	64.00	81.00	0.76

Min: Minimum; Max: Maximum; SEM: Standard error of mean.

4.4.8 Physiological status of Japanese quail eggs subjected to paused incubation temperature at late embryogenesis

Presented in table 3.35 is the physiological status of Japanese quail eggs subjected to paused incubation temperature during late embryogenesis, hatchability and chick yield. There were statistical variations ($P<0.05$) in all the mean values of the parameters monitored except in total egg weight, average egg weight, pipped and dead embryos as well as pipped and alive but not able to emerge embryos. The incubation period was 17 days in all the treatments except the eggs in T_{II} that hatched on incubation day 18.

While pipped and alive but not able to emerge embryos occurred only in T_{II}, pipped and dead embryos ranged from 0.67 to 1.67 and fully developed but dead embryos varied between 0.33 and 3.67. The fertile eggs ranged from 7.00 (T_{VI}) to 24.00 (T_{III}), hatched chicks (7.33 to 16.33), average egg weight (9.80 to 10.27g) and average chick weight was between 4.83 and 6.85g. While hatchability was absolutely zero in T_V and T_{VI}, it was as high as 81.25% in T_I, fairly followed by 70.96%,68.04%, and 62.69% in T_{IV}, T_{III} and T_{II} in that order. Meanwhile, chick yield was highest (67.49%) in T_{III}, closely followed by 66.69% (T_{II}), 60.66% (T_I) and 47.72%(T_{IV}).

Table 4.35:Physiological status of Japanese quail eggs subjected to paused incubation temperature during late embryogenesis

	Treatments						
	T_I	T_{II}	T_{III}	T_{IV}	T_V	T_{VI}	
Parameters	**Control (38°C)**	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**SEM**
Total eggs set	102	102	102	102	102	102	-
TEW (g)	1038.18	1048.61	1029.16	1033.48	1022.97	1000.40	15.99
AEW (g)	10.17	10.27	10.09	10.12	10.03	9.80	0.04
IP (days)	17	18	17	17	-	-	-
Unfertile eggs: No evidence of embryo growth							
Infertile eggs	12.33^{a}	3.33^{b}	2.66^{b}	2.67^{b}	9.66^{a}	9.00^{a}	1.17
Caked eggs	4.67^{c}	6.67^{bc}	4.67^{c}	12.33^{a}	8.37^{ab}	13.00^{a}	1.11
NCWFN	6.33^{a}	1.67^{b}	2.67^{b}	8.67^{a}	2.64^{b}	5.00^{a}	0.67
Fertile eggs: Evidence of embryo growth							
EDE	1.67^{b}	4.00^{ab}	2.33^{b}	2.00^{b}	9.67^{a}	7.00^{a}	1.09
FDDE	0.33^{b}	2.33^{a}	3.67^{a}	0.33^{b}	3.67^{a}	0.00^{b}	0.53
PDE	0.00	1.67	1.67	0.67	0.00	0.00	0.29
PAE	0.00	0.33	0.00	0.00	0.00	0.00	0.05
Hatched chicks	8.67^{b}	14.00^{a}	16.33^{a}	7.33^{b}	0.00^{c}	0.00^{c}	1.68
Fertile eggs	10.67^{b}	22.33^{a}	24.00^{a}	10.33^{c}	13.33^{b}	7.00^{c}	1.91
Fertility (%)	31.40^{b}	65.70^{a}	70.57^{a}	30.37^{b}	39.20^{ab}	20.59^{b}	5.83
Hatchability and chick yield							
Hatchability (%)	81.25^{a}	62.69^{a}	68.04^{a}	70.96^{a}	0.00^{b}	0.00^{b}	9.25
TCW (g)	53.39^{b}	95.64^{ab}	110.70^{a}	53.15^{b}	0.00^{c}	0.00^{c}	11.47
ACW(g)	6.17^{a}	6.85^{a}	6.81^{a}	4.83^{a}	0.00^{b}	0.00^{b}	0.79
Chick yield (%)	60.66^{a}	66.69^{a}	67.49^{a}	47.72^{a}	0.00^{b}	0.00^{b}	7.88

abc: Mean values on the same row with different superscript differ statistically at 5% probability test; SEM: Standard error of means; TEW: Total egg weight; AEW: Average egg weight; IP: Incubation period; NCWFN: Not clear whether fertile eggs or not; EDE: Early dead embryos; FDDE: Fully developed but dead embryos; PDE: Pipped and dead embryos; PAE: Pipped and alive but not able to emerge embryos; TCW: Total chick weight; ACW: Average chick weight.

4.4.9 Post-hatchperformance of Japanese quail chicks subjected to paused incubation temperature at late embryogenesis

4.4.9.1 Feed intake by Japanese quail chicks subjected to paused incubation temperature at late embryogenesis

Daily feed intake by Japanese quail chicks subjected to paused temperature during late embryogenesis is expressed in table 4.36. There were statistical variations ($P<0.05$) in all the mean values of the parameters measured across the treatments. It was observed that the population size was gradually reducing weekly due to consistent chick mortality across the treatments. Nevertheless, the feed intake value was increasing with age among all the birds across the treatments. In week 2, feed intake per group of chicks ranged from 196.67g (T_{IV}) to 443.02g (T_{III}), feed intake per chick varied from 7.89g (T_{II}) to 9.99g (T_{IV}). A similar trend was maintained at 3 to 6 weeks of age, when the feed intake value per group as well as per chick was superior in T_{III}.

Table 4.36: Feed intake by Japanese quail chicks subjected to paused incubation temperature during late embryogenesis

		Treatments						
		T_I	T_{II}	T_{III}	T_{IV}	T_V	T_{VI}	
		Control						
Week	**Parameters**	**38°C**	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**SEM**
1	No. of chicks	46	44	49	22	-	-	-
	Feed intake/group (g)	ND	ND	ND	ND	ND	ND	ND
	Feed intake/chick (g)	ND	ND	ND	ND	ND	ND	ND
2	No. of chicks	23	31	44	18	-	-	-
	Feed intake/group (g)	206.81^{b}	231.05^{b}	443.02^{a}	196.67^{b}	0.00c	0.00^{c}	4.97
	Feed intake/chick (g)	8.94^{a}	7.89^{a}	9.86^{a}	9.99^{a}	0.00^{b}	0.00^{b}	1.08
3	No. of chicks	23	28	43	18	-	-	-
	Feed intake/group (g)	210.71^{bc}	253.86^{b}	411.00^{a}	199.35^{c}	0.00^{d}	0.00^{d}	7.69
	Feed intake/chick (g)	9.64^{a}	9.02^{a}	9.76^{a}	10.87^{a}	0.00^{b}	0.00^{b}	0.65
4	No. of chicks	23	19	43	14	-	-	-
	Feed intake/group (g)	272.85^{b}	218.78^{bc}	463.07^{a}	171.07^{c}	0.00^{d}	0.00^{d}	9.20
	Feed intake/chick (g)	11.71^{a}	11.49^{a}	10.80^{a}	9.01^{a}	0.00^{b}	0.00^{b}	1.44
5	No. of chicks	22	14	37	12	-	-	-
	Feed intake/group (g)	257.07^{b}	204.00^{c}	421.57^{a}	156.71^{c}	0.00^{d}	0.00^{d}	9.27
	Feed intake/chick (g)	11.99^{a}	14.09^{a}	11.88^{a}	12.98^{a}	0.00^{b}	0.00^{b}	2.11
6	No. of chicks	19	11	33	10	-	-	-
	Feed intake/group (g)	292.00^{b}	150.21^{c}	453.14^{a}	164.78^{c}	0.00^{d}	0.00^{d}	8.76
	Feed intake/chick (g)	15.04^{a}	13.70^{a}	13.69^{a}	16.01^{a}	0.00^{b}	0.00^{b}	1.08

abcd: Mean values on the same row with different superscript differ statistically at 5% probability test; SEM: Standard error of means;ND: Not determined due to irregular incubation periods and 100% mortality in T_{IV} and T_V; Group: Represent total number of birds per treatments.

4.4.9.2 Growth pattern of Japanese quail chicks subjected to paused incubation temperature at late embryogenesis

Table 4.37 presents the weekly growth pattern of Japanese quail chicks subjected to paused incubation temperature at late embryogenesis. The mean values of all the parameters measured differed significantly ($P<0.05$) across the treatments. The weight values were increasing with age, indicating that the chicks were gaining weight except in week 4, when the values dropped tremendously across the treatments, apart from T_{II} that was still increasing, probably due to late hatching. In week 5, a similar trend was maintained across the treatments, implying that the birds have seemingly attained full growth except for T_{II} that was apparently higher (11.96g) compared to a range of 7.79 to 9.99g recorded in T_{III}, T_{IV}, and T_{I}.

Table 4.37: Growth pattern of Japanese quail chicks subjected to paused incubation temperature at late embryogenesis

		Treatments						
		T_I Control	T_{II}	T_{III}	T_{IV}	T_V	T_{VI}	
Week	**Parameters**	**38°C**	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**SEM**
1	No. of chicks	46	44	49	22	-	-	-
	Weight/group (g)	304.67^{b}	290.32^{c}	330.11^{a}	159.44^{d}	0.00^{e}	0.00^{e}	1.98
	Weight/chick (g)	6.57^{a}	6.67^{a}	6.77^{a}	7.25^{a}	0.00^{b}	0.00^{b}	3.87
	Absolute growth rate	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	No. of chicks	23	31	44	18	-	-	-
	Weight/group (g)	777.66^{c}	938.06^{b}	1601.44^{a}	605.26^{d}	0.00^{e}	0.00^{e}	92.19
	Relative growth rate	33.23^{b}	30.95^{c}	34.93^{a}	32.77^{a}	0.00^{d}	0.00^{d}	2.10
	Absolute growth rate (g)	26.36^{b}	24.96^{c}	28.42^{a}	26.00^{b}	0.00^{d}	0.00^{d}	6.39
3	No. of chicks	23	28	43	18	0.00^{b}	0.00^{b}	
	Weight/group (g)	1140.61^{c}	1214.23^{b}	2232.06^{a}	865.14^{d}	0.00^{e}	0.00^{e}	10.48
	Relative growth rate	48.47^{b}	43.26^{c}	50.97^{a}	50.07^{a}	0.00^{d}	0.00^{d}	2.10
	Absolute growth rate (g)	15.36^{b}	13.96^{c}	16.42^{a}	15.00^{b}	0.00^{d}	0.00^{d}	9.23
4	No. of chicks	23	19	43	14	-	-	-
	Weight/group (g)	1333.11^{b}	1120.91^{c}	2662.90^{a}	965.75^{d}	0.00^{e}	0.00^{e}	14.95
	Relative growth rate	57.94^{b}	58.93^{b}	61.68^{a}	62.63^{a}	0.00^{c}	0.00^{c}	7.32
	Absolute growth rate (g)	10.36^{c}	15.96^{a}	10.42^{b}	12.49^{b}	0.00^{d}	0.00^{d}	4.05
5	No. of chicks	22	14	37	12	-	-	-
	Weight/group (g)	1589.46^{b}	1017.17^{c}	2977.04^{a}	965.15^{d}	0.00^{e}	0.00^{e}	22.95
	Relative growth rate	69.91^{a}	68.79^{a}	68.88^{a}	70.89^{a}	0.00^{b}	0.00^{b}	4.11
	Absolute growth rate (g)	9.99^{b}	11.96^{a}	7.79^{d}	8.37^{c}	0.00^{e}	0.00^{e}	8.44

abcde: Mean values on the same row with different superscript differ statistically at 5% probability test; SEM: Standard error of means; Group: Represent total number of birds per treatments.

4.4.9.3 Blood profiles of Japanese quail chicks subjected to paused incubation temperature at late embryogenesis

The blood profiles of Japanese quail chicks subjected to paused incubation temperature at late embryogenesis is shown in table 4.38. There were significant differences ($P<0.05$) in all the parameters evaluated, except in monocytes which were recorded only in T_{III} and eosinophil that varied between 0.50% (T_{III}) and 1.33% (T_{IV}). Packed cell volume ranged from (22.83%) in T_{IV} to 50.50% in T_{II}, red blood cells (1.72 to 3.42x10^{12}/L), white blood cells (2.98 to 9.55x 10^{9}/L), haemoglobin (7.22 to 16.82g/dL), neutrophil (37.83 to 79.17%) and lymphocytes was lowest (10.67%) in both T_I and T_{IV}, compared to 24.83% and 19.83% recorded in T_{II} and T_{III}, respectively.

Table 4.38: Blood profiles of Japanese quail chicks subjected to paused incubation temperature at late embryogenesis

	Treatments						Statistics			
Parameters	T_I Control (38°C)	T_{II} 36°C	T_{III} 37°C	T_{IV} 38°C	T_V 39°C	T_{VI} 40°C	Min	Max	Overall mean	SEM
No. of birds	5	5	6	3	0	0	-	-	-	-
PCV (%)	29.83^{b}	50.50^{a}	48.33^{a}	22.83^{b}	0.00^{c}	0.00^{c}	0.00	60.00	25.25	4.12
RBC ($x10^{12}$/L)	2.38^{ab}	3.42^{a}	3.42^{a}	1.72^{b}	0.00^{c}	0.00^{c}	0.00	4.30	1.82	2.30
WBC ($x10^{9}$/L)	6.52^{ab}	9.10^{a}	9.55^{a}	2.98^{b}	0.00^{c}	0.00^{c}	0.00	14.60	4.69	3.21
HB(g/dL)	9.87^{b}	16.82^{a}	16.18^{a}	7.22^{b}	0.00^{c}	0.00^{c}	0.00	20.40	8.34	1.36
Neut (%)	55.33^{ab}	75.00^{a}	79.17^{a}	37.83^{b}	0.00^{c}	0.00^{c}	0.00	87.00	41.22	6.64
Lym (%)	10.67^{b}	24.83^{a}	19.83^{ab}	10.67^{b}	0.00^{c}	0.00^{c}	0.00	37.00	11.00	1.94
Mono (%)	0.00	0.00	0.17	0.00	0.00	0.00	0.00	1.00	0.03	0.03
Eosino%	0.67	0.17	0.50	1.33	0.00	0.00	0.00	5.00	0.44	0.18

abc: Mean values on the same row with different superscript differ statistically at 5% probability test; Min: Minimum value; Max: Maximum value; SEM: Standard error of means; PVC: Packed cell volume; RBC: Red blood cells; WBC: White blood cells; HB: Haemoglobin; Neut:Neutrophils; Lym: Lymphocytes; Mono: Monocytes; Eosin: Eosinophil.

4.4.9.4 Serum biochemistry of Japanese quail chicks subjected to paused incubation temperature at late embryogenesis

Provided in table 4.39 is the serum biochemistry of Japanese quail chicks subjected to paused incubation temperature at late embryogenesis. There were significant differences ($P<0.05$) in all the parameters determined and it was observed that T_{III} values were predominantly superior in each case. Nonetheless, the alanine aminotransferase ranged from 4.67 to 10.17U/L, the total protein varied from 24.76 to 48.76g/L and albumin was from 12.07 to 18.88g/L. The creatinine varied from 20.93 to 52.57Umol/L whereas, cholesterol was 127.99mg/dL, 121.75mg/dL, 102.63mg/dL and 101.07mg/dL in T_{III}, T_I, T_{II}, and T_{IV} accordingly.

Table 4.39: Serum biochemistry of Japanese quail chicks subjected to paused incubation temperature at late embryogenesis

	Treatments						Statistics			
	T_I	T_{II}	T_{III}	T_{IV}	T_V	T_{VI}			Overall	
Parameter	**Control 38C**	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**Min**	**Max**	**mean**	**SEM**
No. of birds	5	5	6	3	0	0	-	-	-	-
ALT(U/L)	4.67^{b}	6.17^{ab}	10.17^{a}	5.50^{b}	0.00^{c}	0.00^{c}	0.00	15.00	4.42	0.80
TP (g/L)	31.63^{ab}	38.18^{ab}	48.76^{a}	24.31^{b}	0.00^{c}	0.00^{c}	0.00	52.58	23.81	3.87
ALB (g/L)	14.24^{a}	14.15^{a}	18.88^{a}	12.07^{a}	0.00^{b}	0.00^{b}	0.00	25.53	9.89	1.64
CREAT (Umol/L)	40.02^{ab}	41.08^{ab}	52.57^{a}	20.93^{bc}	0.00^{c}	0.00^{c}	0.00	66.48	25.77	4.39
CHOL (Mg/dL)	121.75^{a}	102.63^{a}	127.99^{a}	101.07^{a}	0.00^{b}	0.00^{b}	0.00	240.80	75.57	13.45

abc: Mean values on the same row with different superscript differ statistically at 5% probability test; Min: Minimum value; Max: Maximum value; SEM: Standard error of means; ALT: Alanine aminotransferase; TP: Total protein; ALB: Albumin; CREAT: Creatinine; CHOL: Cholesterol.

