ISSN 1682-8356
ansinet.com/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

Science Alert
scialert.net

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2019.626.633

Research Article
Effect of High Temperature During First and Second Halves of Incubation on Layer Chicken Embryo Physiology

[1,2]D. Nideou, [1]O. N'nanlé, [1]Y.A.E. Kouame, [3]C. Chrysostome, [1]M. Gbeassor, [4]E. Decuypere and [1]K. Tona

[1]Centre d'Excellence Régional en Sciences Aviaires (CERSA), Université de Lomé. B.P: 1515 Lome, Togo
[2]Département des Sciences Techniques d'Elevage, Institut National Supérieur des Sciences et Techniques d'Abéché, BP: 130 Abéché, Chad
[3]Laboratoire de recherche avicole et de zooéconomie, Faculty of Agricultural Sciences, University of Abomey-Calavi, Cotonou, Benin
[4]Laboratory for Physiology, Immunology and Genetics of Domestic Animals, Department of Biosystems, K.U. Leuven, Belgium

Abstract

Background and Objective: High incubation temperatures accelerate embryonic growth or increase embryonic mortality depending on incubation stage, duration of exposure. The purpose of this study was to evaluate the influence of high incubation temperature on layer chicken embryo. **Materials and Methods:** A total of 1200 hatching eggs were studied in two different experiments and divided into two groups, control and high temperature group. Eggs of control group were incubated at standard incubation temperature of 37.6°C. Eggs of high temperature groups were incubated at 38.6°C during the first 10 days for experiment 1 or 18 days for experiment 2. During incubation samples of eggs were used to determine the weights of remaining albumen, embryo and yolk sac. Also, hatching events and hatch were monitored every two hours between 19 and 21 day of incubation. Blood samples were collected at 18 day-old embryo, internal pipping stage and at hatch for tri-iodothyronine, thyroxine and corticosterone level determinations. **Results:** Results suggested that, the embryos incubated at high temperature during the first 10 days used albumen more rapidly with no effect on hatchability. On contrary, embryos incubated at high temperature during the first 18 days reduced significantly albumen utilization after days 13 of incubation with negative effect on hatchability ($p<0.05$). In addition, high incubation temperature decreased yolk sac weight compared to control groups ($p<0.05$). In experiment 1, the highest T3 and T4 levels were obtained at internal pipping stage. **Conclusion:** A temperature increased by 1°C of the standard during the 18 days of incubation is detrimental for embryo development and hatching performance.

Key words: Blood parameters, embryonic growth, embryonic mortality, hatching eggs, incubation temperature

Citation: D. Nideou, O. N'nanlé, Y.A.E. Kouame, C. Chrysostome, M. Gbeassor, E. Decuypere and K. Tona, 2019. Effect of high temperature during first and second halves of incubation on layer chicken embryo physiology. Int. J. Poult. Sci., 18: 626-633.

Corresponding Author: D. Nideou, Centre d'Excellence Régional en Sciences Aviaires (CERSA), Université de Lomé. B.P: 1515 Lome, Togo
Tel : (+235) 66 25 12 90
Département des Sciences Techniques d'Elevage, Institut National Supérieur des Sciences et Techniques d'Abéché, BP: 130 Abéché, Chad
Tel : (+228) 22 40 60 58

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Irrespective of hatching egg characteristics, incubator temperature is suggested to be the most important factor that influences chick embryo development[1] besides relative humidity, ventilation and turning. Ancel *et al.*[2] reported that the optimum temperature for chicken embryo development is between 37.5-38.0°C. Moreover, it is well known that chicken embryo is more sensitive to higher than to lower temperature and the sensitivity to hyperthermy increases as the embryo age increases[3,4]. In addition, high incubation temperatures accelerate embryonic growth, leading to a shorter duration of incubation[5] but may increase embryonic mortality[6,7]depending on incubation stage, duration of exposure and how high the temperature is. Line and strain differences also affect the tolerance to variations in the standard temperature during incubation[8,9] . On the basis of the previous studies[8,9], it can be hypothesized that selection in domestic chicken for egg production compared with meat production can alter the optimal range of incubation temperature. Moreover, intensive and focused selection over many years has significantly improved broiler growth rate as well as early maturity and high egg production rate of layer chickens. Long-term selection for production traits has also led to an increase in the ratio of demand organs, such as skeletal muscles and fat mass, to supply organs, such as heart, lungs, spleen and liver in broilers Ricklefs[10], Nestor *et al.*[11] and Gavinand Mc Devitt[12] suggesting adequate changes in incubation conditions in order to meet embryo needs. Indeed, many studies[13-17] have focused on intermittent temperature manipulations during late incubation, ranging from 3-12 h day^{-1}. All these studies focused on broiler breeder hatching eggs. Since embryo sensitivity to high incubation temperature is related to its development stage, the present study was designed to investigate the effects of a temperature of 1°C higher than standard incubation temperature during the first 10 and 18 days of incubation on layer-type embryo development and hatching process.

