Egypt. Poult. Sci. Vol. (39) (IV): (825-833) (2019) (**1910-1047**)

Egyptian Poultry Science Journal

http://www.epsj.journals.ekb.eg/

ISSN: 1110-5623 (Print) – 2090-0570 (Online)

INFLUENCE OF THERMAL MANIPULATION DURING EMBRYONIC DEVELOPMENT ON HATCHABILITY TRAITS AND SECONDARY SEX RATIO OF BROILER CHICKS

Ayat R. M. El-Zeniny[1]; Ahmed M. El-Kaiaty[2]; Hewida M. H. El-Allawy[1]; Hosam M. Safaa[2]; Gamal M. Kamel[2]; Alaa K. Alm El-Din[3] and Nafisa A. Abd El-Azeem[1]

[1]Anim. Prod. Dep., Nation.Res. Centre, Giza, Egypt, [2]Anim.Prod. Dep., Fac. of Agric., Cairo Univ., Giza, Egypt, [3]Anim. Prod. Res. Inst., Agric. Res. Centre, Minis. of Agric., Giza, Egypt

Corresponding author: Ayat R. M. El-Zeniny Email: ayat_ramadan2008@yahoo.com

Received: 13 / 10 /2019 Accepted: 13 /11/2019

ABSTRACT: The current study was conducted to investigate the influence of thermal manipulation (TM) during embryogenesis on hatchability traits, secondary sex ratio and hatchling weights of broiler chicks. A total of 1200 fertile eggs obtained from Cobb-500 breeder broilers parent stock were used in this study. The eggs were randomly distributed into 4 experimental groups. Incubation conditions from day 1 to day 18 were 37.5°C and 52% relative humidity for control group (TM0). while incubation temperature of the thermally treated groups during 13-15 days of incubation was increased to 39ºC for 2 hours in the second group (TM2), 4 hours in the third group (TM4) and 6 hours in the fourth group (TM6). At hatch, the hatchability traits and embryonic mortality (EM) were calculated; all hatched chicks were sorted, sexed and individually weighed. The obtained results showed that, Thermal manipulation did not adversely affect hatchability and embryonic mortality rate, but there was a positive significant effect on the normality of hatched chicks. However there were non-significant differences in the secondary sex ratio of hatched chicks. Thermal treatment did not significantly effect on body weight of hatching chicks.

Key Words: thermal manipulation, embryogenesis, hatching parameters, broiler chicks

INTRODUCTION

Incubation climate may have a long lasting influence on poultry performance (Collin *et al.,* 2007; Hulet *et al.,* 2007; Piestun *et al.,* 2008; Tzschentke and Halle, 2009; Shinder *et al.,* 2011). The incubation temperature is the most important climatic factor in incubation (Decuypere and Michels, 1992). Changes in incubation temperature by only 1°C from the standard temperature have a major effect, for instance, on hatching results in turkeys (French, 1994), but the force of this effect depends on the time of application and the duration of changes during embryonic growth (French, 2000). Tzschentke and Halle (2009) demonstrated that short-term warm stimulation (prenatal temperature training) during the late incubation period in broiler chicks improves hatchability and post-hatching performance until age of slaughter.

Previous researches noticed that embryos, exposed to high or low incubation temperatures, improved their ability to adapt to hot or cold environments, respectively, in the post hatch phase. The timing of thermal manipulation has to be correlated to the development of two axes; the hypothalamus-hypophysis-thyroid axis (to change the heat production threshold response), and the hypothalamus-hypophysis-adrenal axis (to avoid increase in stress response) (Minne and Decuypere 1984, Janke *et al.* 2002, Yahav *et al.* 2004 a, b). In other terms, incubation temperature may affect thermoregulation of birds after hatching, subsequently the specificity of epigenetic adaptation, which may happen during early pre- or postnatal embryo development (Nichelmann and Tzschentke 2002), may involve to reduction problems related to the control of thermoregulation during rearing. This reasoning proposes that modulating standard incubation temperatures may be a way to improve post-hatch performance of poultry (Yalcin and Siegel 2003).

The present study was conducted to determine the effects of thermal manipulation during embryonic development on the egg weight loss, hatchability, embryonic mortality rate, normality of hatching chicks, secondary sex ratio and hatching weight of broiler chicks.