4.4.9.5 Sexual maturity, sex reversal, carcass quality and organs weight of Japanese quails subjected to paused incubation temperature at late embryogenesis

Table 4.40 shows sexual maturity, carcass quality and organs weight of Japanese quail chicks subjected to paused incubation temperature at late embryogenesis. There were statistical differences ($P<0.05$) in all the parameters measured, except the formed eggs that were only found in T_{III}. The hens in T_I were the first to start laying eggs at the age of 5 weeks, followed by the birds in T_{II} at 6 weeks old and T_{III} at 7 weeks old. Although there were yolks found in the uteri of the hens sacrificed in T_{IV}, they did not lay any egg at the age of 7 weeks. Fully formed eggs that were purely white (without pigmentation) were found only in the uteri of hens in T_{III}.

There was no case of reversed sex in any of the birds across the treatments and the sex ratio (male: female) was 7:12, 7:4, 10:13 and 0:10 in T_I, T_{II}, T_{III} and T_{IV} in that order. While the live weight ranged from 61.71g (T_{IV}) to 108.85g in T_{III}, the carcass weight was highest (75.63g) in T_{III}, followed by 62.14g (T_I), 51.05g (T_{II}) and the dressing percentage varied between 67.20% (T_{II}) and 69.21% in T_{IV}. The gizzard weight was highest (3.45g) in T_{III} and least (2.02g) in T_{IV}, liver weight ranged from 1.45g (T_{IV}) to 2.32g (T_I) while, the heart weight varied between 0.49 and 0.91g, the right testis mean weight ranged from 0.45 to 0.74g and the left testis weight was between 0.44 and 0.80g.

Table 4.40: Sexual maturity, sex reversal, carcass quality and organs weight of Japanese quail subjected to paused incubation temperature at late embryogenesis

	Treatments						
Parameters	**T_I Control (38°C)**	**T_{II} 36°C**	**T_{III} 37°C**	**T_{IV} 38°C**	**T_V 39°C**	**T_{VI} 40°C**	**SEM**
Sexual maturity							
Age at point of lay (wk)	5	6	7	-	-	-	-
Yolk	1.17^{a}	0.17^{b}	0.92^{ab}	1.42^{a}	0.00^{b}	0.00^{b}	0.14
Formed eggs	0.00	0.00	0.08	0.00	0.00	0.00	0.01
Sex ratio (M : F)	7:12	7:4	10:13	0:10	0	0	-
Sex Reversal M → F	0	0	0	0	0	0	-
F → M	0	0	0	0	0	0	-
Carcass quality (g)							
No. of birds	10	9	12	6	-	-	-
Live weight	90.69^{ab}	75.96^{b}	108.85^{a}	61.71^{b}	0.00^{c}	0.00^{c}	6.62
Bled weight	87.11^{ab}	72.67^{b}	104.22^{a}	59.10^{b}	0.00^{c}	0.00^{c}	6.34
Def. weight	75.23^{ab}	62.87^{b}	91.62^{a}	52.24^{b}	0.00^{c}	0.00^{c}	5.57
Carcass weight	62.14^{ab}	51.05^{b}	75.63^{a}	43.53^{b}	0.00^{c}	0.00^{c}	4.62
D. percentage (%)	68.86^{a}	67.20^{a}	69.05^{a}	69.21^{a}	0.00^{b}	0.00^{b}	4.08
Organs weight (g)							
Gizzard weight	3.02^{ab}	2.34^{ab}	3.45^{a}	2.02^{b}	0.00^{c}	0.00^{c}	0.22
Liver weight	2.32^{a}	1.62^{a}	2.28^{a}	1.45^{a}	0.00^{b}	0.00^{b}	0.17
Heart	0.78^{ab}	0.62^{bc}	0.91^{a}	0.49^{c}	0.00^{d}	0.00^{d}	0.06
Testes weight Right	0.45^{ab}	0.52^{a}	0.74^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.07
Left	0.44^{ab}	0.71^{a}	0.80^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.08

abcd: Mean values on the same row with different superscript differ statistically at 5% probability test; SEM: Standard error of means; D. percentage: Dressing percentage; Def. weight: Defeathered weight.

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4.4.9.6 Mortality of Japanese quail chicks subjected to paused incubation temperature at late embryogenesis

Presented in table 4.41 is the mortality of Japanese quail chicks that were subjected to paused incubation temperature at late embryogenesis. There were no significant differences (P>0.05) in the mean values across the treatments except, during the brooding period (week 1 to 3), when mortality was highest (9.92) in T_{II}, followed by 8.75 (T_{III}), 5.80 (T_{IV}) and 2.17 (T_1). At 3 – 4 weeks of age, more chicks (1.60) died in T_{III}, slightly followed by 1.50 in T_{II} and the least (0.60) was recorded in T_1. At 4 – 5 weeks of age, highest mean mortality value (1.20) was recorded in T_{III} with the least value (0.40) in T_1, whereas at 5 – 6 weeks of age, the mortality varied from 0.40 in T_{III} to 1.00 in T_{IV}.

Table 4.41: Mortality of Japanese quail chicks subjected to paused incubation temperature at late embryogenesis

	Treatments						Statistics		
	T_I	T_{II}	T_{III}	T_{IV}	T_V	T_{VI}			
Age (week)	Control (38°C)	36°C	37°C	38°C	39°C	40°C	Min	Max	SEM
1 - 3	2.17^{ab}	9.92^{a}	8.75^{ab}	5.80^{ab}	0.00^{b}	0.00^{b}	0.00	20.00	1.08
3 - 4	0.60	1.50	1.60	1.33	0.00	0.00	0.00	7.00	0.38
4 - 5	0.40	0.83	1.20	0.67	0.00	0.00	0.00	4.00	0.23
5 - 6	0.80	0.83	0.40	1.00	0.00	0.00	0.00	3.00	0.22

abcde: Mean values on the same row with different superscript differ statistically at 5% probability test;

Min: Minimum value; Max: Maximum value; SEM: Standard error of means.

4.4.9.7 Survival rate of Japanese quail chicks subjected to paused incubation temperature at late embryogenesis

Table 4.42 shows the survival rate of Japanese quail chicks subjected to paused incubation temperature at late embryogenesis. There were significant differences ($P<0.05$) in all the parameters monitored. More hatchlings (16.33) were recorded in T_{III}, slightly followed by 14.00, 8.67 and 7.33 in T_{II}, T_1 and T_{IV} and there was no hatching (absolutely zero hatchability) in T_V and T_{VI}. More chicks (11.33) were lost in T_{II}, compared to a range of 3.67 to 7.00 recorded in T_I, T_{IV} and T_{III}, respectively. Highest mortality rate (81.33%) was observed in T_{II}, followed by 45.57%, 44.03% and 40.00% in T_{IV}, T_{III} and T_I, respectively. The survival rate was best (59.20%) in T_I, slightly followed by 55.97% (T_{III}), 54.43% (T_{IV}) and 18.67% in T_{II}.

Table 4.42: Survival rate of Japanese quail chicks subjected to paused incubation temperature at late embryogenesis

	Treatments						Statistics		
	T_I Control	T_{II}	T_{III}	T_{IV}	T_V	T_{VI}			
Parameters	**38°C**	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**Min**	**Max**	**SEM**
Hatched chicks	8.67^{b}	14.00^{ab}	16.33^{a}	7.33^{b}	0.00^{c}	0.00^{c}	0.00	22.00	1.68
Chick mortality	3.67^{bc}	11.33^{a}	7.00^{b}	5.00^{b}	0.00^{c}	0.00^{c}	0.00	12.00	1.05
Mortality rate (%)	40.80^{b}	81.33^{a}	44.03^{b}	45.57^{b}	0.00^{c}	0.00^{c}	0.00	85.70	7.74
Survival rate (%)	59.20^{a}	18.67^{b}	55.97^{a}	54.43^{a}	0.00^{c}	0.00^{c}	0.00	75.00	6.18

abc: Mean values on the same row with different superscript differ statistically at 5% probability test; Min: Minimum value;Max: Maximum value; SEM: Standard error of means.

4.4.10 Discussion

4.4.11Incubation temperature pausing during late embryogenesis in Japanese quail sex reversal

It was shown that incubation temperature on embryonic days 11, 12 and 13 dropped to 26 – 36^{o}C in all the treatment incubators except the Control. This waswithin the range given by Conway and Thomas (2000) as physiological zero temperature. This observation suggested that the embryo growth and development were only slow and not possibly impaired. This lent more credence to the report of Fasenko (2007) that some cellular metabolic processes still continue in the developing embryo but gross morphological changes like shape and structure maybe arrested. Similarly, this observation conformed to the report of Boerjan (2016) that described physiological zero temperature as incubationtemperaturelow enough to maintain embryonic cell activity at a greatly reduced rate yet reversible level when normalcy is restored.Thus, may not cause developmental abnormalities rather, there might be swift development of the opposite sex features, that were initially rudimentary leading to sex reversal. However, this was not observed in the study possibly because of the relatively short period of 5 hours that the incubation temperature was paused.

On other embryonic days, the incubation temperature was observed to fluctuate within a range of 0.03 – 0.80^{o}C across all the treatments including control, which was higher than 0.03 – 0.05^{o}C that Poultry Hub (2018) reported as normal in avian eggs incubation. This seemingly uncontrollable circumstance could be due to partial heat production, even when the thermostat has shut down the heating device and partly due to ambient weather conditions that apparently determined the rate of heat loss in the incubators. During the incubation temperature pausing, that lasted for 5 hours on each of the embryonic days 11, 12 and 13, it was observed that the temperature gradually declined swiftly over time, until both incubation and ambient temperatures were at equilibrium. The observed ambient temperature range of 29.4 – 36.8^{o}C across the treatments, was similar to the predicted range of 20 – 21^{o}C expected to prevail during that season of the year (June/July) in Jos, Plateau State (EUMETSAT, 2018). Therefore, the microclimatic conditions required, that necessitated the experimental site were apparently observed. The incubation and ambient

relative humidity values were close, suggesting that the water supplied in the incubators was probably insufficient and maybe there was poor air circulation within the incubators. This observation apparently did not affect the growth and development of Japanese quails as incubation period was still within 16 and 18 days reported to be normal (Sellier *et al*., 2006; Romao *et al*. (2009b). The incubated egg weight was comparable to 9.62g reported in Japanese quails by Al-Shammari *et al*. (2019) hence the eggs used in this study were probably normal. Although, there was hatching failure in T_V and T_{VI} with very few hatchlings in other treatments, hatchability of approximately 60 – 80% was within a range speculated to be normal in avian species (Duman and Şekeroğlu, 2017; Sousa de Araújo *et al*., 2017). However, the chick yield was relatively poor in T_{IV}, while others were similar to 60 – 70% reported to be normal in Japanese quails (Romao *et al*., 2009b; The Poultry Site, 2014a; Aviagen 2015). The observed hatching failure in T_V and T_{VI} could be largely due to the incubation temperature manipulation that eventually led to irreversible growth and development even death of the embryos *in ovo* (Shubber *et al*., 2012). Also, it could be partly due to low egg fertility recorded in T_{IV}, T_V and T_{VI}. This evidently supported the report of Boerjan (2016) that wide fluctuation in incubation temperature, will result in death of embryos in Japanese quails. The unhatched eggs when opened showed that some of them were "caked" probably due to crack leading to excessive moisture loss and "infertile" probably due to inappropriate sex ratio in the flock, mating failure and nutritional plane of the hens. Whereas, "not clear whether fertile or not" eggs were perhaps due to failure of the zygote to cleave, differentiate resulting in death of the zygote thus, difficult to determine whether the eggs were fertile or not.

In some other unhatched eggs opened, it was observed that the embryos were at different phases of growth and development, that were described as either "early dead embryos", "fully developed but dead embryos", "pipped and dead embryos" or "pipped and alive but not able to emerge embryos". These physiological statuses of the developing embryos, were observed in all the treatments except "pipped and alive but not able to emerge embryos" that was only recorded in T_{II} which could be a mere coincidence. According to Romao *et al*. (2009b) and Yilmaz *et al*. (2011), embryo death is a common incidence in avian species during eggs incubation, thus it may not be as a result of the incubation temperature that was manipulated during late embryogenesis in Japanese quails. This

probably buttressed the report of Woodard *et al*. (1973) that most embryonic deaths happen during the first 8 days of incubation, due to the inability of the developing embryo to form a vital organ or a malfunction of its development.The brooding temperature was within the range of 19 – 33^{o}C, recommended for avian chick brooding and the relative humidity during the brooding period was close to that of the ambient, but both values were within 30 – 70% recommended for avian species brooding (Bourne, 2016). In any case, the observations were within ambient temperature of 20 – 27^{o}C and relative humidity of 66 – 83%, predicted to prevail at that time of the year (June/July) in Jos, Plateau State ((EUMETSAT, 2018)), thus, the microclimatic conditions required for this study was probably achieved. Consequently, the unique nature of results recorded seemingly buttressed the observation of Renwick and Washburn (1982), who revealed that brooding broiler chicks under cool temperatures (26.7 – 32.2^{o}C), raised mortality rate and reduced feed efficiency. It was stated that fast-growing chicks were mainly sensitive to low temperatures and those under low temperature conditions had limited capacity to meet their oxygen requirements leading to the high incidence of ascites (Olkowski *et al*., 2005; Druyan *et al*., 2007). The feed intake was increasing with the age of the birds and the values were comparatively lower than 23.3 – 30g (week 1), 38 – 57g (week 2) and 56.7 – 92g (week 3) as well as 85 – 127.3g (week 4) and 87 – 166.3g (week 5) reported (Rashid *et al*., 2016) in Japanese quails of similar age and treatment conditions. But, the recorded values were close to 10.56g, 10.39g, 8.93g and 8.87g in weeks 3, 4, 5 and 6 reported (Adesina, 2013) when Japanese quails were fed sundried edible frog meal. This observation probably culminated in similar trend in growth rate but at 4 weeks old, it was observed that the growth rate gradually declined, suggesting that the birds probably attained "growth spurt" which Hossner (2005) and Versele-Laga, (2018) stated is bound to occur in animals' lifetime. Therefore, inclusion level of protein source may be reduced at 4 weeks of age in Japanese quails however the energy level may be maintained or increased in order to meet the energy requirement of the birds at this growth phase.

The blood profiles of the birds subjected to incubation temperature during late embryogenesis, revealed that values of packed cell volume, red blood cells, white blood cells, haemoglobin, neutrophils, lymphocytes, monocytes and eosinophil were similar to the values reported in normal healthy Japanese quails (Fudge, 2000; Samour, 2006;

Bickford, 2007). However, packed cell volume and red blood cells were close to 47.21% and 3.51 x 10^{12}/L, respectively, but haemoglobin was lower than 20.59g/dL reported in Japanese quails subjected to high environmental temperature (Fathi *et al*., 2019). The serum alanine aminotransferase, total protein, albumin, creatinine and cholesterol values were within a range reported to be normal in healthy avian species (Sakas, 2002; Mnisi and Mlambo, 2017). On the other hand, cholesterol was less than 198.47mg/dL recorded in Japanese quails reared under high environmental temperature (Fathi *et al*., 2019). Since the physiological integrity of the birds was probably intact with little or no detrimental effects, paused incubation temperature during late embryogenesis in Japanese quails, may not hamper vital organs functions. The hens started laying eggs later than 5 weeks of age reported by Al-Shammari *et al*. (2019) as age at first egg drop thus, altered incubation temperature during late embryogenesis in Japanese quails may influence sexual maturity.The apparent delay in the hens in T_{II} and T_{III} to start laying eggs and no laying at all in T_{IV} at the age of 7 weeks may purely be as a result of the treatment, more so that formed eggs were only found in T_{III} at the same age. Since yolk was found in all the treatments, it probably indicated that their reproductive potentials were only distorted but not impaired. This observation contradicted several reports, that egg lay commenced at 4 – 6 weeks of age in Japanese quails (Woodard *et al*., 1973; Randall, 2008; Al-Shammari *et al*. 2019; Krishnan (2019). It was shown that there was no cock among the birds in T_{IV} yet there were more hens in all the other treatments except T_{II}. This apparently strengthened the reports of Göth and Booth (2005) and Eiby *et al*. (2008) that more hens hatched in avian species at high incubation temperature. There was no case of sex reversal in any of the treatments, suggesting that incubation temperature paused for 5 hours during late embryogenesis, may not reverse sex in Japanese quails. The carcass qualityindices revealed that the live weight, carcass weight and dressing percentage were within the values reported (Sellier *et al*., 2006; Romao *et al*., 2009a) in normal healthy Japanese quails at a similar age. However, the live weight was less than 223.9g reported in Japanese quails raised in high ambient temperature yet the dressing percentage, liver and heart values were similar (Fathi *et al*., 2019). As a result, the organs were apparently not affected in all the birds across the treatments, indicating that incubation temperature alteration during embryogenesis, may probably not elicit detrimental effects on

physiological systems in Japanese quails. The hatching failure in T_V and T_{VI} could be largely due to the relatively high incubation temperature and partly due to the incubation temperature pausing for 5 hours on embryonic days 11, 12 and 13 disagreeing with the observation of Elmehdawi (2013), that no adverse effects of incubation temperature alteration on hatchability was recorded when broiler breeder eggs were subjected to low and high incubation temperatures. The disparities could be possibly due to the incubation temperature fluctuations range and probably due to the avian species used in the studies. Nonetheless, the observation apparently buttressed the reports of Romao *et al*. (2009b), who recorded poor hatchability in Japanese quail eggs incubated at different temperatures and Mani *et al*. (2008), when Japanese quail eggs were stored for 20 days before incubating at high incubation temperature.Even with the hatching failure in Japanese quail eggs incubated at 39^{o}C (T_V) and 40^{o}C (T_{VI}), the hatchability at other incubation temperatures was within a range of 76 to 88.55% recorded by Abdelfattah (2019) in Japanese quails subjected to incubation thermal manipulations. Therefore, high incubation temperature when paused at late embryogenesis may affect Japanese quail eggs hatchability.