MATERIALS AND METHODS

Experimental design: A total of 2,400 hatchable eggs of Isa Brown layer breeders were provided by Belgabroed (Merksplas, Belgium) for the experiment. The 2,400 eggs were grouped into two different experiments of 1,200 eggs each. For each experiment, the eggs were divided into two groups, control group (Cont) and high temperature group of 600 eggs each. Prior to set for incubation, the eggs were numbered, weighed and divided into 4 replicates of 150 eggs each. Eggs of control group were incubated at standard incubation temperature of 37.6°C throughout incubation time. Eggs of high temperature groups were incubated at 38.6°C during the first 10 day (Temp10) in experiment 1 and 18 days (Temp18) in experiment 2. At day 18 of incubation, the eggs were candled and those with evidence of living embryos were transferred to hatching baskets. On sampling days of incubation, 12 eggs were collected from each replicate opened to extract and weigh the remaining albumen, embryo and yolk sac. Also, hatching events such as internal pipping (IP), external pipping (EP) and hatch were monitored every two hours between 462 and 516 h of incubation. At the end of incubation, all unhatched eggs were broken and classified as "fertile" or infertile. For fertile eggs, dead embryos were classified as early dead (from setting to 7th day of incubation) or late dead (8th day to end of incubation). Blood samples were collected at embryonic day (ED) 18, IP stage and at hatch for tri-iodothyronine (T3), thyroxine (T4) and corticosterone level determinations.

Remaining albumen, embryo and yolk sac weight: From day 11-17 of incubation, samples of 12 eggs per treatment with evidence of living embryo were broken from the small end in order to extract and weigh remaining albumen and embryo every two days. The eggs sampled at day 17 were also used to record yolk sac weight. Samples of 12 eggs were also opened at day 18 and at IP stage to determine yolk sac and embryo weights.

Hatching process: Between 462 and 516 h of incubation, the transferred eggs were checked individually every 2 h to monitor the hatching events. During this period, the times of the occurrence of IP, EP and emergence from individual egg were recorded. These data were used to calculate the total incubation duration up to IP, EP and hatch. Durations of IP (incubation time up to EP-incubation time up IP), EP (incubation time up to hatch-incubation time up EP) and hatch (incubation time up to hatch-incubation time up IP) events were calculated. After 516 h of incubation, the number of hatched chicks were recorded. Eggs that failed to hatch were broken to distinguish infertile eggs from dead in shell embryos. These data were used to calculate hatchability in relation to the number of fertile eggs.

Blood parameters analysis: Blood samples were collected from 35 eggs from each treatment group at ED 18, IP and from chicks at hatch for T3, T4 and corticosterone determinations.

The T3 and T4 concentrations were measured in plasma samples by RIA as described previously[18,19]. Intra-assay coefficients of variation were 4.5 and 5.4% for T3 and T4, respectively. Antisera and T3 and T4 standards were purchased (Byk-Belga, Belgium). Corticosterone was measured using a commercially available double antibody RIA kit from IDS Ltd (Boldon, England). All samples were run in the same assay in order to avoid inter-assay variability[20,21].

Statistical analysis: The general linear models procedure was used to analyze remaining albumen, embryo and yolk sac weight; plasma T3, T4 and corticosterone concentrations; IP and EP duration and hatching time in relation to temperature treatment. The model was as follows:

$$Y_i = \mu+\alpha_i+\varepsilon_i$$

where, Yi is remaining albumen, embryo and yolk sac weight; plasma T3, T4, corticosterone, incubation time up to IP, EP and hatch, or duration of IP, EP and hatch of egg from temperature i, μ is overall mean, αi is main effect of temperature i and εi is random error term from temperature treatment.

Embryonic mortality and hatchability were analysed using a proc logistic procedure. A degree of significance of 5% was used.