MATERIAL AND METHODS

This experiment was carried out at the hatchery (Pass-Reform, Holland) of Ismailia–Misr Poultry Company, in winter season through January and February. One thousand two hundreds fertile eggs were obtained from Cobb-500 breeder stock at 35 weeks of age. All eggs were almost similar in weight and produced in the same production day. Broken, distorted or irregular, too small, too large and unclean eggs were excluded and checked the correct position of all eggs in tray. Eggs were randomly divided into 4 groups (300 eggs for each, with six replicates of each). A representative sample of eggs were numbered in each tray and individually weighted before starting incubation and during thermal manipulation every day of treatment to calculate the egg weight loss. Before starting incubation, the average initial eggs weight of the treatment groups was nearly similar with no observed significant differences. At day 13 of incubation, all eggs were subjected to candling to calculate the fertility %. Incubation conditions from day 1 to day 18 were 37.5°C (99.5 ºF) and 52% relative humidity for control group (TM0) at setter. In the thermally treated groups at 13-15 days of incubation, temperature

was increased to 39ºC (102ºF) for 2 hours (TM2), 4 hours (TM4) or 6 hours (TM6). Immediately after the thermal treatments terminated, incubation conditions were restored to the regular levels (37.5°C and 52% relative humidity). All eggs in setter were turned with 45º angles automatically each hour. At day 18 of incubation, the eggs were transferred to hatching baskets. At hatch, hatchability traits were calculated; all hatched chicks were sorted, sexed and individually weighed. The unhatched eggs were cracked open and visually examined to determine the embryonic death stage (early, middle and late) and pipped chicks.

STATISTICAL ANALYSIS

Data were analyzed using the General Linear Model (GLM) procedure, **SAS (2003)**. All the characteristics were performed in conformity by one way analysis and factorial analysis model. The significant differences among treatments means were separated by Tukey's Studentized Range Test (HSD) according to **Tukey (1949)**.

Model applied was:

a- one way analysis

$Yij = \mu + Ti + eij$

Where: Yij =Observed value of a given dependent variable, μ= Overall adjusted mean, Ti = Fixed effect of treatments, i= 1, 2....4. eij = Random error associated to each observation.

b- factorial analysis

$Yijk = \mu + Xi + Zj + (XZ)ij + eijk$

Where: Yijk = observation, μ = overall mean, Xi = thermal manipulation effect, Zj = sex effect, (X Z)ij = interaction between thermal manipulation and sex, eijk = experimental errors

RESULTS

Descriptive statistics of egg weight (EW) at 0, 12, 13, 14 and 15 days of incubation were presented in Table (1). Before starting incubation, all treatment groups were similar in the average of egg weight, so no significant differences were observed. Also, no significant differences were observed on egg weight (EW) and egg weight loss (EWL) at 12, 13, 14 and 15 days of incubation. This may be due to the duration of thermal treatment was too short to affect the egg weight loss. Hatchability was 87.18%, 83.11%, 89.89% and 86.27% for TM0, TM2, TM4 and TM6 groups, respectively (Table 2). This showed that thermal treatment (39ºC at 13-15 days of incubation) reduced hatchability (%) in groups TM2 and TM6 compared to the control group, but the difference was not significant. Also, the embryonic mortality was 7.02%, 6.30%, 5.06% and 8.14%, which mean that thermal treatment reduced embryonic mortality in groups TM2 and TM4 compared to the control group, while TM6 group which had the highest embryonic mortality % than all groups, but the differences were not significant.

As well as the effect of thermal manipulation on normal and abnormal chick's percentage were presented in Table (2). With regarding to normal chick percentage (%), data shows that TM2 group present the lowest value (92.88) compared to (94.53, 95.21, and 97.80) for TM0, TM4 and TM6, respectively. TM6 achieved the highest normal chick percentage (97.80%) and lowest abnormal chick percentage (2.20%). While TM4 group recorded the highest value of hatchability percentage (89.89%) compared to TM0, TM2 and TM6 groups (87.18%, 83.11% and 86.27% respectively).

The presented results show that there was no significant effect of thermal manipulation on the secondary sex ratio (Table 2). But TM2 and TM4 groups

were numerically higher male percentage than TM6 and control group; however TM6 group had lower male percentage and higher female percentage than the control group.

There was no significant effect of thermal manipulation on hatching body weight of chicks (Table 3). However, the body weight at hatch of TM4 group was the highest but without significant differences. The current results showed that there was a significant effect of sex on body weight at a day of hatch though; male hatched chicks were significantly heavier than females.