Although, there was chick mortality in all the treatments except T_V and T_{VI} where there was no hatching, the seemingly depressed survival rate in T_{II}, could be a mere coincidence and not probably due to the treatments. Consequently, pausing incubation temperature for 5 hours during late embryogenesis may not affect post-hatch performance in Japanese quails. But, the none hatchability in T_V and T_{VI} perhaps, suggested that incubation temperature should not be paused for as long as 5 hours particularly at high incubation temperature (39 – 40^{o}C) in Japanese quails.

4.5 Results

4.5.1 Efficiency test of the fabricated incubators

4.5.2 Temperature (without alteration) and relative humidity of Japanese quail eggs incubation

Presented in table 4.43 are the temperature (without alteration) and relative humidity during Japanese quail eggs incubation. There were statistical variations ($P<0.05$) across the treatments in all the mean values of the parameters examined. It was revealed that the incubation temperature values were within the desired calibrated treatments values. The ambient temperature ranged between 30.43^{o}C in T_{EV} and 32.88^{o}C in T_{AI}. Incubation relative humidity values varied from 43.62%% (T_{AI}) to 60.11% (T_{DIV}) whereas, the ambient relative humidity was highest (60.01%) in T_{CIII}, followed by 58.96% (T_{DIV}), 58.88% (T5), 49.35% (T_{AI}), 48.93% (T_{FVI}) and 48.65% in T_{BII}, accordingly.

Table 4.43: Temperature without alteration and relative humidity of Japanese quail eggs incubation

	Treatments						Statistics			
	T_{AI}	T_{BII}	T_{CIII}	T_{DIV}	T_{EV}	T_{FVI}				
Parameters	**Control (38°C)**	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**Min**	**Max**	**Mean**	**SEM**
IT (°C)	38.26[c]	36.22[f]	37.07[e]	38.03[d]	38.98[b]	40.02[a]	35.50	40.90	38.09	0.06
AT (°C)	32.88[a]	32.52[a]	33.22[a]	31.27[b]	30.43[c]	32.74[a]	25.20	37.50	32.18	0.11
IRH (%)	43.62[d]	53.42[b]	52.03[b]	60.11[a]	49.70[c]	48.60[c]	28.00	68.00	51.25	0.34
ARH (%)	49.35[b]	48.65[b]	60.01[a]	58.96[a]	58.88[a]	48.93[b]	32.00	68.00	54.13	0.33

abcd: Mean values on the same row with different superscript differ statistically at 5% probability test; SEM: Standard error of means; IT: Incubation temperature; AT: Ambient temperature; IRH: Incubation relative humidity; ARH Ambient relative humidity; Min: Minimum value; Max: Maximum value.

4.5.3 Physiological status of Japanese quail eggs incubated without incubation temperature alteration

Table 4.44 gives the physiological status of Japanese quail eggs incubated at 36^{o}C, 37^{o}C, 38^{o}C, 39^{o}C and 40^{o}C without altering the incubation temperature, hatchability and chick yield. There were statistical differences ($P<0.05$) in all the parameters measured except,average egg weight that varied from 9.23 to 9.65g andchick yield that ranged from 43.77 to 68.95%across the treatments.

The incubation period was delayed (24 days) in T_{BII} yet, it was as short as 17 days in T_{AI} and 18 days in T_{CIII}, T_{DIV}, T_{EV} and T_{FVI}, respectively. Infertile eggs ranged from 2.66 (T_{CIII}) to 11.33 (T_{AI}), early dead embryos were more (6.00) in T_{EV}, slightly followed by 5.00 in T_{CIII} and was least (3.00) in $T_{BII}$and T_{FVI}. There were more fertile eggs (17.34 and 17.00) in $T_{AI}$and T_{DIV}, followed by a range of 10.00 to 13.33 in $T_{BII,}$ T_{CIII}, T_{EV} andT_{FVI}. There were more hatched chicks (6.33) in T_{AI}slightly followed by5.00, 3.33, 3.07, 1.67 and 0.33 recorded in T_{DIV}, $T_{CIII,}T_{EV}$, T_{FVI} and T_{BII} in that order.

Average chick weight varied from 4.04g (T_{BII}) to 6.62g (T_{CIII}), hatchability was best (36.51%) in T_{AI},followed by 30.70% (T_{EV}), 29.41% (T_{DIV}), 24.98% (T_{CIII})and3% (T_{BII}). While fertility rate ranged from 27.77 to 48.17%, chick mortalitywas recorded in all the treatments but the highest value (5.67%) was in T_{AI}.

Table 4.44: Physiological status of Japanese quail eggs incubated at 36 – 40°C without incubation temperature alteration, hatchability andchick yield

	Treatments						
	T_{AI}	T_{BII}	T_{CIII}	T_{DIV}	T_{EV}	T_{FVI}	
Parameters	**Control (38°C)**	**36°C**	**37°C**	**38°C**	**39°C**	**40°C**	**SEM**
Total eggs set	108	108	108	108	108	108	-
TEW (g)	336.25^{ab}	332.26^{b}	345.65^{ab}	347.50^{a}	346.79^{a}	333.56^{ab}	16.84
AEW (g)	9.37	9.23	9.60	9.65	9.63	9.27	0.06
IP (days)	17	24	18	18	18	18	-
Unfertile eggs: No evidence of embryo growth							
Infertile eggs	11.33^{a}	9.67^{b}	2.66^{d}	9.00^{b}	5.67^{c}	8.33^{b}	0.67
Caked eggs	6.67^{b}	7.67^{b}	8.36^{b}	7.33^{b}	18.67^{a}	9.00^{b}	0.78
NCWFN	0.66^{c}	7.66^{b}	11.65^{a}	2.67^{c}	1.66^{c}	5.67^{b}	0.26
Fertile eggs: Evidence of embryo growth							
EDE	3.35^{b}	3.00^{b}	5.00^{a}	3.67^{b}	6.00^{a}	3.00^{b}	1.26
FDDE	2.33^{b}	5.33^{a}	3.33^{b}	5.00^{a}	0.60^{c}	6.00^{a}	1.03
PDE	3.66^{a}	2.34^{a}	1.67^{b}	3.33^{a}	0.33^{b}	2.33^{a}	0.24
PAE	1.67^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.16
Hatched chicks	6.33^{a}	0.33^{c}	3.33^{b}	5.00^{a}	3.07^{b}	1.67^{bc}	1.17
Fertile eggs	17.34^{a}	11.00^{c}	13.33^{bc}	17.00^{a}	10.00^{c}	13.00^{bc}	1.37
Fertility (%)	48.17^{b}	30.55^{b}	37.03^{c}	47.22^{a}	27.77^{bc}	36.11^{b}	2.90
Hatchability and chick yield							
Hatchability (%)	36.51^{a}	3.00^{d}	24.98^{b}	29.41^{b}	30.70^{a}	12.84^{c}	5.03
TCW (g)	36.86^{a}	4.04^{c}	28.07^{ab}	31.81^{a}	22.20^{b}	9.23^{c}	7.98
ACW(g)	5.82^{a}	4.04^{b}	6.62^{a}	6.33^{a}	5.89^{a}	5.52^{a}	0.19
Chick yield (%)	62.11	43.77	68.95	65.59	61.16	59.55	6.45
⁋Mortality (%)	5.67^{a}	0.11^{b}	2.53^{b}	0.93^{b}	0.61^{b}	0.56^{b}	0.31

abcde: Mean values on the same row with different superscript differ statistically at 5% probability test; SEM: Standard error of means; TEW: Total egg weight; AEW: Average egg weight; IP: Incubation period; NCWFN: Not clear whether fertile eggs or not; EDE:Early dead embryos; FDDE: Fully developed but dead embryos; PDE: Pipped and dead embryos; ACW: Average chick weight;PAE: Pipped and alive but not able to emerge embryos; TCW: Total chick weight;⁋Mortality: Data collected only during the first week of brooding.

4.5.4 Discussion

4.5.5 Efficiency test of the fabricated incubators

When hatching failure was recorded in T_{EV} and T_{FVI}, during incubation temperature manipulation at early or late embryogenesis, it was deemed necessary to evaluate the efficiency of the fabricated incubators. The incubation temperature was observed to follow similar trends as observed in the preceding experiments (particularly T_{EV} and T_{FVI}), where fluctuations were recorded instead of precisely the calibrated treatments values of 36 – 40^{o}C(Mauldin and Buhr, 1995; French, 1997). Also, the ambient temperature, incubation and ambient relative humidity values were similar to the observations recorded in the preceding experimentsas recommended by French (2000). The incubation conditions were similar to what Hegab and Hanafy (2019) used and described as "standard" in Japanese quails production. However, the hatchability was less than 67.4% and 78.6% reported in what were described as "small" and "large" Japanese quail eggs.Essentially, it was observed that hatching was recorded in all the treatments including T_{EV} and T_{FVI}, where there was absolutely zero hatchability in the preceding experiments. So, the fabricated incubators set at 36 to 40^{o}C and used in this study, were apparently effective in incubating Japanese quail eggs. Although the incubation period was prolonged up to 24 days in T_{BII}, it was 17 days in $T_{AI}$and uniformly 18 days in T_{CIII}, T_{DIV}, T_{EV} and T_{FVI}confirming earlier findings on poultry incubation periods (Archer and Cartwright, 2018; Sartell, 2018). In any case, the recorded incubation periods were more than 15 – 16 days reported byAbdelfattah (2019), when Japanese quail eggs were subjected to 41°C and sprayed with vitamin C during incubation. Consequently, the fabricated incubators were possibly effective in hatching Japanese quail eggs. The cause of prolonged incubation period in T_{BII} is not clear, but it could be as a result of extreme fluctuations of the incubation temperature probably due to inconsistent power supply. On this premise, unhatched eggs on incubation days 16 – 18, should not be evacuated immediately from the incubators. Instead, they should be left for more time up to the 5th or 6th day later, in order to have more hatchlings. Nonetheless, survivability of such late hatched chicks cannot be guaranteed because of fatigue and exhaustion of the withdrawn unabsorbed yolk that would have supplied nutrients even without feed for up to 3 days post-hatch.

Essentially, “caked”, “infertile” and “not clear whether fertile or not” eggs were observed and physiological statuses “early dead embryos”, “fully developed but dead embryos”, “pipped and dead embryos” or “pipped and alive but not able to emerge embryos” were similar to what was recorded in the preceding experimentsthus, lent more credence to earlier reports (Romao *et al*. (2009b; Yilmaz *et al*., 2011; Akpınar and Günenç 2019;Hegab and Hanafy 2019). More significantly, chick mortality during the first week of brooding was recorded in all the treatments even higher in T_{AI}(control)confirming earlier findings (Musa *et al.* 2013; Taskin and Karadavut, 2014; Hussain *et al*., 2019; Abdelfattah 2019). In view of this, it could be presumed that most likely the incubation temperature manipulation on embryonic days 3, 4, 5, 11, 12 and 13, undoubtedly resulted in the poor hatchability and absolute hatching failure, recorded in the preceding experiments. Since there was chick emergence in all the treatments, particularly in T_{EV} and T_{FVI} the fabricated incubators were probably effective throughout the study period.

CHAPTER FIVE

SUMMARY, CONCLUSION, RECOMMENDATIONSAND CONTRIBUTION TO KNOWLEDGE

5.1 Summary

It has been established that there is inadequate animal protein intake in Nigeria and it is believed that increased commercial poultry production, could boost animal protein availability and intake at affordable rate. Unfortunately, poultry species in domestication can only incubate very few eggs at a time and Japanese quail in particular lack broody behaviour due to high degree of domestication. Therefore, artificial incubation strategies andexpertise are required in commercial poultry production. Artificial incubator could be home-made (backyardproduction) or industrial (commercial capacity), in any case, it should provide the optimum microclimate that will mimic the broody hen strategy in natural incubation.Since a range of 37.2^{o}C – 39.4^{o}C has been reported as incubation temperature in different regions of the world, depending on the poultry species, facilities available and prevailing environmental conditions.Adoption of inappropriate incubation temperature in those regions of the world may have resulted inhigh embryo mortality, hatchability failure, low chick yield,poor survivability and post-hatch performance in poultry species. Japanese quail has been accepted as a laboratory bird but, the sex cannot be identified at day old, leading to compulsory culling of undesired sex in the flock. But it has been reported that manipulated incubation temperature determined sex in Australian brush-turkey therefore, it could be feasible in Japanese quails. On this premise,optimum incubation temperature in Japanese quails was determined and the effects of alteration ofsuch incubation temperature to enhance sex reversal, hatchability and post-hatch performance were investigated.

Five fabricated electric incubators were used to determine the optimum incubation temperature while the sixth electric incubator (imported as control) was included in subsequent experiments to enhance sex reversal, hatchability and post-hatch performance.

The experimental layout was:control: 38^{o}C (imported incubator); Very low incubation temperature: 36^{o}C; Low incubation temperature: 37^{o}C; Medium incubation temperature: 38^{o}C; High incubation temperature: 39^{o}C and Very high incubation temperature: 40^{o}C. All the incubators could automatically switch on/off at the calibrated temperature ± a range of 0.26 to 0.97^{o}C. In each incubator, there were three egg trays that served as the replicates. A total of 4,125 eggs were used throughout the study with 1,605 for optimum incubation temperature determination, 504 for embryo developmental stages, 1,260 for incubation temperature manipulation during early embryogenesis, 612 for incubation temperature manipulation during late embryogenesis and 648 were used to reconfirm optimum incubation temperature determination vis-à-vis efficiency of the fabricated incubators. It was observed that the calibrated incubation temperature fluctuated with a range of 0.03 to 0.97^{o}C, suggesting that it might not be feasible to achieve perfect precision of calibrated incubation temperature value.However, the temperature recorded in each of the treatment incubators, were within the range of 36 to 40.5^{o}C given as incubation temperature believed to be the ideal values in all avian species.

The incubation period was consistently within 16 – 19 days throughout the study period except forVery lowincubation temperature (36^{o}C), where the chicks emerged late even on incubation days 20 – 24.Based on this, after the collection of hatchlings on days 16 to 18, the remainder eggs should not be evacuated immediately, until the 5th or 6th day later in order to have more hatchlings. However, survivability of such late hatchlings cannot be ascertained because of fatigueand exhaustion of the withdrawn unabsorbed yolk that would have supplied more energy.

It was found out that the first stage of embryonic growth in Japanese quail was seemingly in concentric layers on the surface of the yolk.During embryogenesis in Japanese quails, it was observed that the yellow yolk could become green, yet normal development and growth continued till term.This maypossibly result in the green excreta sometimes voidedby some day-old chicks early in life. Also, it was noticed that the head of the developing embryo bent dorsally either through the left or right and at pipping, the chick uses the egg tooth to window the eggshell while reversing the head ventrally.Hatchability and chick yield were somewhat affected by manipulating the incubation temperature particularly at 39 and 40^{o}C. Meanwhile, some of the residual eggs opened were observed

to be “caked”, “infertile”, “not clear whether fertile or not” and all these made up the entire unfertile eggs among the incubated eggs. Others residual eggs opened were observed to havedeveloping embryos at different physiological stages described in this study as “early dead embryos”[embryonic days 1 – 7], “fully developed dead embryos”[embryonic days 8 – 15], “pipped and dead embryos” [embryonic days 16 – 17]as well as “pipped and alive embryos but not able to emerge”[embryonic days 17 – 23]. All these stages including the hatched chicks were summed up to form the fertile eggs among the entire incubated ones.Feed intake and growth pattern remained increasing with chick age except for the growth rate that dropped on the 3rd and 4th week of age, implying “growth peak” that could be physiologically described as “growth spurt”. The organs and carcass quality as well as the blood profiles were all within the reference values given as a normal range in adult Japanese quails and other avian species. Although mortality was recorded in all the treatments, which was consistently higher during the brooding period but gradually reduced during the grower phase, it was absolutely 100% in chicks hatched at 39^{o}C and 40^{o}C when the incubation temperature was paused at early or late embryogenesis.