RESULTS

Effect of incubation temperature on albumen utilization: In both experiments, remaining albumen weights decreased rapidly with increasing incubation duration irrespective of temperature treatments as indicated in Fig. 1 ($p<0.01$). In experiment 1, remaining albumen weight was similar at day 11 and at day 17 for both treatments. Between 13 and 15 day, albumen weight was lower in temperature treatment group than in control group ($p<0.05$). In experiment 2, albumen weight did not follow the same trend. Albumen weight for temperature group was similar at 11 day, lower at 13 ($p<0.05$) then higher from 15 day onward ($p<0.05$) compared to the control group.

Effect of incubation temperature on yolk sac weight: The result show that, high incubation temperature decreased yolk sac weight compared to control groups ($p<0.05$). Differences in yolk sac weight due to high incubation temperature disappeared at IP stage. Moreover, the decrease in yolk sac weight due to high temperature compared to control group was more pronounced in experiment 2 (Fig. 3b) than in experiment 1 (Fig. 3a). At IP stage yolk sac weight of temperature group was similar to that of control group in experiment 1 while it was lower in experiment 2 ($p<0.05$).

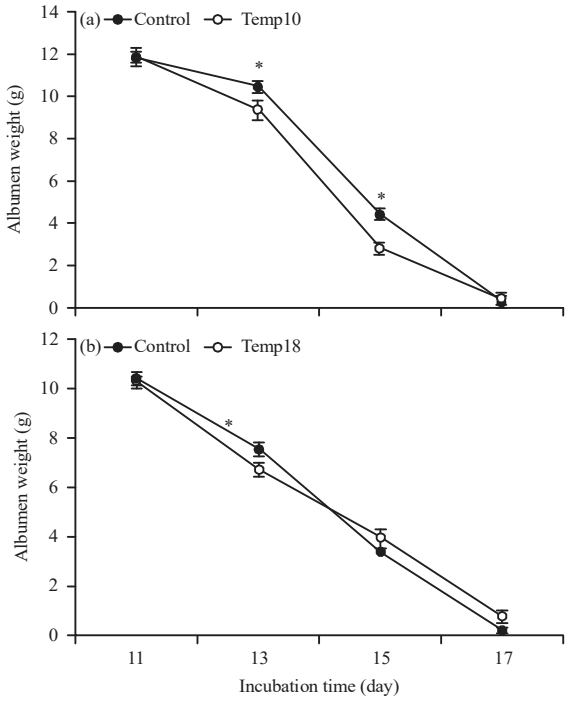

Fig. 1(a-b): Remaining albumen amount according to incubation time and treatment (a) Experiment 1 and (b) Experiment 2

At each developmental stage, *indicates difference between treatments ($p<0.05$)

Effects of high temperature on embryo weight: From ED11 to IP stage, embryo weight increased with incubation time irrespective of treatments as shown in Fig. 3a and b respectively for experiment 1 and 2. In both experiments, embryo weights were higher in temperature groups between day 11 and day 15 of incubation ($p<0.05$). From 17 day-old embryo onward, embryo weights of control groups were similar to those of temperature treatments in both experiments (Fig. 2a and b).

Effects of high temperature on hatching events and hatchability: The high temperature during incubation shortened the incubation duration ($p<0.01$). In experiment 1, the internal pipping stage (IP), external pipping stage (EP) or hatch occurred respectively 12, 14 or 9 h earlier ($p<0.05$) in temperature group compared to the control group. The same trend was observed in experiment 2.

In experiment 1, hatching performance was not affected by incubation temperature treatment (Table 1). But in experiment 2, hatchability of temperature treatment group was lower and late mortality rate was higher than that of control group ($p<0.01$) while early mortality rate was not affected in both experiments (Table 1).

Effects of high temperature on T3, T4 and corticosterone levels: The result show that, in experiment 1, the highest T3 (Fig. 4a) and T4 (Fig. 5a) levels were obtained at IP stage ($p<0.05$). In experiment 2, the levels of T3 (Fig. 4b) in the high temperature group were higher than control group at IP stage but lower at hatch compared to control group ($p<0.05$). T4 levels (Fig. 5b) in the high temperature group were lower at hatch ($p<0.05$) but similar at IP compared to the control group. In experiment 1 temperature treatment during the first half of incubation did not significantly affect T3 and T4 levels at hatch. T3 levels at IP for temperature treatment group up to10 day of incubation was lower than that of control group

