Effect of Thermal Manipulation during Embryogenesis on Hatching Traits

Al SARDARY, S. and MOHAMMAD, Sh.[1]
Animal Resource Department
Salahaddin University
Agriculture College-Erbil
IRAQ
[1]shireen.dhahir@yahoo.com

Abstract: - This study was conducted to investigate the effect of thermal manipulation (TM) during different embryogenesis stages of broiler breeder eggs at embryonic days (ED): T1 (1-5), T2 (8-12), T3 (14-18), T4 (19-21) and T5 was control (no TM) on hatching results and subsequent chick characteristics. Three thousand Ross-308 eggs from twenty seventh weeks old breeder flock were used. Eggs were randomly divided into five treatments with four replicates per each treatment (150 eggs / replicate). Two identical incubators were used. In the first incubator all eggs were incubated at 37.7 ºC and 60-65% relative humidity (RH). The eggs thermally treated eggs were transferred into the second incubator and was kept at 38.7 ˚C and 60-65% RH. After four (4) hours (hrs) of thermal treating during all embryonic stages of all TM groups, the eggs were returned to the first incubator, immediately. It was found that: thermal manipulation did not affect the hatchability percentage of total and fertile set eggs, normal birds percentage, chick quality and body temperature (°C) at hatch. Thermally manipulation improved significantly male percentages and male/female ratio mainly during ED 19-21 than control group. In conclusion, high incubation temperatures altered sex ratio in favor of more male percentage without affecting hatchability and chick quality.

Key-Words: - Thermal Manipulation, Embryogenesis, Hatching Traits, Sex Ratio.

1 Introduction

Temperature has been commonly acknowledged to be the most influential factor concerning embryonic growth and development during all stages of incubation from storage to incubation to hatching [1], because without the appropriate temperature, embryonic development will not occur and modest changes in temperature can significantly affect the chick that emerges [2].

Thermal manipulation (TM) can be defined as exposing embryos to high or low temperature during embryogenesis to increase their ability to adapt hot or cold environment by altering the thermotolerance of broiler chickens during life [3]. The main factors in the fine tuning of thermal changes during embryogenesis responsible for improvement of thermotolerance are the critical period of embryogenesis and the level and the duration of thermal changes [4].

2 Materials and Methods

This experiment was conducted in Evan private hatchery; which is located in Erbil-Kirkuk main road, during the period from 15/7 to 7/8/2013, to investigate the effect of thermal manipulation during embryogenesis on hatching traits.

Eggs were weighed as replicate groups by an electronic digital balance (accuracy of ± 5gm) before setting it in an incubator and weight ranges was between (53.8-54.5 g) with average of (53.9 g). Two identical Petersime incubators with a maximum capacity of 16800 eggs were used. The first incubator adjusted in a standard condition of incubation of 37.7 °C temperature and 60-65% RH and turned in angle 90° once per hour, while the second incubator adjusted at 38.8 °C temperature and 60-65% RH and turned 90°/hour, which was used for applying the thermal manipulation during different stages of embryogenesis. The eggs of replicates in all treatments transported from first to the second incubator and/or hatcher exposed to temperature for four hours from 8.00 to 12.00 during different embryogenesis stages of studied treatments, , the heat exposing on treatments were as follow:

T1, T2 and T3 represents the thermal manipulation started from ED (1-5), ED (8-12) and ED (14-18) of incubation periods, respectively.

T4: Thermal manipulation started from ED (19-21) of hatching period. After transporting eggs to the

hatching machine, when the eggs of this treatment exposed to 38.3, 38.1 and 38.0 °C during 19th, 20th and 21st days of hatching, respectively for four hours daily with 75-80% RH, till the time of hatching.

T5: Control: no TM= exposed to standard conditions (37.7 °C and 65-70% RH) in incubator and (37.3, 37.1 and 37.0 °C during 19th, 20th and 21st days of hatching, respectively with 75-80% RH) in hatchery.