Sex ratio was approximately 1 rooster: 1 hen across the treatments except the chicks that were hatched at40^{o}C, where it was seemingly 1 rooster: 2 hens during the first experiment when the optimum incubation temperature was being determined. Although three cases of sex reversal: two from “female to male” was recorded only at 39^{o}C and one from “male to female” only in control (38^{o}C)were recorded, there was no case of sex reversal when the incubation temperature was manipulated at early or late embryogenesis. Sexual maturity was attained at the age of 5weeks, except in chicks hatched at 36^{o}C and 37^{o}C, when the incubation temperature was manipulated at early or late embryogenesis that probably resulted in the hens laying eggs at 6 to 7 weeks of age. The eggs found *in utero* among the sacrificed hens were absolutely white without pigmentation.

5.2 Conclusion

Incubation period and hatchability were best at 37 – 40^{o}C, chick yield was best at 38 to 39^{o}C, and dressing percentage was best at 36^{o}C whereas, survivability was best at 38 to 40^{o}C but at 39°C hatching commenced on incubation day 16.Therefore,39°Cmay be recommended as optimum incubation temperature and could be adopted in Nigeria, to

enhance Japanese quail eggs hatchability and chick performance.Incubation temperature at 36 to 40^{o}C may influence embryo growth and development, but may not adversely reduce chick yield in Japanese quails.

Alterationof incubation temperature at 39 and 40^{o}C for 5 hours during early or late embryogenesis may result in hatchability failure yet, may not bias sex ratio nor reverse sex in Japanese quails.Since the survival rate did not follow a particular trend among the treatments, both hens and roosters matured at 5 weeks of age, yolk and formed eggs were found in all the sacrificed hens and testes in the roosters, incubation temperature range of 36 to 40^{o}C may not adversely depressed post-hatch performance in Japanese quails.

5.3 Recommendations

Prototype of locally fabricated electric incubators was developed and used in this research work.Therefore,manufacturing of efficient commercial capacity of electric poultry eggs incubators in Nigeriacould be recommended in order to boost gross domestic products. However, the incubators could not conserve heat for a long time therefore, use of insulators and lagging materials in the fabricated incubators are recommended in order to conserve heat for at least 6 hours in case of power failure. A novel categorisation of incubated Japanese quail eggs' physiological status could be proposed as "**Unfertile:no evidence of embryo growth**" to include "infertile eggs","caked eggs" and "not clear whether fertile or not eggs" and "**Fertile:evidence of embryo growth**" to include "early dead embryos", "fully developed but dead embryos", "pipped and dead embryos", "pipped and alive embryos but not able to emerge"as well as hatched chicks. These could be recommendedpossibly in all poultry species in order to describe the exact stage of embryo growth in avian research.Assisting Japanese quail chick that is unable to complete pipping process may be recommended to enhance more chicks' survivability during hatching.

5.4 Contributions to knowledge

1. Low temperature (36°C) prolonged incubation period, enhanced feed intake and carcass weight in Japanese quails, while high temperature (40°C) enhanced survivability. But at 39°C, hatching commenced on day 16. Therefore,39°C may be recommended as optimum incubation temperature for Japanese quails.
2. Incubation temperature at 36 – 40°C may affect embryo growth and development but may not adversely reduce chick yield in Japanese quails.

3. Paused incubation temperature at 39 – 40°C for 5 hours during early or late embryogenesis may result in hatchability failure yet, may not bias sex ratio nor elicit sex reversal in Japanese quails.
4. Post-hatch performance of Japanese quails subjected to incubation temperature at 36 – 40°C may not be compromised.
5. This study has established a practical basis for intensive production of Japanese quails, thus these findings may be useful to animal production stakeholders particularly as a guide in hatchery operations, class works and avian researches. However, further studies on assisting chicks in pipping process, survivability of late hatched chicks, causes of greenish yolk and pure white eggshell *in utero* may be deemed necessary, in order to boost poultry productivity.

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APPENDICES

Appendix 1: Notice of research ethical approval

NATIONAL VETERINARY RESEARCH INSTITUTE
Federal Ministry of Agriculture and Water Resources
P. M. B. 01, VOM, PLATEAU STATE

Tel: 07055578876
Efax:+442081817080
Website: www.nvri.gov.ng
E-mail: edvr@nvri.gov.ng
nvri1924@yahoo.com

NOTICE OF ETHICAL APPROVAL

AEC/02/41/17

Name of Principal Investigator	**IDAHOR, KINGSLEY O**
Address	Department of Animal Science, Nasarawa State University, Shabu-Lafia
Title of Project	Effect of incubation and brooding on embryonic development, sex reversal and thermo-tolerance in Japanese quail (*Coturnix conurnix japonica)*

This is to inform you that the protocol described in the above project has been reviewed and approved by the **National Veterinary Research Institute, Animal Use and Care Committee (NVRIAUCC).**

This project begins **January 30, 2017** and terminates **December 31, 2017.** Please note that no activity pertaining to this project should be conducted outside the specified dates. You are to notify the Committee in writing if there is a delay in starting or need to extend the termination of the project.

Note also that, there are no changes permitted in the project without prior approval by the **NVRI, AUCC.** The Committee reserves the right to pay compliance visit to the project site without prior notice.

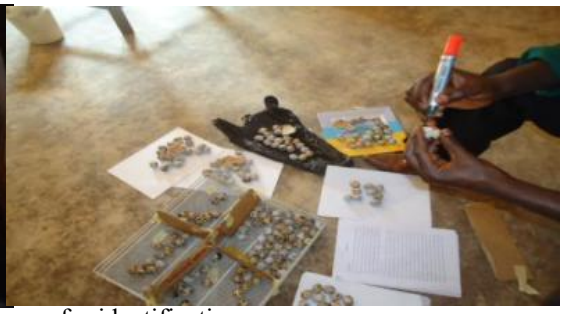
Good luck.

Chika Nwosuh (DVM, PhD, FCVSN)
AUCC Coordinator

Appendix 2:Fabrication of the incubatorsat Dagwom Farm, NVRI

Appendix 3:Experimental poultry eggs incubation procedures

Collection of eggs

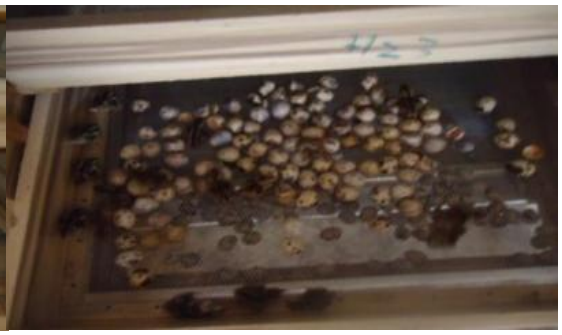


Labelling the eggs for identification

Weighing of egg before setting

Padding of eggs in upright position in the incubator

Transfer of eggs to hatcher at 3 days to pipping

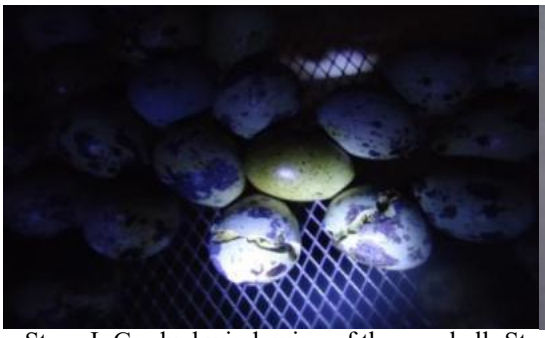


Pipping, hatching and drying of the hatchlings

Transportation of the hatchlings

Brooding the hatchlings

Appendix 4:Stages of Japanese quail chick pipping in the incubator



Stage I: Gradual windowing of the eggshell

Stage II: Widening of cut eggshell

Stage III: Partially emerged chick

Stage IV: Fully emerged and healthy chick

Stage V: Drying of chicks within the incubator

Stage VI: Empty eggshell

Stage VII: Carefully windowed empty eggshell

Stage VIII: Fluffy dried chicks in transport crate

Appendix 5: Brooding, rearing and sexidentification of Japanese quails

Broody Unit

Growers unit

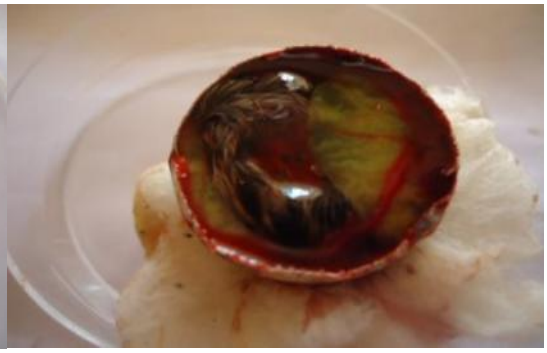
Tagging of Japanese quail based on identified sex using breast and neck region plumage patterns

Female Japanese quail Male Japanese quail

Appendix 6: Serendipitous discovery 1

Abnormal: Green unabsorbed yolk found in Japanese quail's developing embryo

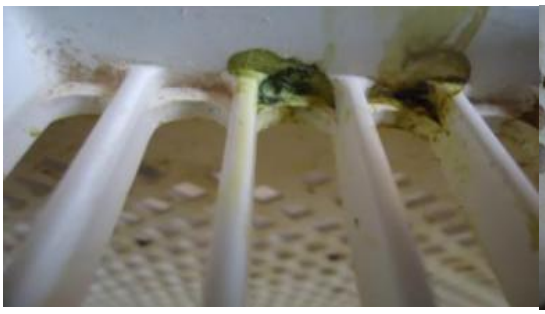
Normal: Yellow unabsorbed yolk found in Japanese quail's developing embryo



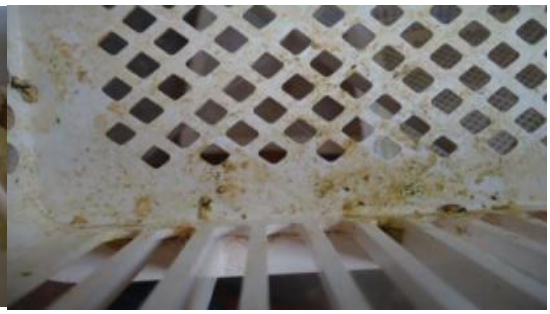
Mass of green unabsorbed yolk found in Japanese quail's developing embryo

Withdrawal of green unabsorbed yolk in Japanese quail's developing embryo

Withdrawal of yellow unabsorbed yolk in Japanese quail's developing embryo

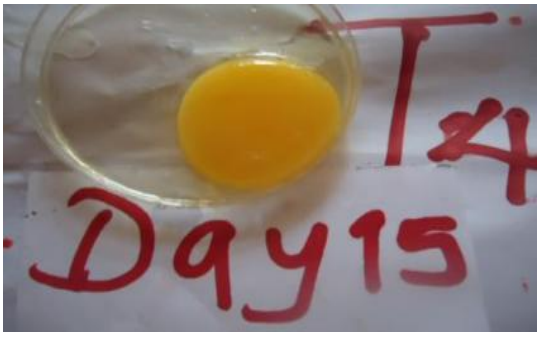


Withdrawn green unabsorbed yolk resulted in green excreta in Japanese quail chick



Withdrawn yellow unabsorbed yolk resulted in brown excreta in Japanese quail chick

Appendix 7: Serendipitous discovery 2



Intact incubated Japanese quail egg on day 15

Intact incubated Japanese quail egg on day16

Caked incubated Japanese quail egg

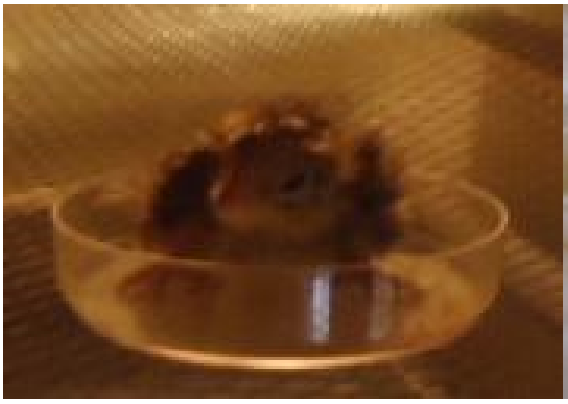
Blastoderm development in concentric layers on incubation day 1

Un-pigmented Japanese quail eggs *in utero*

Appendix 8: Serendipitous discovery 3

Possibility of assisting chickthat is unable to complete pipping process

Opened egg on day 17 to examine the embryo growth stage was returned to the incubator



The examined and returned embryo survived on day 18

This chick at pipping stage was not assisted and was lost in the process therefore, assisted pipping may enhance survivability

This chick in pipping process was assisted and survived

Appendix 9: Physiological status of Japanese quail incubated eggs

Not clear whether fertile or not incubated Japanese quail egg

Early dead embryo

Fully developed but dead embryos

Pipped and dead embryo

Pipped and alive but not able to emergeembryos

Appendix 10: Blood collection and biochemical analysis

Appendix 11: Carcass quality determination

Appendix 12:Japanese quailoffal could become delicacies


Japanese quail gizzards



Japanese quail yolkUteri and yet-to-be-laid eggs


Japanese quail heads



Spices to taste


Cooking the offal


Spread it to cool


Food is ready!



Delicious Japanese quail cooked offal

Appendix 13: Visitation at the experimental site by a team from the Department of Animal Science, Faculty of Agriculture, University of Ibadan, Ibadan and the Staff of NVRI, Poultry Division, Vom

Appendix 14: Presentation of some of the experimental results at the 8th Annual Conference of the ASAN-NIAS Joint Annual Meeting, held at the ICC, Umuahia, Abia State, Nigeria in September, 2019

Paper 1: Effectiveness of locally fabricated electric incubators, use of stethoscope and sensitive weighing balance in Japanese quail incubated eggs fertility determination. *Proceedings of the 8th ASAN-NIAS Joint Annual Meeting*, Umuahia. Pp. 1 – 7.

AB 001

EFFECTIVENESS OF LOCALLY FABRICATED ELECTRIC INCUBATORS, USE OF STETHOSCOPE AND SENSITIVE WEIGHING BALANCE IN JAPANESE QUAIL INCUBATED EGGS FERTILITY DETERMINATION

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ABSTRACT

Japanese quails in domestication lack broody behaviour hence, the adoption of artificial incubation for commercial production. Yet, little is known about the possibility of fabricating electric incubators for poultry eggs incubation in Nigeria. More essentially, there is no information on the possibility of using stethoscope and sensitive weighing balance to detect viable poultry eggs *in ovo* during incubation. Thus, this study was designed to investigate the effectiveness of locally fabricated electric incubators, use of stethoscope and sensitive weighing balance in Japanese quail incubated eggs fertility determination. A total of 300 Japanese quail eggs were used in three separate studies, 100 each to test run newly fabricated electric poultry eggs incubators, testing incubated Japanese quail eggs for auscultation and deflection on sensitive weighing balance. It was shown that though the incubated eggs were normal with high hatchability suggesting the fabricated incubators were probably efficient, auscultation and deflection were not observed during the study. Therefore, efficient electric incubators could be manufactured in Nigeria but stethoscope and sensitive weighing balance may not be practicable in determining Japanese quail incubated eggs fertility *in ovo*.

Keywords: Auscultation, egg candling, Japanese quail eggs, *in ovo*

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DESCRIPTION OF PROBLEM

There is inadequate animal protein intake in Nigeria thus, increased commercial poultry production is imperative. Intensive Japanese quails production could boost animal protein intake due to its short generation interval with a potency of laying 250 – 320 eggs per year and the males mature at about 30 to 60 days old hence, the possibility of 3 to 4 generations per year (Woodward *et al*., 1973). According to Nakane and Tsudzuki (1999), Japanese quail body size and the precocial nature of the chicks make it a potential research bird. Although, Fah (2009) reported that Japanese quails originated from Eastern Asia, it was introduced into Nigeria by NVRI in 1992 (Haruna *et al*., 1997). It has been observed that Japanese quails do not sit on the eggs in order to hatch and brood the chicks in domestication. Thus, artificial incubation expertise is required for commercial production. Artificial incubator could be homemade or commercial but should typically have heating source, air circulator (fan), temperature regulator (thermostat) as well as water trough and egg trays. Unfortunately, there is no known locally fabricated electric incubator in Nigeria. Worse still, egg candling is not practiced in some hatcheries where imported incubators are used, probably due to the cumbersome nature of the activities involved and partly due to inability of the operator to recognise viable embryos in the

incubated eggs when pin-hole-light source is used. Therefore, alternative techniques perceived to be less cumbersome with little or no expertise may be necessary hence, this study was targeted at fabricating electric poultry egg incubator locally, as well as using stethoscope and sensitive weighing balance to determine viable Japanese quail embryos during incubation.