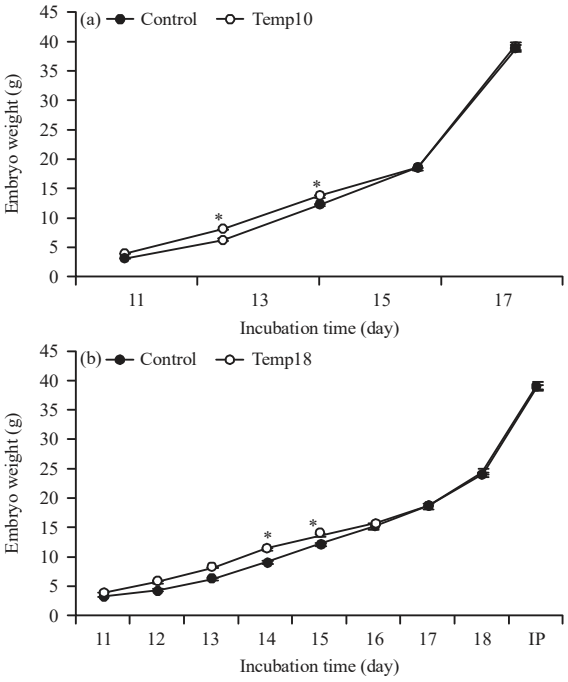

Fig. 2(a-b): Embryo weights according to incubation stage and treatment in experiment 1 (a) and experiment 2 (b)
At each developmental stage, *indicates difference between treatments ($p<.05$)

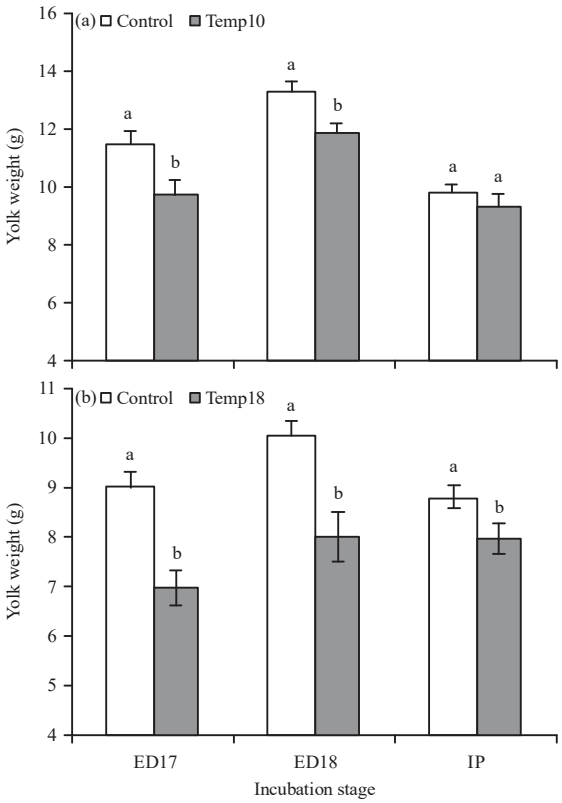

Fig. 3(a-b): Yolk sac weights according to incubation stage and treatment in experiment 1 (a) and experiment 2 (b)
At each developmental stage, data sharing no common letter are different ($p<.05$)

Table 1: Incubation duration up to IP, EP and hatch and hatching performance according to treatments

	Experiment 1		Experiment 2	
Hatching events	Control	Temp10	Control	Temp18
Incubation duration up to IP (h)	485.60±0.52^a	473.01±0.55^b	476.46±0.49^a	460.06±0.30^b
Incubation duration up to EP (h)	495.12±0.41^a	485.14±0.56^b	484.37±0.52^a	470.16±0.67^b
Incubation duration up to Hatch (h)	504.64±0.36^a	495.07±0.46^b	494.26±0.37^a	479.97±0.64^b
IP duration (h)	13.92±0.33^a	11.42±0.36^b	10.27±0.41	11.20±0.48
EP duration (h)	10.88±0,26	10.33±0,31	10.39±0.35^a	8.99±0.47^b
Hatch duration (h)	24.30±0.34^a	22.48±0.36^b	21.14±0.33^a	19.41±0.48^b
Hatchability	87.50^a	87.57^a	87.50^a	80.99^b
Early mortality	6.82^a	7.32^a	6.94^a	4.93^a
Late mortality	5.68^a	5.12^a	5.56^b	14.79^a

^{a,b}Within row and for each experiment, data sharing no common letter are different ($p<0.05$)