On the day 21, chicks got out of the hatching trays and put in cartoon boxes assigned to replicates of each treatment, during picking chicks up chick quality was scored according to [5]. Number of hatched chicks including the normal, weak, abnormal and dead chicks was counted after hatch and all unhatched eggs were opened to identify visually in fertile eggs and to determine stage of embryonic mortality simultaneously according to replication. The percentages of hatchability (total and fertile), embryonic mortality (early, middle and late), culling and normal chicks were calculated. Hatchery sanitation was strictly maintained during the experimental period.

The experiments executed as a complete randomized design (CRD), all data analyzed using the [6]. Duncan`s multiple range tests were used to compare differences among treatment means [7].

3 Results and Discussion

The results of Table (1) displayed that from hour 478, 480 and 482 that T1 had significantly ($P \leq 0.05$) higher hatchability than T2 and T3. While at hours 480 and 482 it was noted that T2 had significantly ($P \leq 0.05$) higher hatchability than T3. At hour 484, T1, T4 and control group showed significantly ($P \leq 0.05$) higher hatchability than T3. At hour 490, control group had significantly ($P \leq 0.05$) higher hatchability than T2, T3 and T4, also it was clear that T4 had significantly ($P \leq 0.05$) higher hatchability than T3. At hour 496, T4 and control group had significantly ($P \leq 0.05$) higher hatchability than T1. From hour 502 till 528, the results showed that there were no significant differences among all thermally manipulated groups as compared to each other and compared to control group. Also the results in this table displayed that T3 and T4 reached peak of hatchability at hour 520 that were prior to T1, T2 and control group.

Results in T1 were agreed with those of [8, 9] who reported that elevating incubation temperature during first week of incubation caused a reduction in the duration and time of hatchability, which may be due to that a small rise in the temperature of incubation can have a significant and sustained effect on the motility of the embryos, might predict that increasing temperature might subtly reduce the length of incubation. Also it clear that the results of T2 and T3 were in agreement with [10, 11] who reported that a delay in hatch time as a result of warm stimulation (1-2˚C) above standard during, may be due to TM during the development of the thyroid axis (7-16 ED) appeared to have lowered the thyroid gland functional set-point in the embryo, thereby lowering the pre hatching metabolic rate which may lead to delay hatching rates. Another possible reasons that explain the delay in hatching may be due to reduced thyroid hormone levels; decreased liver glycogen reserves, resulting in a decrease in the availability of blood glucose during the hatching process, hyperglycemia, or both in the days before hatch might have been reflected in the slightly delayed start of the hatching process [12]. In contrast to the results of T4 were the results of [13].

The faster hatching rate observed in thermal manipulation during 1-5 days of incubation may be due to the fact that temperature manipulations during the first week of incubation will shorten the total duration of incubation according to Van't Hoff's rule regarding biochemical reactions [14]. However, stimuli applied in the last phase, tend to increase the incubation period due to the cancellation (over ruled) of the Van't Hoff reaction by physiological processes of the embryo.

Table (2) shows that there were no significant differences among all thermally manipulated groups as compared to each other and with control in egg weight, body temperature at hatch, hatchability of both total and fertile eggs and normal chicks percentages. As well as, result indicated that T1 resulted significantly ($P \leq 0.05$) higher culling chicks percentage compare to T4 and control group, but there were no significant differences among all thermally groups with control in early and intermediate embryonic mortality. While control group had significantly ($P \leq 0.05$) lower late embryonic mortality percentage than T1 and T4. As well as, control group had significantly ($P \leq 0.05$) lower total embryonic mortality percentage than T1 and T2, also T2 had significantly ($P \leq 0.05$) lower total embryonic mortality percentage than T1.

The results of body temperature at hatch were in agreement with the results of [15, 16, 17]. In contrast [18, 19] were disagreed with the results of body temperature at hatch.