MATERIALS AND METHODS

ELECTRIC INCUBATORS FABRICATION

Designed electric incubators were invented with the capacity of 1,500 Japanese quail eggs fitted with sensitive thermoregulatory devices to switch off and on at appropriate interval yet, maintained temperature of as low as 1°C and as high as 150°C. Fabrication of five units of electric incubators was carried out at the Fabrication Division, Dagwom Farm, NVRI, Vom, Plateau State, strictly according to the design specifications using locally sourced materials. The materials used were HBF plywood, acrylic glass, a 315W heater and three-tier wire gauze egg-tray that was turned at 180° with a pedal outside the incubator. Electric bulbs (40W and 100W) were placed strategically within the incubators for lighting system and heat supply with a fan mounted on the inner top to enable even warm-air circulation. Hygrothermometer with a sensitive probe capable of sensing inside/outside temperature and relative humidity was placed at the middle of the incubator. Thermostat and relay devices were connected to the heater for temperature regulation and four rollers were fixed on the base to ease movement of the incubators. The fabricated incubators were perforated for air inlet/outlet. A switch was connected to power the electric bulbs and another to operate the fabricated incubators.

EXPERIMENTAL DESIGN AND TREATMENT LAYOUT

A total of three hundred Japanese quail eggs believed to be fertile were acquired from a renowned farm in Jos, Plateau State. Sixty eggs were weighed together in a bowl using sensitive weighing balance (KERRO, Taiwan, Electronic Compact Scale, BL5002), labelled for individual identification, fumigated using 30ml of formalin and 10g of $KMnO_4$ mixture at 26.5°C for 30 minutes and set on the incubation trays padded with cotton wool to hold the eggs in pointed-end down position.

STUDY I: TEST RUN OF FABRICATED ELECTRIC INCUBATORS

The five fabricated incubators were switched on for 7days and the temperature was regulated at 38°C. The water compartment was filled all the time to maintain the required relative humidity. The temperature and the relative humidity were monitored with a hygrothermometer (HTC-2). A total of 100 Japanese quail eggs were set in each of the incubators such that each incubator had 20 eggs. In this study, the eggs used were not labelled and were not touched throughout the incubation period.

STUDY II: USE OF STETHOSCOPE TO DETERMINE EGG FERTILITY DURING INCUBATION

One hundred separate Japanese quail eggs were labelled appropriately for individual record and were set on the middle tray within the same incubators such that each incubator had 20 eggs. Each of the 20 eggs was picked carefully and placed on the stethoscope for auscultation every other day (i.e. days 1, 3, 5, 7, 9, 11, 13, 15) during the incubation period. On each day, all the eggs were returned to the incubators immediately and where auscultation sound was observed the eggs were suspected to be fertile and were proven correct or otherwise at hatch.

STUDY III: USE OF SENSITIVE WEIGHING BALANCE TO DETERMINE EGG FERTILITY DURING INCUBATION

One hundred separate Japanese quail eggs were labelled appropriately for individual record and set on the same incubators such that each treatment had 20 eggs. Each of the 20 eggs was picked carefully, placed on the sensitive weighing balance and continuous deflection was presumed to be due to the living embryo. This was done every other day (i.e. days 1, 3, 5, 7, 9, 11, 13, 15) during the incubation period and on each day, all the eggs were returned to the incubators immediately and egg fertility was confirmed at hatch.

DATA COLLECTION AND ANALYSES

Egg weight was taken using sensitive weighing balance (KERRO, Taiwan, Electronic Compact Scale, BL5002) and incubation period was taken as the period between setting the eggs in the incubators and emergence of the chicks. Chick weight was determined using sensitive weighing balance, chick yield was estimated by dividing chick weight by egg weight multiplied by 100%, hatchability was determined by dividing the number of chicks hatched by the number of fertile egg incubated multiplied by 100% and egg fertility was assessed by dividing the number of fertile eggs by the number of eggs incubated multiplied by 100%. At the end of the incubation period, all the un-hatched eggs were carefully windowed to check for embryo, confirm egg fertility and record the physiological status of the developing embryo as described by Idahor *et al*. (2018). All the data collected were subjected to analysis of variance procedure of SPSS (2010) and mean values were separated using Duncan Multiple Range Test of the same software package.

RESULTS AND DISCUSSION

Hatchability of Japanese quail eggs incubated at 38°C in fabricated incubators, those tested with stethoscope and sensitive weighing balance to determine egg fertility during incubation are presented in Tables 1, 2 and 3. In Table 1, there were no statistical differences ($P>0.05$) in all the parameters measured except in early dead embryos that were significantly higher ($P<0.05$) in incubator 1 and total chick weight that was superior in incubators 1 and 2 respectively. Meanwhile, there were significant differences ($P<0.05$) in pipped and dead embryos, pipped and alive but not able to emerge embryos, hatched chicks and hatchability in Table 2. Similarly, there were statistical variations ($P<0.05$) in all the parameters measured in Table 3 except chick weight, egg fertility and chick yield. In all the studies however, the egg weight was similar to 7.69 – 10.8g (Ajide, 2011) in Japanese quails but less than 10 – 12g speculated to be the normal size of Japanese quail eggs (FAO, 2013). The incubation period was within 16 – 19 days reported to be normal in Japanese quails (Sellier *et al*., 2006). The chick weight, chick yield and hatchability values were similar to those reported elsewhere (Farghly *et al*. 2015; Romao *et al*., 2009; Sellier *et al*., 2006). Consequently, the eggs were probably normal and suitable for use in the study.

There were hatched chicks in all the fabricated incubators thus they were probably efficient in Japanese quail eggs incubation. In all the eggs tested with stethoscope during the incubation, no one indicated auscultation, probably due to the small size of the developing Japanese quail embryo heart. Similarly, none of the eggs deflected the sensitive weighing balance during the incubation, possibly due to the size of the developing Japanese quail embryo. Therefore, use of stethoscope and sensitive weighing balance may not be feasible in determining living embryo in Japanese quails during egg incubation.

CONCLUSIONS AND APPLICATIONS

All the fabricated incubators hatched an appreciable number of Japanese quail eggs incubated. Therefore, poultry eggs electric incubators could be manufactured in Nigeria. Since the incubated Japanese quail eggs did not auscultate nor deflect sensitive weighing balance, these techniques may not be practicable in testing poultry embryo viability *in ovo*.

Table 1: Hatchability of Japanese quail eggs incubated at 38°C in fabricated incubators

Parameters	Fabricated incubators (±STD)					SEM
	1	2	3	4	5	
No. of eggs set	20	20	20	20	20	-
TEW (g)	200.2±1.09	206.1±3.14	201.6±2.14	200.9±0.11	208.8±0.55	0.11
AEW (g)	9.87±0.14	10.21±0.14	9.93±0.60	10.00±0.23	10.42±0.43	0.04
IP (days)	19	18	17	18	18	-
Infertile eggs	1.68±1.36	1.02±1.32	1.67±0.22	1.34±0.22	1.33±0.44	0.05
Caked eggs	1.74±0.58	2.40±0.44	1.31±0.41	2.11±0.16	1.21±0.19[b]	0.11
NCWFN	1.04±0.51	0.67±0.72	1.02±1.44	1.72±0.17	1.62±0.16	1.35
EDE	2.21±0.53[a]	0.33±0.76[b]	1.16±0.77[ab]	1.02±0.88[ab]	1.67±0.18[b]	1.05
FDDE	0.55±0.38	1.33±1.44	0.54±0.54	1.88±0.17	1.25±0.11	1.06
PDE	0.36±0.18	1.12±1.14	0.00±0.44	0.88±0.19	1.16±0.16	0.12
PAE	1.33±0.03	1.03±2.18	1.03±0.12	0.00±0.13	2.64±0.14	0.17
*Fertile eggs	18.39±1.15	17.11±1.34	16.47±0.34	15.69±0.11	17.68±0.14	0.83
Hatched chicks	13.67±1.15	14.66±1.22	13.70±0.19	12.34±0.15	12.34±0.14	0.05
TCW (g)	93.89±2.24[a]	97.78±0.44[a]	83.72±0.14[b]	79.22±0.19[b]	84.89±0.17[b]	12.81
ACW(g)	6.87±0.41	6.95±0.23	6.44±0.33	6.67±0.56	7.10±0.19	0.07
Hatchability (%)	64.82±4.14	69.88±0.73	68.33±0.65	61.68±0.88	61.62±0.64	1.35
Egg fertility (%)	90.14±1.82	85.51±0.88	80.44±0.55	78.63±0.54	88.68±0.34	0.64
Chick yield (%)	68.72±0.98	68.12±0.23	65.03±0.73	66.56±0.62	68.34±0.29	0.44

ab: Mean values on the same row with different superscript differ statistically at 5% probability test; STD: Standard deviation; SEM: Standard error of means; TEW: Total egg weight; AEW: Average egg weight; IP: Incubation period; NCWFN: Not clear whether fertile eggs or not; EDE: Early dead embryos; FDDE: Fully developed but dead embryos; PDE: Pipped and dead embryos; PAE: Pipped and alive but not able to emerge embryos; TCW: Total chick weight; ACW: Average chick weight. *Fertile eggs = the sum of "early dead embryos", "fully developed but dead embryos", "pipped and dead embryos", "pipped and alive but not able to emerge embryos" and "fully developed, pipped and emerged chicks".

Table 2: Hatchability of Japanese quail eggs tested with stethoscope to determine egg fertility during incubation

Parameters	Fabricated incubators (±STD)					SEM
	1	2	3	4	5	
No. of eggs set	20	20	20	20	20	-
TEW (g)	181.2±2.10	184.1±1.77	198.6±1.48	179.9±0.99	188.8±0.89	0.41
AEW (g)	9.07±0.12	9.21±0.16	9.93±0.44	9.00±0.72	9.42±0.88	0.24
IP (days)	19	18	19	18	18	-
Infertile eggs	4.21±1.11	1.02±1.19	1.67±0.88	1.34±0.77	1.33±0.44	0.05
Caked eggs	2.08±1.14	2.40±1.54	1.31±.88	2.11±0.43	1.21±0.19^{b}	0.11
NCWFN	2.22±0.69	0.67±0.23	1.02±0.77	1.72±0.92	1.62±0.33^{a}	1.35
EDE	0.00±0.00^{b}	0.33±0.22^{ab}	1.16±0.14^{a}	1.02±0.14^{a}	1.67±0.14^{a}	1.05
FDDE	2.44±0.23	1.33±0.54	0.54±0.48	1.88±0.19	1.25±0.23	1.06
PDE	0.00±0.00^{b}	1.12±0.14^{a}	0.00±0.00^{b}	0.88±0.11^{b}	1.16±0.33^{a}	2.05
PAE	1.00±0.72^{a}	1.03±0.45^{a}	1.03±0.79^{a}	0.00±0.00^{b}	2.64±0.56^{a}	2.11
*Fertile eggs	13.02±4.16	15.32±2.84	12.69±2.17	16.77±1.74	14.33±1.88	1.44
Hatched chicks	10.70±2.14^{b}	12.22±1.55^{a}	11.92±1.66^{b}	14.66±3.44^{a}	9.65±2.94^{ab}	3.05
TCW (g)	70.62±0.44^{ab}	80.67±0.74^{a}	83.30±0.88^{a}	97.82±0.49^{a}	66.05±2.44^{b}	9.05
ACW(g)	6.66±3.64	6.57±3.44	7.04±2.88	6.69±1.88	6.88±3.55	1.20
Hatchability (%)	81.67±1.14^{a}	71.76±2.74^{a}	74.37±1.44^{a}	92.52±2.14^{a}	54.24±1.19^{b}	8.08
Egg fertility (%)	65.00±6.14	75.00±3.34	63.35±4.54	80.00±2.24	71.60±2.94	0.22
Chick yield (%)	74.00±3.74	71.42±3.14	70.70±3.94	74.33±3.82	73.19±3.09	0.67

ab: Mean values on the same row with different superscript differ statistically at 5% probability test; STD: Standard deviation; SEM: Standard error of means; TEW: Total egg weight; AEW: Average egg weight; IP: Incubation period; NCWFN: Not clear whether fertile eggs or not; EDE: Early dead embryos; FDDE: Fully developed but dead embryos; PDE: Pipped and dead embryos; PAE: Pipped and alive but not able to emerge embryos; TCW: Total chick weight; ACW: Average chick weight. *Fertile eggs = the sum of "early dead embryos", "fully developed but dead embryos", "pipped and dead embryos", "pipped and alive but not able to emerge embryos" and "fully developed, pipped and emerged chicks".

Table 3: Hatchability of Japanese quail eggs tested with sensitive weighing balance to determine egg fertility during incubation

Parameters	Fabricated incubators (±STD)					SEM
	1	2	3	4	5	
No. of eggs set	20	20	20	20	20	-
TEW (g)	198.4±0.8 8	196.0±0.4 4	180.0±0.7 8	179.9±0.93	195.4±0.6 7	0.39
AEW (g)	9.92±1.36	9.82±0.77	9.01±0.99	9.77±0.14	9.89±0.99	0.36
IP (days)	19	18	17	18	17	-
Infertile eggs	2.21±0.48	0.0±0.54	1.67±0.84	1.34±0.99	1.33±0.65	1.74
Caked eggs	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	1.31 ± 0.14^{a}	2.11 ± 0.94^{a}	1.21 ± 0.55^{a}	2.49
NCWFN	3.00 ± 0.19^{a}	0.00 ± 0.24^{c}	1.02 ± 0.66^{b}	1.72 ± 0.98^{b}	1.62 ± 0.67^{b}	3.22
EDE	2.00 ± 0.99^{a}	3.67 ± 0.86^{a}	1.16 ± 0.44^{b}	1.02 ± 0.14^{b}	1.67 ± 0.14^{b}	1.88
FDDE	1.67 ± 0.14^{a}	2.38 ± 0.94^{a}	0.54 ± 0.55^{b}	1.88 ± 0.93^{a}	1.25 ± 0.18^{a}	2.43
PDE	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.88 ± 0.14^{b}	1.16 ± 0.14^{a}	0.97
PAE	1.00 ± 0.17^{a}	2.67 ± 0.13^{a}	1.03 ± 0.19^{a}	0.00 ± 0.00^{b}	2.64 ± 0.44^{a}	0.47
Fertile eggs	$18.02\pm0.7\ 4^{a}$	$19.45\pm0.9\ 4^{a}$	$13.39\pm0.6\ 4^{b}$	16.90 ± 0.24^{b}	$15.33\pm0.1\ 4^{ab}$	3.08
Hatched chicks	$14.56\pm0.9\ 4^{a}$	$12.22\pm0.1\ 7^{a}$	$11.92\pm0.5\ 4^{a}$	14.66 ± 0.37^{a}	9.65 ± 0.73^{b}	2.88
TCW (g)	$87.06\pm3.5\ 6^{a}$	$72.09\pm6.8\ 8^{b}$	$72.44\pm4.1\ 4^{b}$	94.08 ± 3.94^{a}	$63.36\pm4.9\ 9^{c}$	6.795
ACW(g)	5.97±4.14	6.04±4.84	5.88±2.55	6.44±1.19	6.65±2.14	3.18
Hatchability (%)	$77.78\pm0.9\ 4^{a}$	$63.16\pm0.1\ 4^{b}$	$84.61\pm0.1\ 9^{a}$	82.35 ± 0.44^{a}	$64.09\pm0.8\ 8^{b}$	6.49
*Egg fertility (%)	90.00±0.23	95.00±0.47	67.00±0.19	85.00±0.24	75.00±0.54	0.85
Chick yield (%)	59.60±0.44	61.22±0.64	65.33±0.54	65.91±0.44	66.67±0.88	0.92

abc: Mean values on the same row with different superscript differ statistically at 5% probability test; STD: Standard deviation; SEM: Standard error of means; TEW: Total egg weight; AEW: Average egg weight; IP: Incubation period; NCWFN: Not clear whether fertile eggs or not; EDE: Early dead embryos; FDDE: Fully developed but dead embryos; PDE: Pipped and dead embryos; PAE: Pipped and alive but not able to emerge embryos; TCW: Total chick weight; ACW: Average chick weight. *Fertile eggs = the sum of "early dead embryos", "fully developed but dead embryos", "pipped and dead embryos", "pipped and alive but not able to emerge embryos" and "fully developed, pipped and emerged chicks".