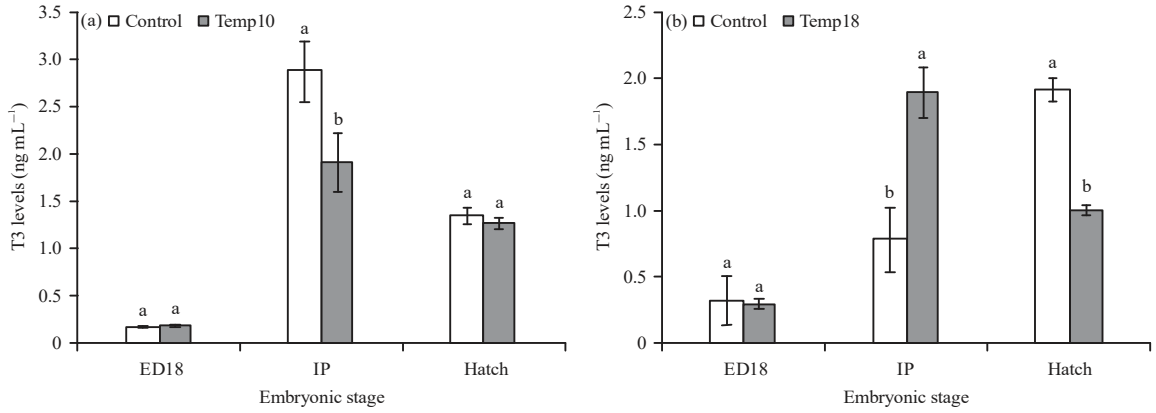

Fig. 4(a-b): Concentrations of T3 according to treatment in (a) Experiment 1 and (b) Experiment 2

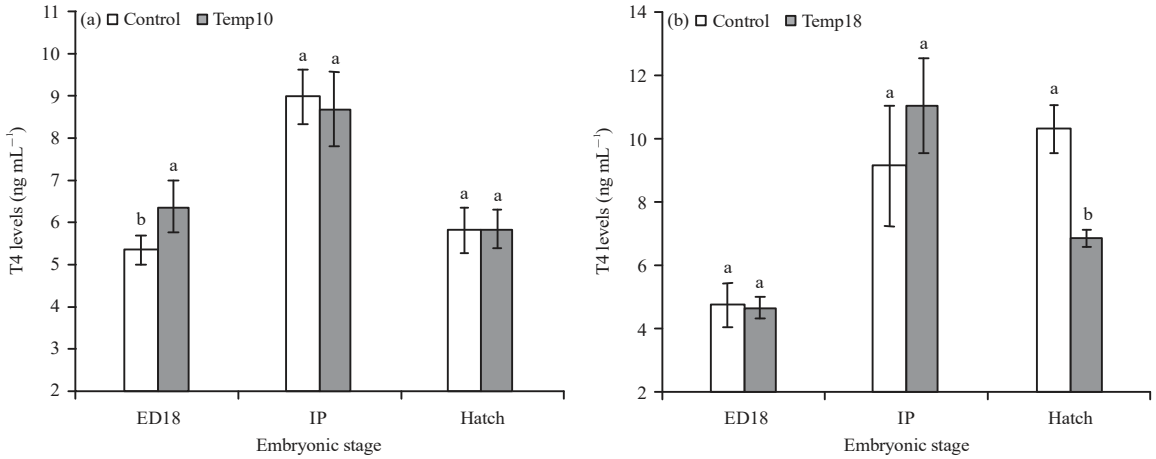

Fig. 5(a-b): Concentrations of T4 according to treatment in (a) Experiment 1 and (b) Experiment 2