Regarding hatchability (total and fertile) these results were in agreement with those of [9, 13, 16, 18, 19, 20, 21] the non significant hatchability observed in thermally manipulated broilers may be due to the fact that related to the level of increase in temperature and exposure time [22]. In contrast, the results of [17, 20, 23] revealed that thermal manipulation of chicken

eggs of 1°C above standard during embryogenesis affect hatchability significantly.
The results of [9, 20, 24, 25] were in agreement with results of early and intermediate embryonic mortality percentage. In contrast [15, 26, 27] resulted that increasing 1-2 C during 16-18 days of incubation, increased late embryonic mortality compare to control. While [21, 12] were confirmed T3 in late embryos dead. Whereas [25, 28] these results was in contrast with T4 in late embryos dead rate.
Increased early and late embryonic mortality may be related to high incubation temperature either cause embryos grow quickly and their morphological changes are striking in early-stage development, because embryos are very sensitive to environmental conditions [29], or may be due to excessive water loss by eggs and consequent dehydration [24].
Table (3) shows that there was no significant effect of thermal manipulation on chick quality score (Tona), chick length (cm), chick yielding (%) and average body weight at hatch (gm) when comparing thermally manipulated groups to each other and to control.
The results of chick quality were in agreement with those of [13, 30]. The results of chick length in Table (3) were agreed with [16, 25]. In contrast to T1, T3 and T4 were the results of [1, 26] for chick length at hatch.
The chick yield results of [12] were agree with T3. In contrast chick yield [28] disagreed with T4 results.
The results of [9, 16, 31], they confirmed the results of chick weight at hatch. In contrast [11] disagreed T2 and T3 results in chick weight.
Table (4) shows that all thermally manipulated groups had significantly ($P \leq 0.05$) higher male percentage and male/female ratio than control group. While all thermally manipulated groups had significantly ($P \leq 0.05$) lower female percentage than control group. Results of T4 was in agreement with those of [13, 16, 32]. In addition, T1 results agreed with [9, 33]. In contrast [20] reported that no evidence was found for sex-biased embryo mortality in commercial broilers Also [16] found that thermal stimulation of 1°C above the optimum temperature of incubation for 2 h/d from ED 18-21 resulted a non significant numerically higher sex ratio in favor of the treatment group, concluded that the discrepancy might be due to high variation between replicates that masked a possible treatment effect on secondary sex ratio at hatch.
Sex ratio has a relationship with environmental temperature as reported that avian male embryos are more vulnerable to environmental conditions than females [34]. Male embryos were more resistance high temperature than females throughout the incubation period, thus female hatchability decreased and the percentage of male offspring increased [35, 36]. The mechanism offered for altering avian sex ratio was based on temperature-dependent sex-biased embryo mortality [9]. The reason of increased male percentage in T1, possibly was due to the significant differences in embryonic development between females and males lead to the variation in embryonic mortality during the first week of incubation [29]. The reasons of sex ratio skewing may be due to increased incubation temperature, perhaps due to during late embryo development possibly as a result of elevated plasma blood glucose concentrations and strain by treatment alterations in insulin-like growth factor concentration [12]. Alternation in the concentration of hormones linked to metabolism and growth of embryos might affect vitality [37] especially in the males at piping time and result in more males percentage and it may have also increased hatchability of male chicks [30, 16].

4Conclusions and Recommendations

In light of the present results of exposing the broiler breeder eggs to intermittent thermal manipulation (TM) by 1˚C above the optimum incubation temperature during different embryogenesis periods, it was concluded that:
1- Thermal manipulation at early embryogenesis period (ED 1-5) accelerated the hatching of the chicks prior to hatchability peak compared to control and other treated groups, while TM at ED (14-18) and ED (19-21) reached hatchability peak earlier than control and other treated groups.
2- Thermal manipulation groups resulted significantly higher male percentages and higher male/female ratio with superiority for ED 19-21 group than control.
3- Tona score for chick quality of all treated groups was higher than control group.
So we recommend that:
1-In the case of high demand on broiler chicks generally, it is recommended to apply thermal manipulation at ED (14-18) and ED (19-21).
2-In the case of high demand on male chicks, it is recommended to practice thermal manipulation at ED 19-21.
3- According to the results of chick quality we advise thermal manipulation generally.