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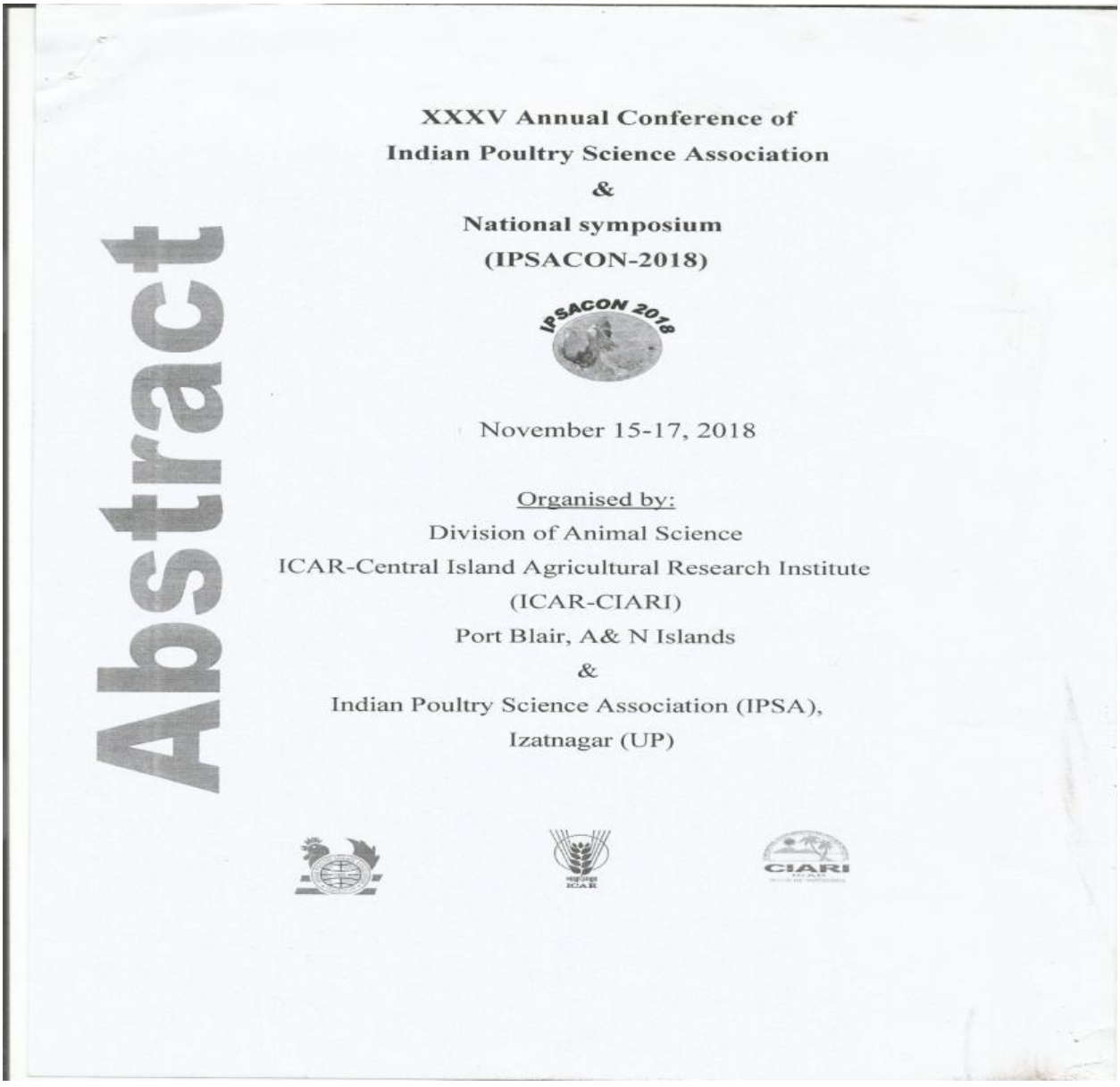
Appendix 15: Presentation of some of the experimental results at the XXXV Annual Conference of Indian Poultry Science Association, held at Port Blair, India in November, 2018

Paper 2: Post-hatch performance of Japanese quails subjected to paused incubation temperature during late embryogenesis. *Book of Abstracts, XXXV Annual Conference of Indian Poultry Science Association*, Port Blair, India. Pp. 222.



Abstract

XXXV Annual Conference of
Indian Poultry Science Association
&
National symposium
(IPSACON-2018)

November 15-17, 2018

Organised by:
Division of Animal Science
ICAR-Central Island Agricultural Research Institute
(ICAR-CIARI)
Port Blair, A& N Islands
&
Indian Poultry Science Association (IPSA),
Izatnagar (UP)

IPSACON2018

XXXV Annual Conference of Indian Poultry Science Association

RURAL POULTRY PRODUCTION:

CHALLENGES FOR SUSTAINABLE ENTREPRENEURSHIP DEVELOPMENT

15-17, November 2018

Organised by: Indian Poultry Science Association &

ICAR-Central Island Agricultural Research Institute, Port Blair, A&N Islands

To,
Idahor K. O

Department of Animal Science, Nasarawa State University, Keffi, Shabu-Lafia Campus, Nigeria

Dear Madam,

I am pleased to inform you that the abstract titled "**Posthatch performance of Japanese quails subjected to paused incubation temperature during late embryogenesis**" authored by Idahor K. O., Sokunbi O. A. OmidiwuraB. R. O., AgboolaA. F., Shamaki D. and Nwosuh C. I. has been accepted for the **oral presentation** during IPSACON2018 to be held at ICAR-CIARI, Port Blair from 15-17 November 2018. The details for the presentation are given below

Abstract no.: PPH-13

Theme: Poultry Physiology and Housing management: Impact of climate change and Integrated Farming System

Mode of presentation: Oral

You are therefore invited to participate and present the work during IPSACON 2018 conference.

With Regards

Arun Kumar De

Chairman

Abstract Committee

Venue:
ICAR-Central Island Agricultural Research Institute, Port Blair
Email: ipsacon2018@gmail.com,
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PPH-08 Effect of moulting on calcium up take and basal epithelial ion transport and hormonal profile in white leghorn hens: A Comprehensive study
David C G, R K Gorti, P A Hearwin, R.U Suganthi, A Mech, K P Suresh and I J Reddy

PPH-09 Effect of systems of rearing on performance of Rajasri pullets
Naga Raja Kumari. K, M. Mahammad Ali, and Narendranath. D

PPH-10 Study on effect of temperature and relative humidity on weight loss of Rajasri hatching eggs during incubation
Murali .L, M. Mahammad Ali[1], Naga Raja Kumari. K[2] and Narendranath. D[3]

PPH-11 Effect of acute heat stress exposure on production performance of indigenous chicken breeds
JagadhaneVinay M., J.S. Tyagi, Gopi, M., Kolluri, G., Beulah V. Pearlin, Mukthar, A. and Jag Mohan

PPH-12 Cytological alteration in immune organs of broiler chicken due to inorganic arsenic
Subhashree Das, Debasis Bhattacharya1, Asit Kumar Bera1, Tanmoy Rana, Partha Das, Srikanta Samanta, Diganta Pan, Sandip K Bandyopadhyay

PPH-13 Post-hatch performance of Japanese quails subjected to paused incubation temperature during late embryogenesis
Idahor K. O.,Sokunbi O. A.OmidiwuraB. R. O., AgboolaA. F., Shamaki D. and Nwosuh C. I.

PPH-15 Chicken embryogenesis: Influence of egg quality traits onembryo morphology
J.O. Alabi, S.K. Bhanja, A. Goel, M. Mehra and A.O. Fafiolu

PPH-16 Effect of short period of incubation during egg storage (spide) on hatching performance of broiler breeder eggs
Shamsudeen, P., A. Poorani, K. Mani and D. Ilaya Bharathi

PPH-18 Effect of stocking density on behaviour of Turkeys in summer evenings
R. Sirohi, P. K. Shukla, A. Bhattacharyya, Y. Singh, D. N. Singh, Mamta and A. Kumar

PPH-21 Effect of thermal conditioning on cell mediated immune response of different chicken strains
S. Sivaramakrishnan, A.V. Omprakash, S. Ezhilvalavan, K.G. Tirumurugaan, G. Srinivasan and A. Varun

PPH-22 Ammonia gas in the poultry houses –an overview
I.U. Sheikh, M.T.Banday, A.A. Khan, I.A. Baba, S. Adil, Shaista S. Nissa and Bushra Zaffer

PPH-23 Rural poultry: Component of Integrated Farming System for livelihood security in Bay Islands, India
T.P. Swarnam, A.Velmurugan, T.Subramani, M.S.kundu, I.Jaisankar and R.Kirubasankar,

PPH-26 Hot humid climate negatively affects the poultry production system.
P. Perumal, K. Muniswamy, A. K. De, D. Bhattacharya, Jai sunder, T. Sujatha, S. K. Ravi, R. R. Alyethodi, M. S. Kundu and A. Kundu

PPH-27 Poultry farming in Tsunami waterlogged lands of Andaman & Nicobar Islands – As an climate change induced sea level rise coping mechanism of Coastal regions of India
B. Gangaiah, Abbubaker, M. S. Kundu, M. N. V. Laxmi, T. Subramani, Sirisha Adamala and K. R. Kiran

PPH-28 Seasonal influence on fertility and hatchability of Nicobari Fowl: A retrospective study
S.K. Ravi, T. Sujatha, A. Kundu, P. Perumal, Jai Sunder, A.K. De, K. Muniswamy,

Post hatch performance of Japanese quails subjected to paused incubation temperature during late embryogenesis

K. O. Idahor, O. A. Sokunbi, B. R. O. Omidiwura, A. F. Agboola, D. Shamaki and C. I. Nwosuh

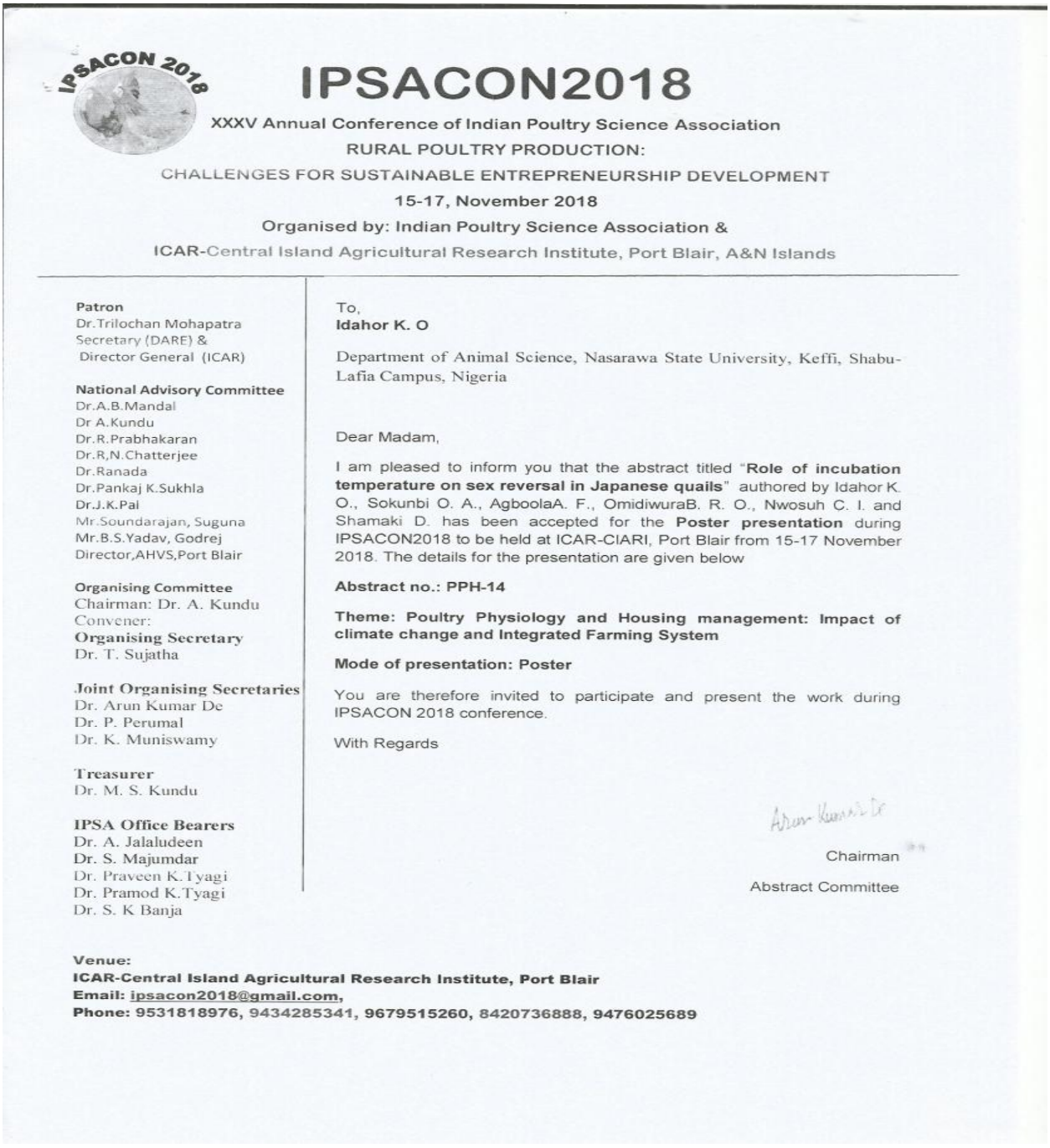
Nasarawa State University, Keffi, Shabu-Lafia Campus, Nigeria
Faculty of Agriculture and Forestry, University of Ibadan, Ibadan, Nigeria
National Veterinary Research Institute, Vom, Nigeria

PPH -13 Oral Email: koidahor@nsuk.edu.ng

Abstract

Japanese quails in domestication do not sit on eggs in other to incubate them hence, the use of artificial incubator that simulates broody hen strategy. Although the artificial incubator can be regulated, it could be too low or too high for embryo growth and development, resulting in sex reversal, early or late hatching with attendant high mortality and poor posthatch performance. Therefore, would incubation temperature set at 36°C be too low or 40°C be too high when paused for 5 hours during incubation days 11, 12, and 13 to reverse sex or elicit poor posthatch performance in Japanese quails? Based on this, six incubators set at 38°C (Control: T1), 36°C (T2), 37°C (T3), 38°C (T4), 39°C (T5) and 40°C (T6) representing the treatments were used in this study. On incubation days 11, 12 and 13, the temperature was paused for 5 hours except the Control. Control incubator was imported, others were locally fabricated and in each incubator, 102 Japanese quail eggs were set and incubation period, hatchability, daily feed intake, weekly weight gain and blood profiles were determined. Results indicated that incubation period was 17days in Control, T3 and T4 and 18days in T2. Hatchability was superior (83.20±14.70%) in Control, followed by 66.97±18.97% (T3), least (58.97±52.35%) in T4 and absolutely zero in T5 and T6. Daily feed intake and weekly weight gain increased with age, there was mortality among all the hatchlings and no reversed sex was recorded. Packed cell volume ranged from 22.83±25.20% to 50.50±5.24%, red blood cells (1.72±1.88 x 10^{12}/l to 3.42±0.28 x 10^{12}/l), white blood cells (2.98±3.49 x 10^{9}/l to 9.55±1.58 x 10^{9}/l) and haemoglobin (7.22±7.98g/dl to 16.82±1.71g/dl). Total protein was highest (48.76±3.33g/l) in T3 and least (24.31±26.74g/l) in T4, creatinine varied from 20.93±24.01µmol/l to 52.57±13.33µmol/l and cholesterol was highest (127.99±29.25mg/dl) in T3 and lowest (101.07±11.08mg/dl) in T4. Basically, values in all the parameters measured, were within the range given in healthy Japanese quails of similar age. Therefore, paused incubation temperature at 36°C to 40°C during late embryogenesis, may not elicit early or late hatching, sex reversal or poor performance in Japanese quails.

Paper 3: Role of incubation temperature on sex reversal in Japanese quails. *Book of Abstracts,*XXXV Annual Conference of Indian Poultry Science Association, Port Blair, India. Pp. 223.