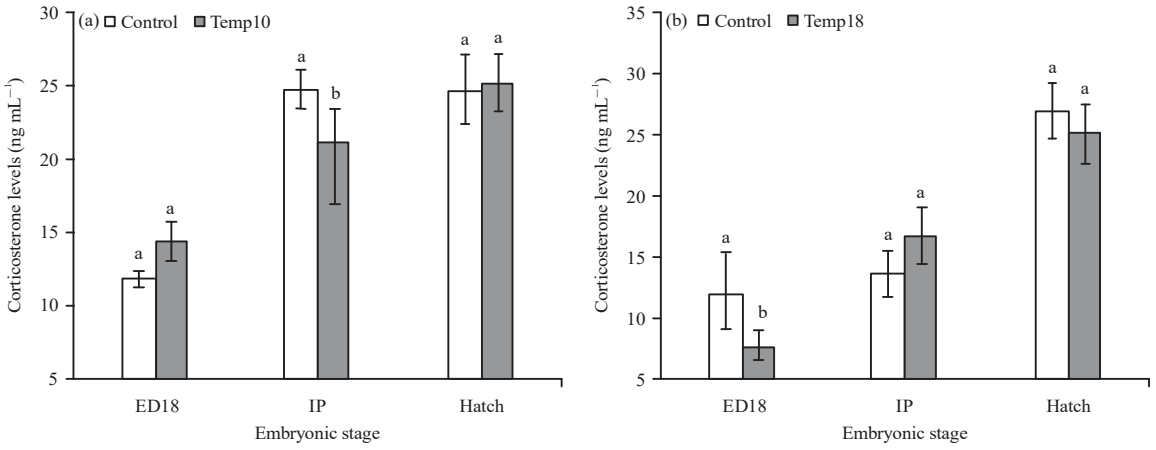

Fig. 6(a-b): Corticosterone levels according to treatment in (a) Experiment 1 and (b) Experiment 2

($p<0.05$). In experiment 1, corticosterone levels (Fig. 6a) were lower at ED18 ($p<0.05$) compared toIP but similar at hatch. At IP and hatch, high temperature during the first half of incubation did not affect significantly corticosterone levels compared to control group. In experiment 2, corticosterone levels (Fig. 6b) increased with developmental stage in both groups with significant lower levels at ED18 in high temperature group compared to the control ($p<0.05$).

DISCUSSION

This study clearly demonstrated the effects of high temperature increased by 1°C of the standard during the first and second half of incubation compared with standard incubation temperature on embryonic development, hatching process and hatchability. It was pointed out that exposure stage of layer breeders hatching eggs to high incubation temperature leads to differential responsiveness of the embryo.

Albumen utilization by developing embryo and growth rate were influenced by high temperature. The effects of high temperature however were related to the stages of the development of the embryo. High incubation temperature by 1°C during the first 10 days enhanced albumen utilization and more embryonic growth rate compared to those incubated at 37.5°C up to 18 d. It is general agreed that slight increase of temperature during the first week of incubation leads to short incubation duration[21,22]. This decrease of incubation duration may suggest that high incubation temperature during the first 10 days in this study improves embryo growth rate while a continuous higher incubation temperature of 1°C until 18 days slows down again developmental processes[22]. This study demonstrates that there is a natural process of albumen use that may be related to the growth of the embryo. During incubation, albumen proteins move into the amniotic fluid and in yolk and are absorbed by the embryo. Thus, level of incubation temperature seems to be an additional impetus to influence the timely use of the albumen. Our results on this aspect are consistent with those previously reported by Willemsen *et al.*[23]. Indeed, significant amount of remaining albumen and lower body weights of embryo from control group up to 16 days suggests that lower use of albumen may be the consequence of lower embryo growth rate. Even though until day 18 albumen was completely used, incubation durations were significantly shorter for eggs incubated at high temperature suggesting that high incubation temperature and its duration influence other internal factors besides albumen use. Hence, high temperature during the first 10 day resulted in increased use of the albumen and embryo growth.

The high temperature on its own may have a stimulatory effect on embryo growth rate but depending on developmental stage. Previous reports indicated that depending on the period and mainly the daily duration of temperature manipulation, hatchability can be decreased[14,24,25], increased[14,26], or not influenced[13,15,24,26,27,28]. These interactions between temperature treatments and incubation stages suggest that there are period during incubation when increasing the temperature by 1°C may be detrimental for the embryo. There was a significant amount of remaining albumen at day 17 of incubation together with lower hatchability and viability of embryos with high temperature up to 18 day of incubation. Higher temperature of 1°C during the plateau phase of embryo metabolism (O_2 and CO_2 exchange between day 14-15) and IP may exhaust embryonic resources (glucose e.g.). These may be detrimental for embryo development and thus, result in increased late embryo mortality, decreased hatchability and weaker chicks. In both experiments, embryos subjected to the high-temperature treatment consumed more yolk which can be partially explained by increased energy need of these embryos probably because of high metabolism as consequence of high temperature.