Table 1: Effect of thermal manipulation on hatchability percentage (%) at different hatch time (hour) of broiler chicks (Means ± S.E).

Hatch time (hour)**	Treatment*					L.S.
	T1	T2	T3	T4	Control	
478	10.66±3.29^{a}	2.50±0.99^{b}	1.50±1.10^{b}	7.50±2.60^{ab}	4.83±1.28^{ab}	*
480	31.66±7.60^{a}	13.50±2.85^{b}	5.83±2.00^{c}	21.83±5.46^{ab}	24.16±2.16^{ab}	*
482	37.33±8.59^{a}	17.33±3.65^{b}	8.16±2.62^{c}	28.50±6.67^{ab}	28.00±2.68^{ab}	*
484	52.33±7.26^{a}	34.83±4.52^{ab}	21.50±3.75^{b}	40.16±8.11^{a}	50.99±3.66^{a}	*
490	73.83±4.80^{ab}	67.99±1.69^{bc}	60.16±2.47^{c}	70.66±2.35^{b}	79.83±1.47^{a}	*
496	78.33±3.73^{b}	79.16±2.14^{ab}	79.33±0.47^{ab}	82.83±1.42^{a}	83.99±0.47^{a}	*
502	79.49±4.00^{a}	83.00±3.15^{a}	84.50±2.29^{a}	84.50±1.85^{a}	85.00±0.69^{a}	**N.S**
508	79.49±4.00^{a}	83.33±3.41^{a}	85.33±1.90^{a}	84.50±1.85^{a}	85.00±0.69^{a}	**N.S**
514	79.49±4.00^{a}	83.33±3.41^{a}	85.66±1.81^{a}	84.50±1.85^{a}	85.00±0.69^{a}	**N.S**
520	79.49±4.00^{a}	83.33±3.41^{a}	85.83±1.83^{a}	85.50±1.79^{a}	85.66±0.63^{a}	**N.S**
526	80.16±4.04^{a}	83.50±3.32^{a}	85.83±1.83^{a}	85.50±1.79^{a}	85.83±0.73^{a}	**N.S**
528	80.00±4.15^{a}	83.50±3.32^{a}	85.83±1.83^{a}	85.50±1.79^{a}	85.66±0.63^{a}	**N.S**

$^{a, b, c}$: means within each row had the different subscript were differ significantly (P≤0.05).

Table 2: Effect of thermal manipulation on hatching parameters of broiler chicks.

Parameters	Treatment*					L.S.
	T1	T2	T3	T4	Control	
Egg weight(gm)	53.8±0.3^{a}	53.8±0.1^{a}	53.8±0.1^{a}	54.5±0.1^{a}	53.8±0.4^{a}	**N.S**
Body Temperature at hatch (°C)	39.31±0.11^{a}	39.46±0.10^{a}	39.53±0.09^{a}	39.31±0.09^{a}	39.31±0.11^{a}	**N.S**
Hatchability of total egg (%)	80.0±4.1^{a}	83.5±3.3^{a}	85.8±1.8^{a}	85.5±1.7^{a}	85.6±0.6^{a}	**N.S**
Hatchability of fertile egg (%)	89.0±2.6^{a}	90.3±2.3^{a}	91.3±0.9^{a}	91.2±0.6^{a}	92.6±0.3^{a}	**N.S**
Normal chicks (%)	99.4±0.3^{a}	99.8±0.1^{a}	99.8±0.2^{a}	100.0±0.0^{a}	100.0±0.0^{a}	**N.S**
Culling chicks (%)	0.6±0.3^{a}	0.2±0.1^{ab}	0.2±0.2^{ab}	0.0±0.0^{b}	0.0±0.0^{b}	*
Early embryonic mortality (%)	4.9±1.9^{a}	3.8±1.4^{ab}	4.6±0.8^{a}	2.8±0.6^{b}	3.4±0.8^{ab}	*
Intermediate embryonic mortality (%)	2.1±0.4^{a}	2.0±0.5^{a}	0.9±0.7^{b}	1.2±0.5^{ab}	1.6±0.4^{ab}	*
Late embryonic mortality (%)	4.1±1.0^{a}	3.8±0.6^{ab}	3.1±0.5^{ab}	4.6±1.3^{a}	2.5±0.6^{b}	*
Total embryonic mortality(%)	11.0±0.4^{a}	9.7±0.3^{b}	8.7±0.3^{bc}	8.6±0.3^{bc}	7.5±0.3^{c}	*