IPSACON2018

XXXV Annual Conference of Indian Poultry Science Association

RURAL POULTRY PRODUCTION:

CHALLENGES FOR SUSTAINABLE ENTREPRENEURSHIP DEVELOPMENT

15-17, November 2018

Organised by: Indian Poultry Science Association &

ICAR-Central Island Agricultural Research Institute, Port Blair, A&N Islands

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To,
Idahor K. O

Department of Animal Science, Nasarawa State University, Keffi, Shabu-Lafia Campus, Nigeria

Dear Madam,

I am pleased to inform you that the abstract titled "**Role of incubation temperature on sex reversal in Japanese quails**" authored by Idahor K. O., Sokunbi O. A., AgboolaA. F., OmidiwuraB. R. O., Nwosuh C. I. and Shamaki D. has been accepted for the **Poster presentation** during IPSACON2018 to be held at ICAR-CIARI, Port Blair from 15-17 November 2018. The details for the presentation are given below

Abstract no.: PPH-14

Theme: Poultry Physiology and Housing management: Impact of climate change and Integrated Farming System

Mode of presentation: Poster

You are therefore invited to participate and present the work during IPSACON 2018 conference.

With Regards

Chairman

Abstract Committee

Venue:
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Poultry physiology & housing management-impact of climate change/Integrated farming system

PPH-14 Role of incubation temperature on sex reversal in Japanese quails
Idahor K. O.,Sokunbi O. A., AgboolaA. F., OmidiwuraB. R. O.,Nwosuh C. I. and Shamaki D.

PPH-17 Relationship of time of oviposition with egg weight and egg production in broiler breeder
Shamsudeen, P., J. Jeyasri, K. Mani and D. Ilaya Bharathi

PPH-19 Effect of stocking density on the growth of turkeys during summer
R. Sirohi, P. K. Shukla, A. Bhattacharyya, Y. Singh, Mamta and D.N. Singh

PPH-20 Effect of stocking density on the feed consumption and feed conversion ratio of turkeys during summer
R. Sirohi, P. K. Shukla, A. Bhattacharyya, Y. Singh, D.N. Singh and S.S. Kashyap

PPH-24 Backyard poultry in Integrated Farming System for food and nutritional security of tribal farmers in Nicobar Islands, India
Sanjay Kumar Pandey, T.P. Swarnam, Zachariah George, A.Velmurugan, Sanjeev Kumar Singh, and Nagesh Ram

PPH-25 Chromium: Heat stress redeemer and performance enhancer in Poultry
P.A. Bala, D. Bhattacharya, M.S. Kundu, S.K.Ravi, T. Sujatha, J. sunder, A.K. De, R. R. Alyethodi & A. Kundu

Session-III

Post-harvest management and processing –improvement in poultry products

PHM-05 Evaluation of internal egg quality traits of Kadaknath chicken
Preeti Ekka, K. Mukherjee, Mohan Singh, , Deepti Kiran Barwa and Om Prakash Dinani

PHM-17 Development of Functional Egg Spiral Roll Incorporating Certain Selected Cereals and Natural Preservatives
Dnyanada Jadhav, C. K. Beura and A. K. Biswas

PHM-18 Hatchery performance of In-ovo betaine supplemented eggs
Monika, M., V.K.Saxena, J.J.Rokade, M.Gopi, Shyamkumar, T.S., Gautham, K. and Narayan, R.

PHM-21 Meat quality and oxidative stability are improved by flaxseed meal and turmeric rhizome powder inclusion in broiler chicken diets
Nasir Akbar Mir, Faneshwar Kumar, Praveen K. Tyagi, Kapil Dev, Pramod K. Tyagi, IndrajitBera, A.B. Mandal

Session-V

Role of incubation temperature on sex reversal in Japanese quails

K. O. Idahor, O. A. Sokunbi, A. F. Agboola, B. R. O. Omidiwura, C. I. Nwosuh and D. Shamaki

Nasarawa State University, Keffi, Shabu-Lafia Campus, Nigeria
Faculty of Agriculture and Forestry, University of Ibadan, Ibadan, Nigeria
National Veterinary Research Institute, Vom, Nigeria

PPH -14 Poster Email: koidahor@nsuk.edu.ng

Abstract

Japanese quail chick sex cannot be identified at day old until about 3weeks of age, resulting in compulsory culling of undesired sex in the flock, in order to maintain the recommended sex ratio. This may be quite disappointing and unpromising hence, the need to predetermine Japanese quail sex during incubation by manipulating the temperature as reported in Australian brush turkey and reptiles. If embryogenesis could be disrupted by diseases, climatic conditions and other factors, triggering development of the rudimentary sex characters in livestock, incubation temperature manipulation may reverse sex in Japanese quails. Hinged on this premise, a total of 1, 260 Japanese quail eggs were set in six incubators (210 eggs each), adjusted to maintain 38°C (Control: T1), 36°C (T2), 37°C (T3), 38°C (T4), 39°C (T5) and 40°C (T6) representing the treatments. On incubation days 3, 4 and 5, the temperature was paused to physiological zero temperature (26 – 28°C) for 5hrs, essentially to disrupt growth and development of the embryos, in other to reverse the destined sex. However, the Control: T1 (imported incubator) was not paused but other locally fabricated ones were paused. During the 6wks study, incubation period, hatchability, chick weight, chick yield, mortality, sex ratio and sex reversal were monitored. At 3wks of age, the sex was identified using plumage pattern and at sexual maturity, it was re-identified painstakingly, sacrificed and the reproductive organs carefully examined. Results showed that incubation period was 18days across the treatments except T2 (19days), hatchability was best (63.78±50.96%) in Control, followed by T3 (46.61±17.71%) and absolutely zero in T6. Chick weight varied between 5.46±0.12g and 6.48±0.82g, chick yield was inferior (20.49±35.49%) in T4 compared to 67.73±1.79% observed in T2. Male to female ratio was 40:37, 10:9, 10:11 in Control, T2 and T3 respectively. While mortality rate was highest (2.07±2.06%) in Control, followed by 1.58±0.93% (T3) and 1.54±0.77% (T2), it was 100% in T4 and T5. In Control, sex reversal from male to female chick was recorded. Therefore, incubation temperature alteration for 5 hours during early embryogenesis may not play significant role in Japanese quail sex reversal.

Appendix 16: Presentation of some of the experimental results at the XVth Meeting of the Avian Immunology Research Group held at St. Catherine's College, Oxford, UK in September 2018.

Paper 4: Possibility of using high incubation temperature to elicit thermo-tolerance traits in Japanese quail chicks hatched at varied incubation temperatures in a tropical environment.*Book of Abstracts, XVth Meeting of Avian Immunology Research Group*, St Catherine's College, Oxford, UK. Pp. 141– 142.

UNIVERSITY OF OXFORD

AIRG OXFORD

Dear Idahor K.O.,

ACCEPTANCE LETTER TO PRESENT PAPERS AT THE AIRG 2018 CONFERENCE

I am writing this email to indicate that your submissions: 1. "Possibility of using high incubation temperature to elicit thermotolerance traits in Japanese quail chicks hatched at varied incubation temperatures in a tropical environment" and 2. "Haematological and serum indices of Japanese quail chicks subjected to incubation temperature equivalent to avian species body temperature" to the AIRG conference have been accepted as **poster presentations**. I know for some that this may be a disappointment but we have had a very large number of submissions and it was simply not possible to schedule all that requested oral presentations.

Many aspects of the submissions were taken into account during the selection process, including trying to spread the geographic distribution of abstracts in oral versus poster categories. In no way does the selection for a poster reflect the science, and I am so sorry not to have been able to accommodate more into the schedule. There will be at least one significant prize for best poster.

Poster boards will have a maximum size of A0 in landscape format.

Looking forward to seeing all of you in September.

Best wishes,
Adrian L. Smith
Associate Professor of Infectious Disease,
Department of Zoology,
Sir Peter Medawar Building for Pathogen Research,
University of Oxford,
South Parks Road,
Oxford,
UK
OX1 3SY
Tel: 01865 271 195

E. tenella parasites conferred partial protection against E. maxima challenge, at levels comparable to those obtained using EmAMA1 recombinant protein vaccination. This provides evidence that vaccination with transgenic Eimeria can induce cross protection against challenge with a heterologous coccidial species. Taken further, this technology has the potential to streamline the production of coccidiosis live vaccines through the generation of parasite lines that co-express immunoprotective antigens derived from multiple species of Eimeria.

#67 Genetic diversity of the major histocompatibility complex region in Australian specific pathogen free chickens

Mauricio JC Coppo[1*], Dulari Thilakarathne[1], Joanne M Devlin[1] and Carol A Hartley[1]

[1]Asia-Pacific Centre for Animal Health, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Victoria, Australia.

***mjcoppo@unimelb.edu.au**

The adoptive transfer of leukocytes from immunised to naïve individuals can be a powerful tool to investigate the role of selected leukocyte subsets in the immune response against avian pathogens. Such methodological approach requires the transfer of cells between histo-compatible individuals. Unfortunately, the lack of inbred chicken flocks combined with strict quarantine regulations has difficulted the application of these methodologies in avian immunological research in Australia. The objective of the current study was to characterise the MHC diversity in the Australian specific pathogen free (SPF) flock. DNA extracted from specimens collected from SPF chickens or embryos were subjected to PCR targeting the markers LEI0258 and MCW0371 located in the BF/BL region of the chicken MHC. The analysis of DNA amplicons using polyacrylamide gel electrophoresis and the TapeStation DNA analysis system was combined with DNA sequence analysis of the LEI0258 marker to provide detailed information about the diversity of MHC haplotypes present in Australian SPF chickens. Results from this study provide useful information about the genetic heterogeneity of the Australian SPF birds and serve as a stepping stone towards future adoptive leukocyte transfer studies aimed at better understanding the cell-mediated immune responses against important poultry pathogens in Australia.

#68 Possibility of using high incubation temperature to elicit thermotolerance traits in Japanese quail chicks hatched at varied incubation temperatures in a tropical environment

Idahor K. O. [1*], Shamaki D.[2], Nwosuh C. I.[3], and Sokunbi O. A[3].

[1]Department of Animal Science, Nasarawa State University, Keffi, Shabu-Lafia Campus, Nigeria; [2]National Veterinary Research Institute, Vom, Plateau State, Nigeria; 3 Animal Science Department, Faculty of Agriculture and Forestry, University of Ibadan, Nigeria

***omokingida@yahoo.com**

It has been established that most avian species body temperature range from 38 – 43oC and it has been reported that ambient temperature close to body temperature of any animal species could result in physiological dysfunction. However, the animal body system can tolerate this through the mechanism of homeostasis, resulting in balance internal milieu. With the present heat wave as a result of global warming and its attendant effects on poultry productivity, deliberate efforts to instigate thermotolerance traits using high incubation temperature become imperative. Generally, Japanese quails have been accepted as laboratory bird in most avian research. Hence, the present study was aimed at investigating the effects of high incubation temperature on egg hatchability and chick survivability. A total of 1,725 Japanese quail eggs were set in five locally fabricated incubators representing T2 (36oC), T3 (37oC), T4 (38oC), T5 (39oC) and T6 (40oC) with imported incubator as Control (38oC). Results showed that the eggs in T5 hatched on incubation day 16, followed by T4 on day 17 while T6 and T3 hatched on day 18. Control eggs hatched on day 19 with late hatching on day 20 in T2. Hatchability values were not statistically different ($P>0.05$) among the treatments but the highest (36.6±13.8%) was recorded in T5 and T3. This was slightly followed by 35.9±28.7 and 34.1±7.09% in T4 and Control respectively with the least value (18.9±26.6%) in T2. Similarly, chick yield values did not differ significantly across the treatments but the highest value (66.9±8.2%) was observed in T4, followed by 65.6±3.02% in T5 and 62.6 ±0.89% in Control. Survival rate values were significantly different ($P<0.05$) among the treatments with the highest value (80.5±4.79%) in Control.

Paper 5: Haematological and serum indices of Japanese quail chicks subjected to incubation temperature equivalent to avian species body temperature. *Book of Abstracts, XVth Meeting of Avian Immunology Research Group*, St Catherine's College, Oxford, UK. PP 142– 143.

UNIVERSITY OF OXFORD

AIRG OXFORD

Dear Idahor K.O.,

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Looking forward to seeing all of you in September.

Best wishes,
Adrian L. Smith
Associate Professor of Infectious Disease,
Department of Zoology,
Sir Peter Medawar Building for Pathogen Research,
University of Oxford,
South Parks Road,
Oxford,
UK
OX1 3SY
Tel: 01865 271 195

followed by 66.5±6.35% in T6 and 46.1±10.0% in T5. Therefore, high incubation temperature could be used to develop thermotolerance traits in order to mitigate climate change effects on poultry productivity.

#69 Haematological and serum indices of Japanese quail chicks subjected to incubation temperature equivalent to avian species body temperature

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Japanese quails hardly sit on the eggs and hatch them hence the adoption of artificial incubation techniques. In most avian egg incubation research conducted in the temperate region, the artificial incubation temperature was always set at 37±0.3°C mimicking natural incubation temperature. But in the tropics, the operational egg incubation temperature in most hatcheries, often vary between 37oC and 39°C. This temperature regimen may affect embryo development and post hatch performances. Since Japanese quails are often used as laboratory bird in most avian research, it becomes necessary to evaluate blood profiles of Japanese quails subjected to high incubation temperature. At 6 weeks of age, 8 birds in 1:1 sex ratio were randomly picked from each treatment designated as T1 (Control/38°C), T2 (36°C), T3 (37°C), T4 (38°C), T5 (39°C) and T6 (40°C) except in T2 and T5 were 1 female and 6 birds (2 male:1 female ratio) were the only survivors. Blood samples were collected in bottles with and without EDTA and were processed for haematological and biochemical parameters. Results indicated that PCV values were not statistically different (P>0.05) in T5 (45.2±3.0%) and T6 (44.8±3.69%), but were significantly different (P0.05) among the treatments. It was observed that some of these values were similar to 27 – 42% PCV and 2.2 – 4.0 X 1012/L RBC reported as normal values in healthy avian species. Similarly, the recorded total protein (26.7±2.64 – 53.4±0.0g/l), albumin (13.9±3.03 – 17.5±0.0g/l), creatinine (39.3±0.0 – 54.3±15.7Umol/l), alanine aminotransferase (6.0±0.0 – 26.6±16.7U/l) and cholesterol (153.9±23.5 – 200.1±0.0Mg/dl) values were within 100 – 300mg (cholesterol) and others reported in healthy avian species. Since high incubation temperature did not seemingly elicit physiological responses in Japanese quail chicks, it may be used to improve thermotolerance in avian species in order to withstand prevailing heat wave in the world.

Appendix 17: Presentation of some of the experimental results at the 43rd Annual Conference of the Nigerian Society for Animal Production, held at the Federal University of Technology, Owerri, Imo State, Nigeria in March, 2018

Paper 6: Embryo physiological status, chick yield and hatchability of Japanese quail (*Coturnix coturnix japonica*) eggs incubated in fabricated electric incubators at different temperatures. *Proceedings 43rd Ann. Conf., NSAP*, FUT Owerri. Pp. 315 – 318.

APRW -56

Embryo Physiological Status, Chick Yield and Hatchability of Japanese Quail (*Coturnix coturnix japonica*) Eggs Incubated in Fabricated Electric Incubators at Different Temperatures

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Abstract

Five electric incubators were fabricated and set at 36°C, 37°C, 38°C, 39°C and 40°C representing the treatments. A total of 300 hundred Japanese quail eggs were divided into 60 each per incubator and embryo physiological status, chick yield and hatchability were monitored. It was observed that the fabricated incubators were seemingly effective but could not retain heat and moisture, possibly because lagging materials were not used. This probably resulted in no hatching among the eggs set at 36°C,even when the egg fertility was between 58.3 and 80.0%.Therefore, the efficiency of these fabricated incubators may depend on constant electricity supply. Meanwhile, eggs incubated at 37 to 40°Chatched but the highest hatchability (66.7%) was recorded in those incubated at 39°C. In a similar trend, the chick yield was best (55.8g) in chicks hatched at 39°C, thus 39°C could be recommended for quail chick production.

Keywords: Japanese quail, electric incubators, egg

Introduction

Poultry eggs incubation temperature and relative humidity have been reported to affect embryo development, hatchability and post-hatch performance (Noiva *et al.*, 2014; Deeming and Ferguson, 1991). Essentially, inconsistent reports on incubation temperature for quail eggs ranging from 37.2 – 39.4°C (ECQ, 2015, Pam, 2015; Musa *et al.*, 2007) may confuse and discourage prospective farmers. Quail hens in domestication do not sit on eggs and hatch them therefore commercial production would require artificial incubation that simulates near perfect natural phenomenon.

Hence, this study was targeted at determining the optimum incubation temperature for efficient quail productivity.

Materials and Methods

A total of three hundred Japanese quail eggs were collected from a clutch laid on a day, allowed to cool at room temperature for 24 hrs and were fumigated with 30 ml formalin + 10 g $KMnO_4$ at 26.5°C in a fumigation box for 30 minutes. Five locally fabricated electric incubators with capacity of about 1,500 quail eggs per incubator were used in this study. Water was provided in plastic trays in each incubator and refilled regularly in order to maintain the required relative humidity. The incubators were test-run for 7 days and the temperature set at 36°C, 37°C, 38°C, 39°C and 40°C respectively, representing the treatments and in each treatment 60eggs were set.