The temporal variations in T3 and T4 between IP, EP and hatch[29] together with momentaneous blood sampling at IP and hatch may explain discrepancies in T3 and T4 levels between both experiments as well as between control and temp18 group, because of shifts in timing of these events due to temperature treatment. The higher T3 in temp18 group in experiment 2 at IP may be related to such a shift in advancing pipping and hatching phenomena. Ockleford *et al.*[30] reported that advancing the hatching time results in an increase of T3 concentration in chicks. Since corticosterone is required for the peripheral conversion of T4-T3 during prenatal life[19,20], the significantly higher T3, T4 and corticosterone levels in chicks hatched from eggs incubated at standard incubation conditions suggest a continued peripheral conversion of T4 to T3, hence a possible higher metabolism post-hatch and a more robust day-old chick in the control group vs temp18.

CONCLUSION

It can be concluded that higher incubation temperature during the first 10 days of incubation enhances embryo growth and therefor reduces incubation time without detrimental effects on hatching performance. But, higher temperature during the first 18 days of incubation reduces incubation time with negative effects on hatching performance. It is recommended that incubation temperature be regulated according to the needs of the embryo to improve hatching performances. However, effects of incubation on post-hatch performance of layer-type chicks should be investigated.

SIGNIFICANCE STATEMENT

This study discovered the effects of temperature increase of 1°C higher than standard incubation temperature during the first 10 or 18 days of incubation on layer-type embryo development and hatching process. This is beneficial for

researchers to uncover the higher temperature requirement for embryo development and hatching performance. This study will help the researchers to uncover the consequences of 1°C (temperature) higher than standard incubation temperature during the first 10 or 18 days of incubation on embryo development and hatching performance that are yet to be discovered. Thus a new theory a detrimental and beneficial higher temperature duration for embryo development and hatching performance may be arrived at.

ACKNOWLEDGMENTS

This study was supported by World Bank grant IDA 5424 through the "Centre d'Excellence Régional sur les Sciences Aviaires (CERSA)" of University of Lomé (Togo).

REFERENCES

1. Meijerhof, R., 2000. Embryo temperature as a tool in the incubation process. Incubation and Fertility Research Group [WPSA Working Group 6 (Reproduction)], St. Edmand's Hall, Oxford, UK.
2. Ancel, A. and A.H.J. Visschedijk, 1993. Respiratory exchanges in the incubated egg of the domestic guinea fowl. Respir. Physiol., 91: 31-42.
3. Wilson, H.R., 1991. Physiological Requirements of the Developing Embryos. In: Temperature and Turning, Tullett, S.G. (Ed.). Avian Incubation but terworth-Heinemann, London, pp: 145-156.
4. French, N.A., 1997. Modeling incubation temperature: The effects of incubator design, embryonic development and egg size. Poult. Sci., 76: 124-133.
5. Kaplan, S., G.L. Kolesari and J.P. Bahr, 1978. Temperature dynamics of the fertile chicken egg. Am. J. Physiol., 234: 183-187.
6. Decuypere, E., 1979. Effects of incubation temperature patterns on morphological, physiological and reproduction criteria in Rhode Island Red birds. Agricultura, 27: 65-68.
7. Suarez, M.E., H.R. Wilson, B.N. McPherson, F.B. Mather and C.J. Wicox, 1996. Low temperature effects on embryonic development and hatch time. Poult. Sci., 75: 924-932.
8. Thompson, J.B., H.R. Wilson and R.A. Voitle, 1976. Influence of high temperature stress of 16-day embryos on subsequent hatchability. Poult. Sci., 55: 892-894.
9. Decuypere, E. and H. Michels, 1992. Incubation temperature as a management tool: A review. World's Poult. Sci. J., 48: 28-38.
10. Ricklefs, R.E., 1985. Modification of growth and development of muscles of poultry. Poult. Sci., 64: 1563-1576.
11. Nestor, K.E., Y.M. Saif, D.A. Emmerson and N.B. Anthony, 1995. The influence of genetic changes in body weight, egg production and body conformation on organ growth of Turkeys. Poult. Sci., 74: 601-611.
12. Gavin, A. and R.M. McDevitt, 1999. Intraspecific variation in muscle and organ growth in three strains of chicken with differential genetic selection for fast growth rate. Br. Poult. Sci., 10.1080/00071669986602
13. Moraes, V.B.M., R.D. Malheiros, V. Bruggeman, A. Collin and K. Tona *et al*., 2003. Effect of thermal conditioning during embryonic development on aspects of physiological response of broilers to heat stress. J. Thermal Biol., 28: 133-140.
14. Collin, A., M. Picard and S. Yahav, 2005. The effect of duration of thermal manipulation during broiler chick embryogenesis on body weight and body temperature of post-hatched chicks. Anim. Res., 54: 105-111.
15. Collin, A., C. Berri, S. Tesseraud, F.E.R. Rodon and S. Skiba-Cassy *et al*., 2007. Effects of thermal manipulation during early and late embryogenesis on thermotolerance and breast muscle characteristics in broiler chickens. Poult. Sci., 86: 795-800.
16. Yalcin, S., M. Cabuk, V. Bruggeman, E. Babacanoglu, J. Buyse, E. Decuypere and P.B. Siegel, 2008. Acclimation to heat during incubation: 3. Body weight, cloacal temperatures and blood acid-base balance in broilers exposed to daily high temperatures. Poult. Sci., 87: 2671-2677.
17. Tona, K., O. Onagbesan, V. Bruggeman, V. Collin and C. Berri *et al*., 2008. Effects of heat conditioning at day 16 to 18 of incubation or during early broiler rearing on embryo physiology, post-hatch growth performance and heat tolerance. Arch. Geflugelk, 72: 75-83.
18. Huybrechts, L.M., R. Michielsen, V.M. Darras, F.C. Buonomo, E.R. Kühn and E. Decuypere, 1989. Effect of the sex-linked dwarf gene on thyrotrophic and somatotrophic axes in the chick embryo. Reprod. Nutr. Dev., 29: 219-226.
19. Darras, V.M., T.J. Visser, L.R Berghman and E.R Kuhn, 1992. Ontogeny of type I and type III deiodinase activities in embryonic and posthatch chicks: Relationship with changes in plasma triiodothyronine and growth hormone levels. Comp. Biochem. Physiol. Part A: Physiol., 103: 131-136.
20. Decuypere, E., C.G. Scanes and E.R. Kühn, 1983. Effects of glucocorticoids on circulating concentrations of thyroxine (T_4) and triiodothyronine (T_3) and on peripheral monodeiodination in pre- and post-hatching chickens. Horm. Metab. Res., 15: 233-236.
21. Meeuwis, R., R. Michielsen, E. Decuypere and E.R. Kühn, 1989. Thyrotropic activity of the ovine corticotropin-releasing factor in the chick embryo. Gen. Comp. Endocrinol., 76: 357-363.
22. Tzschentke, B., 2007. Attainment of thermoregulation as affected by environmental factors. Poult. Sci., 86: 1025-1036.