$^{a, b, c}$: means within each row had the different subscript were differ significantly (P≤0.05).

Table 3: Effect of thermal manipulation on chick quality.

Parameters	Treatment*					L.S.
	T1	T2	T3	T4	Control	
Total score (Tona)	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	99.0±1.0^{a}	98.0±1.1^{a}	**N.S**
Chick length (cm)	18.4±0.0^{a}	18.5±0.0^{a}	18.5±0.1^{a}	18.4±0.1^{a}	18.3±0.1^{a}	**N.S**
Yielding (%)	67.2±0.4^{a}	67.8±0.2^{a}	68.4±0.4^{a}	68.9±0.7^{a}	69.4±1.3^{a}	**N.S**
Body weight at hatch (gm)	36.2±0.2^{a}	36.5±0.2^{a}	36.8±0.2^{a}	37.5±0.3^{a}	37.4±0.8^{a}	**N.S**

$^{a, b, c}$: means within each row had the different subscript were differ significantly (P≤0.05).

Table 4: Effect of thermal manipulation on sex ratio (%) of broiler chicks.

Sex ratio (%)	Treatment*					L.S.
	T1	T2	T3	T4	Control	
Male	50.63±0.124^{b}	50.71±0.117^{b}	50.48±0.179^{b}	51.65±0.173^{a}	49.80±0.371^{c}	*
Female	49.37±0.123^{b}	49.29±0.117^{b}	49.52±0.179^{b}	48.35±0.173^{c}	50.20±0.371^{a}	*
Male/ Female	1.03±0.005^{b}	1.03±0.005^{b}	1.02±0.007^{b}	1.07±0.007^{a}	0.99±0.015^{c}	*

$^{a, b, c}$: means within each row had the different subscript were differ significantly ($P \leq 0.05$).

References:

[1] Lin, Y.A. (2012). Effect of preheating temperature, early incubation temperature, and different turning frequency, on embryonic development and broiler performance. Msc thesis Raleigh, North Carolina.

[2] Collins, K.E. (2013). Incubation temperature effects on broiler chicken embryos. MSc thesis in University of Georgia. Athens, Georgia.

[3] Shinder, D.; M. Ruzal; M. Giloh; S. Druyan; Y. Piestun and S. Yahav (2011). Improvement of cold resistance and performance of broilers by acute cold exposure during late embryogenesis. Poultry Science. 90:633-641.

[4] Yahav, S. ; A. Collin; D. Shinder and M. Picard (2004a). Thermal manipulations during broiler chick embryogenesis: Effects of timing and temperature. Poultry Science. 83:1959-1963.

[5] Tona, K. ; F. Bamelis; B. de Ketelaere; V. Bruggeman; V. M. B. Moraes; J. Buyse; O. Onagbesan and E. Decuypere (2003). Effects of egg storage time on spread of hatch, chick quality and chick juvenile growth. Poultry Science. 82.736-741.

[6] SAS, Statistical analysis system (2005). User's guide for personal computer. Release 8.2 SAS Institute Inc. Cary, NC, USA.

[7] Duncan, D. B. (1955). Multiple range and multiple F. Biometrics 11:1-42.