DISCUSSION

Thermal treatment during incubation did not significantly affect hatchability and embryonic mortality rate. This result agree with Alkan *et al.* (2013), who reported that there was no significant difference between control and thermal manipulated groups in respect to hatchability and embryonic mortality. However, previous results on hatchability and embryonic mortality rates are contradictory. Decuypere and Michels (1992) reported that incubation climate can significantly influence hatchability in poultry. Changes of only 1°C from the optimum have a major impact on hatching results in turkey (French 1997). However, the strength of this influence depends on the timing frame used and duration of changes in incubation temperature during embryogenesis (French 2000). Collin *et al.* (2005) and Yahav *et al.* (2004b) reported that the thermal manipulation significantly improved hatchability than control group. In contrast, Yalcin and Siegel (2003) and Badran *et al.* (2012) found that increasing incubation temperature had no significant effect on hatchability percentage. Also, Tzschentke and Halle (2009) incubated broiler eggs from day 1 to day 17 under normal incubation conditions (37.2 to 37.4 °C), then divided them into three hatch incubators (control: 37.2 to 37.4°C; chronic warm incubation: 38.2 to 38.4°C, 24 h daily; short-term warm stimulation: 38.2 to 38.4°C, 2 h daily). They reported that neither chronic nor short-term increase in incubation temperature had a negative effect on hatchability. Also Halle *et al.* (2012) reported that the short-term warm stimulation reduced the percentage of hatched ducklings.

Thermal manipulation had a significant effect on the normality of chicks at hatch. However Halle *et al.* (2012) demonstrated that the vitality of the hatched ducklings not affected by temperature treatment in the incubation.

Thermal manipulation had no significant effect on the secondary sex ratio of hatched chicks. However Tzschentke and Halle (2009) in previous study in broiler chicks demonstrated that after short-term warm load (1°C over standard for 2 h daily during the last 4 days of incubation) the secondary sex ratio shifted in favour of male chickens, but the body weight of the one-day-old male chicks was similar to the control group and the short-term temperature stimulated group. Similar to those results, in Pekin ducks short-term cold temperature stimulation during the last days of incubation changed the secondary sex ratio in favour of female ducklings, but the male one day-old ducklings had a significant higher body weight compared to the male ducklings of the control group, improved performance parameters up to the age of slaughter. Also hatchability was not negatively influenced (Halle *et al.*, 2012).

Thermal manipulation had no significant effect on body weight of hatched chicks (BW0). Similarly, Collin *et al.* (2005)

reported that the different durations of thermal manipulation during E16 to E18 did not affect the body weight of the hatched chicks. Previous results on body weight differences between males and females after hatching were conflicting. Some authors (Khan *et al.* 1975, Whiting and Pesti 1983) found that male chicks to be heavier than females, but others (Burke 1992, Reis *et al.* 1997) found that no gender differences in body weight of day-old hatchlings. But in this study, sex had a significant effect on body weight of the hatched chicks.

In conclusion, thermal manipulation during embryonic development of broiler chicks applied at 13-15 days of incubation did not adversely affect hatchability and embryonic mortality rate, but there was a significant effect on the normality of hatched chicks. However there were no significant differences in the secondary sex ratio of hatched chicks. Thermal treatment did not affect significantly on body weight of hatching chicks.

Table (1): Effect of thermal manipulation (TM)[1] during incubation phase on egg weight (EW) and egg weight loss (EWL) of Cobb-500 chicken embryos at 0,12,13,14 and 15 days of incubation

Item	N[2]	TM0[1]	TM2[1]	TM4[1]	TM6[1]	SEM	Prob.
Egg weight (g)							
EW0	90	65.02	64.79	63.83	64.41	0.391	0.1498
EW12	90	60.41	60.09	59.17	59.71	0.383	0.1261
EW13	90	60.08	59.84	58.89	59.47	0.383	0.1421
EW14	77	59.70	59.08	58.44	58.95	0.410	0.1443
EW15	65	59.33	58.37	58.21	58.56	0.463	0.2552
Egg weight loss							
EWL12	90	4.62	4.71	4.66	4.70	0.082	0.8747
EWL13	90	4.95	4.96	4.94	4.94	0.085	0.9984
EWL14	77	5.32	5.16	5.27	5.28	0.098	0.7395
EWL15	65	5.64	5.62	5.69	5.71	0.116	0.9426

[1] thermal manipulation at 13, 14 and 15 days of incubation for 2 (TM2), 4 (TM4) and 6 hours (TM6) at 39ºC, control group (TM0) hatched at 37.5ºC.
[2]n = number of observations per each treatment.

Table (2): Fertility % of treated eggs from Cobb-500 breeder broilers at 35 weeks of age and effect of thermal manipulation (TM)[1] during incubation phase on embryonic death, normality hatched chicks, hatchability % and secondary sex ratio of hatched chicks

Item	**TM0[1]**	**TM2[1]**	**TM4[1]**	**TM6[1]**	**SEM (n=6)[2]**	**Prob.**
Fertility % at day 13 of incubation	94.00	92.67	96.67	93.67	1.204	0.1447
Embryonic death						
Early Death	2.19	1.32	0.00	2.02	0.706	0.1484
Middle Death	0.41	0.41	0.77	0.45	0.438	0.9236
Late Death	4.43	3.69	3.53	4.41	1.221	0.9302
Pipped chicks	0.00	0.88	0.77	1.26	0.467	0.3055
Embryonic mortality rate	7.02	6.30	5.06	8.14	1.354	0.4546
Normal Chicks %	94.53^{ab}	92.88^{b}	95.21^{ab}	97.80^{a}	1.125	0.0409
Abnormal Chicks %	5.47^{ab}	7.12^{a}	4.79^{ab}	2.20^{b}	1.125	0.0409
Hatchability %	87.18	83.11	89.89	86.27	1.768	0.0886
Male %	48.18	51.37	52.40	46.77	2.058	0.2096
Female %	51.82	48.63	47.60	53.23	2.058	0.2096