23. Willemsen, H., B. Kamers, F. Dahlke, H. Han and Z. Song *et al*., 2010. High- and low-temperature manipulation during late incubation: Effects on embryonic development, the hatching process and metabolism in broilers. Poult. Sci., 89: 2678-2690.
24. Deeming, D.C., 1989. Characteristics of unturned eggs: Critical period, retarded embryonic growth and poor albumen utilisation. Br. Poult. Sci., 30: 239-249.
25. Joseph, N.S., A. Lourens and E.T. Moran Jr., 2006. The effects of suboptimal eggshell temperature during incubation on broiler chick quality, live performance and further processing yield. Poult. Sci., 85: 932-938.
26. Piestun, Y., D. Shinder, M. Ruzal, O. Halevy, J. Brake and S. Yahav, 2008. Thermal manipulations during broiler embryogenesis: Effect on the acquisition of thermotolerance. Poult. Sci., 87: 1516-1525.
27. Yahav, S., A. Collin, D. Shinder and M. Picard, 2004. Thermal manipulations during broiler chick embryogenesis: Effects of timing and temperature. Poult. Sci., 83: 1959-1963.
28. Yahav, S., R.S. Rath and D. Shinder, 2004. The effect of thermal manipulations during embryogenesis of broiler chicks (*Gallus domesticus*) on hatchability, body weight and thermoregulation after hatch. J. Therm. Biol., 29: 245-250.
29. Piestun, Y., D. Shinder, M. Ruzal, O. Halevy and S. Yahav, 2008. The effect of thermal manipulations during the development of the thyroid and adrenal axes on in-hatch and post-hatch thermoregulation. J. Therm. Biol., 33: 413-418.
30. Ockleford, E.M., T.F. Davison and M.A. Vince, 1983. Changes in plasma iodohormone concentrations during the day before hatching in *Gallus domesticus*. Comp. Biochem. Physiol. Part A: Physiol. 75: 139-140.