[8] Albokhadaim, I. F.; I. B. Ismail; S. M. El-Bahr and T. Althnaian (2010). The effect of manipulations of incubation temperature on embryonic and post-hatching growth of native Saudi chickens. Pakistan Journal of Biological Sciences. 13 (17):852-856.

[9] Piestun, Y., S. Druyan, J. Brake, and S. Yahav (2013a). Thermal treatments prior to and during the beginning of incubation affect phenotypic characteristics of broiler chickens posthatching. Poultry Science 92: 882–889.

[10] Tona, K. ; O. Onagbesan; V. Bruggeman; A. Collin; C. Berri; M. J. Duclos; S. Tesseraud; J. Buyse; E. Decuypere and S. Yahav (2008). Effects of heat conditioning at d 16 to 18 of incubation or during early broiler rearing on embryo physiology, post-hatch growth performance and heat tolerance. Archiv für Geflügelkunde. 72 (2): 75-83.

[11] Piestun, Y., O. Halevy, D. Shinder, M. Ruzal, S. Druyan, and S. Yahav (2011). Thermal manipulations during broiler embryogenesis improves post hatch performance under hot conditions. Therm. Biol. 36:469–474.

[12] Willemsen H., Y. Li, E. Willems, L. Franssens, Y. Wang, E. Decuypere, and N. Everaert (2011). Intermittent thermal manipulations of broiler embryos during late incubation and their immediate effect on the embryonic development and hatching process. Poult. Sci. 90 :1302–1312.

[13] Halle, I. and B. Tzschentke (2011). Influence of temperature manipulation during the last 4 days of incubation on hatching results, posthatching performance and adaptability to warm growing conditions in broiler chickens. Japan Poultry Science Association. 48: 97-105.

[14] Tzschentke, B. (2007). Attainment of thermoregulation as affected by environmental factors. Poultry Science, 86: 1025- 1036.

[15] Dalab, Ab,H.S. (2011). Effect of thermal manipulation during embryogenesis on the expression of heat shock protein 70 (HSP-70) in chicken. MSc thesis at Jordan University of Science and Technology.

[16] Elmehdawi, Ab,B.S. (2013). The effects of altering incubation temperature on broiler chick hatchability, chick quality, sex ratio, and subsequent performance under field conditions. PhD. dissertation. Uneversity of Clemson.

[17] Al-Zhgoul, M.B., S.A.H. Dalab, M.M. Ababneh, K.I. Jawasreh, K.A. Al-Busadah, and Z.B. Ismail (2013). Thermal manipulation during chicken embryogenesis results in enhanced Hsp70 gene expression and the acquisition of thermotolerance. Veterinary Science 95:502–507.

[18] Badran, A.M., A. Desoky, E.M. Abou-Eita, and F.K. Stino (2012). Epigenetic thermal adaptation of chickens during late embryonic

development. Egypt Poultry Science. 32:675-689.

[19] Loyau, T., C. Berri, L. Bedrani, S. Métayer-Coustard, C. Praud, M. J. Duclos, S. Tesseraud, N. Rideau, N. Everaert, S. Yahav, S. Mignon-Grasteau and A. Collin (2013). Thermal manipulation of the embryo modifies the physiology and body composition of broiler chickens reared in floor pens without affecting breast meat processing quality. Journal of Animal Science. 91:3674-3685.

[20] Collins, K. E. ; B. J. Jordan; B. L. McLendon; K. J. Navara; R. B. Beckstead and J. L. Wilson (2013). No evidence of temperature-dependent sex determination or sex-biased embryo mortality in the chicken. Poultry Science. 92(12):3096-3102.

[21] Walstra, I., J. Ten Napel, B. Kemp, and H. van den Brand (2010). Temperature manipulation during layer chick embryogenesis. *Poultry Science* 89:1502-1508.

[22] Lourens, A., H. van den Brand, M. J. W. Heetkamp, R. Meijerhof, and B. Kemp (2007). Effects of eggshell temperature and oxygen concentration on embryo growth and metabolism during incubation. Poult. Sci. 86:2194–2199.