The temperature and relative humidity were monitored in the morning, afternoon and evening using a hydro-thermometer (HTC-2) to obtain the mean, minimum and maximum values during the study. Egg and chick weight were measured using sensitive weighing balance (KERRO, Taiwan, Electronic Compact Scale, BL5002) and incubation period was taken as the period between day of setting the eggs in the incubators and emergence of chicks. The chick yield was estimated by dividing chick weight by egg weight multiplied by 100%. Hatchability was determined by dividing the number of chicks hatched by the number of fertile eggs incubated multiplied by 100%.Egg fertility was evaluated by dividing the number of fertile eggs by the number of eggs incubated multiplied by 100% (Adeyina *et al.*, 2015; Aviagen, 2015; Adeyanju *et al.*, 2014). At the end of the incubation period, all the unhatched eggs were carefully windowed on day 23 while still in the incubators, to check for egg or embryo physiological status.

All the data collected were subjected to simple descriptive statistics and analysis of variance according to SPSS (2008).

Results and Discussion

Table 1 shows the temperature and relative humidity values recorded during Japanese quail eggs incubation. While the lowest incubation temperature values varied from 35.5°C – 39.3°C, the maximum were between 36.2 and 40.0°C across the treatments. While the ambient temperature ranged from 27.3 to 29.4°C, incubation relative humidity (28.4 – 30.2%), the ambient relative humidity varied from 10.3 to 21.5%. These values were similar to a range of 34 to 41°C used in quail eggs incubation study, but37°C and 38°C were recommended as the most suitable temperatures (Romao *et al.*, 2009). Although, eggs set at 34°C did not hatch in that study, some chicks hatched at 35°Cwhereas, eggs incubated at 36°C in the present study did not hatch probably due to inability of the incubator to retain heat and moisture hence constant power supply may be necessary.

Table 1: Temperature and relative humidity conditions during Japanese quail eggs incubation

Parameter	Treatment	Mean	Minimum value	Maximum value
Incubation temperature (°C)				
	36	35.9	35.5	36.2
	37	36.9	36.5	37.3
	38	37.9	37.5	38.3
	39	38.8	38.4	39.2
	40	39.6	39.3	40.0
Ambient temperature (°C)				
	36	27.9	27.3	28.6
	37	27.7	27.0	28.3
	38	28.3	27.7	29.0
	39	28.8	28.1	29.4
	40	28.1	27.5	28.8
Incubation relative humidity (%)				
	36	29.2	28.6	29.9
	37	29.1	28.4	29.7
	38	29.6	28.9	30.2
	39	29.6	28.9	30.2
	40	29.6	28.9	30.2
Ambient relative humidity (%)				
	36	10.9	10.3	11.4
	37	13.0	12.4	13.5
	38	21.0	20.4	21.5
	39	17.1	16.5	17.6
	40	20.7	20.1	21.2

Embryo physiological status and hatchability of Japanese quail eggs incubated at different temperatures are presented in table 2. There were significant differences ($p<0.05$) in all the parameters measured across the treatments. Egg fertility value varied from 58.3 – 80.0% with hatchability value as high as 66.7% among the eggs incubated at 39.0°C yet, there was no hatching in eggs incubated at 36°C. This observation agrees with the findings of Romao *et al.* (2009) who reported 46.0 to 70.8% hatchability in quail eggs. It should be noted that quail egg fertility does not depend on the incubation process rather it is determined by reproductive efficacy of the hens and cocks in the same flock. Incubation period ranged from 18 to 20 days, contrary to 16 to 18 days observed by Sellier *et al.* (2006) probably due to inadequate heat circulation in the incubators during the hatching phase. When the unhatched eggs were windowed on day 22, it was observed that some were infertile (1.0 – 14.0) and for others, it was unsure whether fertile or infertile because the yolk were caked (6.0 – 15.0). Meanwhile, embryo development was obvious in some other eggs but they died at the early, late and fully grown stages, while others reached pipping stage but were not able to emerge as chicks. These abnormalities were also observed by Romao *et al.* (2009), who described the conditions as infertile, early embryo death, intermediate embryo death, late embryo death and pipped egg with dead embryo.

Expressed in table 3 is the quail chick yield hatched at different temperatures. There were no statistical differences ($p>0.05$) among the eggs incubated at 37 – 40°C in all the parameters across the treatments but they

were significantly different (P<0.05) from those incubated at 36°C. The egg weight varied from 9.94 – 10.0 g similar to 8.0 – 11.0 g reported by Idahor *et al.* (2015).

Table 2: Embryo physiological status and hatchability of Japanese quail eggs incubated at different temperatures

	Treatment					
Parameters	36°C	37°C	38°C	39°C	40.0°C	±STD
Incubation period (days)	-	20	19	18	18	-
Infertile eggs	14.0^{a}	10.0^{b}	6.0^{c}	7.0^{c}	1.0^{d}	1.0
Fertile eggs	40.0^{d}	35.0^{e}	44.0^{b}	42.0^{c}	48.0^{a}	1.0
Fully grown and alive embryo	17.0^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	1.0
Early dead embryo	20.0^{a}	5.0^{b}	4.0^{b}	5.0^{b}	5.0^{b}	1.0
Late dead embryo	3.0^{e}	14.0^{c}	20.0^{b}	14.0^{d}	33.0^{a}	1.0
Caked eggs	6.0^{c}	15.0^{a}	7.0^{b}	6.0^{c}	7.0^{b}	1.0
Dead at pipping	0.0^{b}	0.0^{b}	2.0^{a}	0.0^{b}	1.0^{ab}	1.0
Alive at pipping$^{\phi}$	0.0^{b}	2.0^{a}	0.0^{b}	0.0^{b}	0.0^{b}	1.0
Hatched eggs	0.0^{d}	14.0^{c}	21.0^{b}	28.0^{a}	13.0^{c}	1.0
Fertility (%)	66.7^{e}	58.3^{d}	73.3^{b}	70.0^{c}	80.0^{a}	1.0
Hatchability (%)	0.0^{e}	40.0^{c}	47.7^{b}	66.7^{a}	27.1^{d}	1.0

^{a,b,c,d,e}: Means with different superscripts differ significantly (p<0.05), $^{\phi}$: Alive at pipping but not able to emerge as chick, STD: Standard deviation.

Table 3: Yield of Japanese quail chicks hatched at different temperatures

	Treatment (±STD)				
Parameters	36°C	37°C	38°C	39°C	40.0°C
Egg weight (g)	10.0±1.0	9.99±0.26	9.97±1.0	9.94±1.0	9.97±1.0
Chick weight (g)	0.0 ± 0.0^{b}	5.94 ± 0.53^{a}	4.96 ± 1.0^{a}	5.55 ± 1.0^{a}	5.09 ± 1.0^{a}
Chick yield (%)	0.0 ± 0.0^{b}	45.5 ± 34.0^{a}	49.7 ± 1.0^{a}	55.8 ± 1.0^{a}	51.1 ± 1.0^{a}

STD: Standard deviation.

Chick weight value was highest (5.94g) among those hatched at 37°C followed by 39°C (5.55g), 40°C (5.09g) and 38°C (4.96g) in that order. Nevertheless, the chick yield was best (55.8%) in chicks hatched at 39°C compared to as low as 45.5g recorded in the chicks hatched at 37°C. These values were lower than 67% (low), 67 – 68% (ideal) and >68% (high) reported by Aviagen (2015).

Conclusion

The fabricated incubators were seemingly effective in maintaining the required temperature and relative humidity for quail eggs incubation. But they were unable to retain heat and moisture, possibly because lagging materials were not used, thus constant electricity supply may be needed. Though many of the eggs incubated were fertile, only a few hatched and others died during embryo development. This probably suggested that the incubators were inefficient hence, required modifications before use at full scale. However, hatchability and chick yield values seemed to be best in incubator set at 39°C therefore, it could be recommended for quail chick production.

Acknowledgement

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Paper 7: Embryo developmental stages in Japanese quail (*Coturnix coturnix japonica*) eggs incubated at 38°C. *Proceedings 43rd Ann. Conf. NSAP, FUT Owerri*. Pp. 1433 – 1436.

Proc. 43rd Annual Conference of the Nigerian Society for Animal Production, March 18th – 22nd 2018, FUT Owerri

POS -28

Embryo Developmental Stages in Japanese Quail (*Coturnix coturnix japonica*) Eggs Incubated at 38°C

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Abstract

A total of twenty seven Japanese quail eggs were incubated at 38°C and were used in monitoring daily egg weight loss as well as gradual embryo metamorphosis into chick. It was observed that the egg weight value before incubation, varied from 8.73 - 12.29g and during incubation between 7.92 and 12.1g, resulting in egg weight loss ranging from 0.09 - 2.55g. Meanwhile, the embryo weight was apparently independent of initial egg weight. Growth on the yolk surface, formation of blood vessels, heart, eye ball and embryo structure were observed on days 1 to 4. Increase in embryo size was evident on day 6 and plumage strips appeared on day 13. On days 14 to 17, the embryo appeared fully grown with the yolk almost used up and the unabsorbed portion gradually withdrawn into the body and on day 18, chicks pipped and emerged. These observations may be useful in providing the required information in *in ovo* study, egg fertility test and quail embryo anatomical study.

Keywords: Quail embryo metamorphosis, incubation period.

Introduction

Domestication of quails started at about the eleventh century, when the Japanese Emperor was cured of tuberculosis after eating quail (Howes, 1964). Today, there are quail strains like *Coturnix coturnix, Coturnix coturnix coturnix and Coturnix coturnix japonica* (Fah 2009). According to Fah (2009), the Japanese quail (*Coturnix coturnix japonica*) originated from Eastern Asia. In 1992, the Asiatic or Japanese quail was introduced into Nigeria by National Veterinary Research Institute, Vom, Plateau State (Musa *et al*., 2007). Since then, a lot of research findings have been reported on quail eggs as well as nutritional requirements and management practices (Adeyina *et al*., 2015; Makinde *et al*., 2015). Yet, little is known about embryo developmental stages hence, this study may be useful in describing the anatomical structure of quail embryo and estimating when an embryo probably stopped developing following unhatched egg break out. The knowledge of quail chick embryo development becomes imperative due to the difficulties in candling incubated quail eggs, necessitating breakout of unhatched eggs to ascertain egg fertility.

Essentially, this study may serve as a guide *in ovo* study, pharmacological operations and introduction of gene expression vectors into specific cell populations.

Materials and Methods

A total of 27 eggs were collected from laying Japanese quails, weighed using digital scale, labelled using permanent ink marker on the shell, stored at room temperature for 24hrs and incubated at 38°C. An egg was removed from the incubator daily at random, reweighed to obtain weight loss and carefully windowed at the broader end. Photographs of the embryo developmental stages were taken using digital camera and eggs without noticeable growth (probably infertile) were discarded. The embryo weight was collected using digital scale on day 14, when the eggs were transferred to the hatchery component of the same incubator till chick emergence.

Results and Discussion

Table 1 shows weight loss of Japanese quail eggs used in the study. The egg windowed on day 12 was the heaviest (12.29 g) followed by those on days 1, 16, 15, 6, 13, 12 and 8 that ranged from 11.57 to 11.17 g. Eggs with the least weight (8.73 and 8.92 g) were used on days 9 and 3. These values were within 8.03 to 11.36 g categorised as small, medium and large eggs by Adeyanju *et al.* (2014).

The eggs used in this study were seemingly moderate in size thus probably measured up to the prerequisite of hatchability. The egg weight loss value was increasing by day, a trend similar to the report of El-Full and Mahmoud (2012) on stored un-incubated quail eggs, except on days 8, 12, 14 and 16 when the values

were apparently distorted. Though the embryo weight value also decreased by day, it did not follow a particular trend which possibly expressed independent relationship between egg weight and chick weight when considering chick yield. This observation perhaps indicated normal metabolic use up of the nutrients contained in the yolk and albumen during embryo development. Therefore, it somewhat buttressed the speculations that egg weight loss is an important parameter for incubation (Adeyanju *et al.*, 2014) and could be used to estimate vital gas exchange (Paganelli *et al.*, 1978; Rahn *et al.*, 1979).

Table 1: Weight loss of Japanese quail eggs incubated at 38°C

Incubation Day	Initial egg weight (g)	Final egg weight (g)	Egg weight Loss (g)	Embryo weight (g)
1	11.71	11.62	0.09	ND
2	12.29	12.1	0.19	ND
3	8.92	8.7	0.22	ND
4	9.67	9.29	0.38	ND
5	9.69	9.26	0.43	ND
6	11.44	10.76	0.68	ND
7	10.56	9.85	0.71	ND
8	11.17	10.59	0.58	ND
9	8.73	7.92	0.81	ND
10	10.34	9.22	1.12	ND
11	10.62	9.39	1.23	ND
12	11.32	10.59	0.73	ND
13	11.35	9.93	1.42	ND
14	9.14	8.3	0.84	3.35
15	11.50	9.53	1.97	3.79
16	11.57	10.36	1.21	7.31
17	10.73	9.11	1.62	5.88
18	9.20	6.65	2.55	Hatching

ND: Not determined

Plates 1 to 18 present the daily embryo development of Japanese quail. On days 1 and 2, growth was observed on the yolk surface and on day 3 network of blood vessels and heart beat was noticeable. On day 4, growth of eye ball was observed and the embryo structure was formed. Gradual increase in the embryo size was noticeable on day 6 with plumage strips appearing on day 13. Growth of feathers, eyes, beak, wings and legs became obvious on day 14 with allantois attaching the yolk to the embryo.

Plate 1: Day 1

Plate 2: Day 2

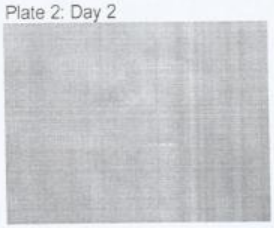

Plate 3: Day 3

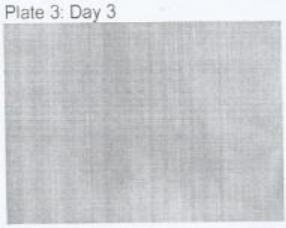

Plate 4: Day 4

Plate 5: Day 5

Plate 6: Day 6

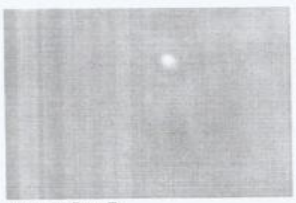
Plate 7: Day 7


Plate 8: Day 8


Plate 9: Day 9


Plate 10: Day 10


Plate 11: Day 11


Plate 12: Day 12


Plate 13: Day 13

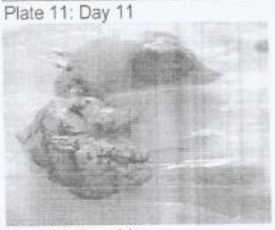
Plate 14: Day 14

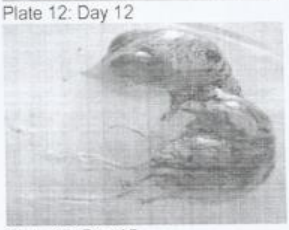
Plate 15: Day 15


Plate 16: Day 16

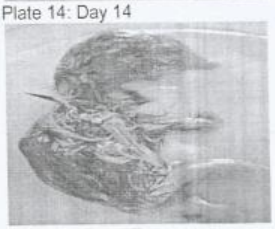
Plate 17: Day 17

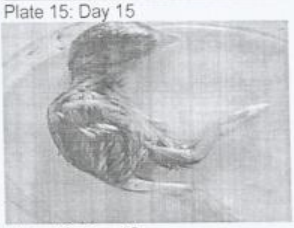
Plate 18: Day 18

Plates 1 – 18: Daily development of Japanese quail embryo incubated at 38°C

On days 15, 16 and 17, the embryo appeared fully grown though with attached unabsorbed yolk which was observed to be gradually reducing probably because it was being withdrawn into the body. Chick emergence

was recorded on day 18 corroborating the report of Sellier *et al.* (2006) that quail incubation period varied from 16 to 18 days. The observed disparity possibly depends on the incubation conditions. The observed embryo developmental stages were similar to those reported by Ramteke *et al.* (2013) in quails.

Conclusion

The incubated eggs were seemingly moderate in size, trend of egg weight loss was almost linear, daily embryo developmental stages were gradual and the incubation period was normal. Therefore, this observation could be useful in hatchery operation, because it apparently provides the required information for anatomical description of quail embryo, better egg fertility test and *in ovo* study.

Acknowledgement

The Authors kindly appreciate TETFUND Abuja for providing Staff Training Fund used in this study, NVRI Vom for providing the experimental facilities and the University of Ibadan, Department of Animal Science for collaborative efforts geared towards manpower development.

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