[1] thermal manipulation at 13, 14 and 15 days of incubation for 2 (TM2), 4 (TM4) and 6 hours (TM6) at 39ºC, control group (TM0) hatched at 37.5ºC.

[2]Standard error of the mean (*n* = number of observations per each treatment).

[a-c]Means for main effect within the same row with different superscripts differ significantly ($P < 0.05$).

Table (3): Effect of thermal manipulation (TM)[1] during incubation phase on body weight at hatch (BW0) of Cobb-500 broiler chicks

Item	BW0
Treatment (TM) [1]	
TM0	44.37
TM2	44.02
TM4	44.60
TM6	43.93
n[2]	121
SEM	0.285
Sex (S)[3]	
1	44.54[a]
2	43.89[b]
n[2]	241
SEM	0.201
TM*Sex	
TM0 *1	44.53
TM2*1	44.30
TM4*1	44.95
TM6*1	44.40
TM0 *2	44.16
TM2*2	43.73
TM4*2	44.26
TM6*2	43.41
n[2]	121
SEM	0.402
General model	0.2201
Main effects	
TM	0.2871
Sex	0.0215
Interactions	
TM* Sex	0.8986

[1] thermal manipulation at 13, 14 and 15 days of incubation for 2 (TM2), 4 (TM4) and 6 hours (TM6) at 39ºC, control group (TM0) hatched at 37.5ºC.

[2] n = number of observations per each treatment.

[3] Sex 1 = male and 2 = female.

[a-c]Means for main or interaction effect within the same column with different superscripts differ significantly ($P < 0.05$).

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الملخص العربى

تأثير المعاملة الحرارية أثناء فترة النمو الجنينى على صفات الفقس و النسبة الجنسيه الثانويه لكتاكيت التسمين

آيات رمضان محمود الزنيني [1] - أحمد محمد القياتي [2] - هويدة محمد حسن الألاوى [1] - حسام محمد صفاء [2] - جمال محمد كامل [2] - علاء كمال علم الدين [3] - نفيسه أحمد عبد العظيم [1]

1- قسم الانتاج الحيواني –المركز القومي للبحوث 2-قسم الانتاج الحيواني –كليه الزراعه –جامعه القاهره

3-معهد بحوث الانتاج الحيواني –مركز البحوث الزراعيه –وزاره الزراعه

تم اجراء هذا البحث لدراسه تاثير رفع درجه الحراره خلال فتره النمو الجنيني علي صفات الفقس و النسبة الجنسيه الثانويه و وزن الكتاكيت الناتجه .تم استخدام 1200 بيضه مخصبه من قطيع امهات دجاج تسمين Cobb 500. تم توزيع البيض عشوائيا إلي 4 مجاميع متماثله فى متوسط وزن البيض. المجموعه الاولى كانت بدون اى معاملات و استخدمت للمقارنه (مجموعه الكنترول) و الثلاثة مجاميع الأخري تم معاملتها حراريا. تم التفريخ علي درجه حراره 37.5 درجه مئويه و رطوبه نسبيه 52 % من اليوم الاول حتي اليوم الثامن عشر من ايام التفريخ في مجموعه الكنترول. وفي الفتره من 13-15 يوم من ايام التفريخ تم زياده درجه حراره التفريخ فى مجاميع المعامله الي 39 درجه مئويه لمده ساعتين (المجموعه الثانيه) و 4 ساعات (المجموعه الثالثه) و 6ساعات (المجموعه الرابعه). عند الفقس تم حساب صفات الفقس و النفوق الجنيني و كذلك تم فرز و تجنيس الكتاكيت الناتجه و تسجيل الوزن فرديا للكتاكيت. أظهرت نتائج الدراسه أن التعديل الحرارى لم يؤثر سلبيا علي نسبه الفقس و معدل النفوق الجنيني و لكن تم تسجيل تاثير معنوي ايجابي علي نسبه الكتاكيت السليمه الناتجه. بالرغم من ذلك لم يوجد تاثيرات ايجابيه علي النسبة الجنسيه الثانويه في الكتاكيت الناتجه و كذلك لم تؤثر المعامله الحراريه معنويا علي اوزان الجسم للكتاكيت الناتجه.