[23] Piestun, Y., S. Druyan, J. Brake, and S. Yahav (2013b). Thermal manipulations during broiler incubation alter performance of broilers to 70 days of age. Poultry Science 92 :1155–1163.

[24] Morita, V.D,S., I. C. Boleli and J. A. de Oliveira (2010). Hematological and incubation parameters of chicks from young breeders eggs: variation with sex and incubation temperature. International Journal of Poultry Science 9 (6): 606-612.

[25] Molenaar, R., R. Hulet, R. Meijerhof, C.M. Maatjens, B. Kemp, and H. van den Brand (2011). High eggshell temperatures during incubation decrease growth performance and increase the incidence of ascites in broiler chickens. Poultry Science. 90 :624–632.

[26] Lourens, A, H., V. D. Brand, R. Meijerhof, and B. Kemp (2005). Effect of eggshell temperature during incubation on embryo development, hatchability, and posthatch development. Poult. Sci. 84:914-320.

[27] Collin, A., C. Berri, S. Tesseraud, F.E. Requena-Rodon, S. Skiba Cassy, S. Crochet, M.J. Duclos, N. Rideau, K. Tona, J. Buyse,V. Bruggeman, E. Decuypere, M. Picard, and S. Yahav (2007). Effects on thermal manipulation during early and late embryogenesis on thermotolerance and breast muscle characteristics in broiler chickens. Poultry Science 86: 795–800.

[28] Joseph, N. S., A. Lourens, and E. T. Moran (2006). The effects of suboptimal eggshell temperature during incubation on broiler chick quality, live performance, and further processing yield. Poult. Sci. 85:932–938.

[29] Wu, J. J. , W. M. Li , Y. P. Feng , R. X. Zhao , C. Wang , Y. Yu , L. G. Yang , and S. J. Zhang (2012). Sex-biased mortality analysis in chick embryos during the entire period of incubation. J. appl. Poult. Res. 21: 508–512.

[30] Tzschentke, B. and I. Halle (2009). Influence of temperature stimulation during the last 4 days of incubation on secondary sex ratio and later performance in male and female broiler chicks. British Poultry Science; 50:634-640.

[31] Werner, C., C. Wecke, F. Liebert, and M. Wicke (2010). Increasing the incubation temperature between embryonic day 7 and 10 has no influence on the growth and slaughter characteristics as well as meat quality of broilers. Anim. 4: 810-816.

[32] Tatge, S., K. Melchior, I. Halle, M. Boerjan and B. Tzschentke (2014). Temperature training before hatching: successful method to optimize hatching results and performance in broiler chickens. *World Poultry Science* Association, Incubation and Fertility Research Group, Lunteren, The Netherlands, 29th-30th September, 2014.

[33] Wang, S.Y., K.J. Yang, K.Y. Wang, S.H. Kuo, C.Y. Liao, and Y.H. Chen (2014). Manipulating incubation conditions to alter sex ratio of Taiwan Country Chickens. *World Poultry Science* Association, Incubation and Fertility Research Group, Lunteren, The Netherlands, 29th-30th September, 2014.

[34] Catalano, R., T. Bruckner and K.R. Smith (2008). Ambient temperature predicts sex ratios and male longevity. Proceedings of the National Academy of Science of the United State of America. 105: 2244-2247.

[35] Hays, F. A. and E. W. Spear (1952). Relation of age of parents to mortality and sex ratio of chicks at eight weeks. Poult. Sci. 31:792-795. Cited by Collins, K.E. (2013).

[36] Hays, F. A. (1952). Sex ratio in Rhode Island Red chicks at hatching. Poult. Sci. 31:1094. Cited by Collins, K.E. (2013).

[37] Decuypere, E. and V. Bruggeman (2007). The endocrine interface of environmental and egg factors affecting chick quality. Poultry Science. 86:1037–1042.