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The effect of duration of thermal manipulation during broiler chick embryogenesis on body weight and body temperature of post-hatched chicks

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Abstract – The significant developments in the genetic selection of fast-growing meat-type broiler chickens, coupled with insufficient development of their visceral systems, have enhanced the interest for thermal manipulations (TM) during susceptible periods of broiler embryogenesis, in order to improve thermotolerance acquisition. The duration of TM may affect both body weight (BW) and body temperature (T_b) of the chicks. This study was aimed at elucidating the effect of different duration periods of TM during broiler embryogenesis on the hatching rate, BW and T_b at hatch and following thermal challenge (41 °C for 6 hours) at the age of 3 days (Challenged C or Naïve N, i.e. non-challenged chicks). Control embryos were incubated at 37.8 °C, whereas the TM-embryos were treated for 3 (D1), 6 (D2), 12 (D3) or 24 (D4) hours per day at 39.5 °C during late embryogenesis from E16 to E18. Different durations of TM did not affect BW of the hatched chicks, but significantly affected hatchability, which was higher in the D3 and D4 treatments compared to the D1 treatment. It further affected the T_b of the treated chicks, which was significantly lower in all treatments than in the controls. During the challenge (C), all 4 treatments (D1C to D4C) exhibited a significantly lower T_b compared to the controls. Eighteen hours post-challenge, D1C chicks maintained significantly lower T_b than D2C, D3C and D4C chicks. The BW of the naïve chicks continued to be similar, whereas that of the challenged ones demonstrated a significantly higher value for D2C and D3C chicks compared to the Controls and D1C's. It can be concluded that out of the four TM durations, the best one to initiate improvement of thermotolerance acquisition requires 3 hours of TM per day during E16 to E18, whereas 6 and 12 hours per day may be the best to reach higher hatchability and initiate growth. However, further research is required to follow both responses during the whole life span of the chicks.

embryogenesis / broiler / thermal manipulation / thermal challenge / body temperature

Résumé – Effet de la durée d'un traitement thermique des embryons de poulets de chair sur le poids vif et la température corporelle des poussins après éclosion. La sélection génétique des poulets de chair à croissance rapide s'est accompagnée d'un développement insuffisant de leurs organes internes qui limite leur capacité à résister à la chaleur. Les manipulations thermiques (MT)

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de l'embryon visent à améliorer l'acquisition de la thermotolérance du poulet. Leur durée pourrait affecter le poids vif (BW) et la température corporelle (T_b) des poussins. Cette étude a pour but de rechercher les effets de différentes durées de MT pendant les jours E16 à E18 de l'embryogenèse sur le taux d'éclosion, T_b et BW des poussins à la naissance (j0), ainsi qu'à 3 jours (j3) pendant une exposition ou non à un coup de chaleur de 6 h à 41 °C. Les œufs témoins sont incubés à 37,8 °C pendant 21 jours, tandis que les embryons traités sont en plus soumis à 39,5 °C pendant 3 (D1), 6 (D2), 12 (D3) ou 24 (D4) h par jour de E16 à E18. Les traitements n'affectent pas BW à j0, mais les taux d'éclosion sont plus élevés chez les embryons D3 et D4 comparés aux D1. Tous les traitements diminuent significativement T_b par rapport aux témoins à j0, résultat retrouvé chez les poussins exposés à un coup de chaleur (C) à j3. Dix-huit heures après le coup de chaleur, les poussins exposés D1C maintiennent une T_b significativement plus faible que celle des poussins D2C, D3C et D4C. Chez les poussins non exposés (N), BW continue à être similaire entre traitements, alors que chez les poussins exposés (C), BW est plus élevé chez les poussins D2C et D3C que chez les témoins et D1C. Il apparaît donc que la meilleure durée de MT dans nos conditions est de 3 h par jour de E16 à E18 pour l'amélioration de la thermotolérance du poussin, et de 6 à 12 h par jour pour l'initiation de la croissance. Des recherches complémentaires sont nécessaires pour évaluer ces deux types de réponses à plus long terme.

embryogenèse / poulet de chair / manipulation thermique / coup de chaleur / température corporelle

1. INTRODUCTION

The significant developments in the genetic selection of fast-growing meat-type broiler chickens, coupled with insufficient development of their visceral systems [8], have reduced their ability to cope with extreme environmental conditions including hot spells. These climatic events induce a depression in feed ingestion and growth performance, with increased mortality rates of economical importance for poultry production. This has enhanced the interest in focusing on thermal manipulations during broiler embryogenesis and the post-hatch period in order to improve thermotolerance acquisition [3, 10, 13, 26, 27]. Thermal manipulations during the chick's early-age, while the body temperature regulation and feedback mechanisms are yet immature [4, 14], cause changes in the thermoregulatory threshold response [21, 22]; it has previously been documented that exposing embryos to high or low temperatures during incubation improves their capacity to adapt to hot or cold environments, respectively, in the post-hatch phase [3, 10, 13].

Thermal manipulations during embryogenesis need fine tuning of timing, level and duration of manipulated temperatures. The

timing of thermal manipulations has to be linked to the development and activation of the hypothalamus-hypophysis-thyroid axis [7, 18] and of the hypothalamic-hypophyseal-adrenal axis [5, 20] in order to change the heat production threshold response and the stress response that might reflect upon thermoregulation. Yahav et al. [26, 27] demonstrated that thermal manipulations during days 16 to 18 of embryogenesis may reach a significant improvement of thermotolerance acquisition, probably related to a reduction in body temperature (T_b) and in plasma thyroid hormone concentrations, assumed to reduce metabolic rate. It was further exhibited that out of 37.8, 39.5 and 41 °C, the best incubation temperature to achieve the same goal was 39.5 °C.

The effects of the duration of altering incubation temperature can be divided according to short or long term. A short-term increase of incubation temperature was found to activate the heat loss mechanism in chick embryos [9], whereas a long-term increase affected the embryo morphology [11], increased the incidence of malpositions and decreased hatchability [6, 15]. However, these studies were conducted under different experimental conditions, and therefore, a specific fine

tuning of duration associated with specific incubation conditions is needed.

This study was aimed at elucidating the effect of different duration periods during broiler chick embryogenesis, while thermal manipulation of 39.5 °C is conducted at E16 to E18, on hatching rate, growth and thermoregulatory response of chicks. Body weight (BW) and body temperature (T_b), a parameter shown to be the most constantly responding to thermal-conditioning in studies [2], were measured at hatch and following thermal challenge at the age of 3 days. This age was found to be the optimal day for post-hatch heat-conditioning [24], initiating both the best improvement of thermotolerance acquisition and growth acceleration.

2. MATERIALS AND METHODS

2.1. Experimental procedure

One thousand fertile Ross PM3 eggs from one breeder flock at optimal period of egg production (40 weeks of age) were used. The eggs were weighed and statistically divided according to JMP® statistics [16] into 5 treatments. The control chicks were maintained at 37.8 °C and 56% relative humidity (rh) during the whole incubation period [1]. In treated birds, thermal manipulation of 39.5 °C and 65% rh was applied at E16, E17 and E18 of embryogenesis using the following durations: D1 – 3 hours; D2 – 6 hours; D3 – 12 hours; D4 – 24 hours per day.

The eggs were incubated in a semi-commercial incubator (La-Nationale, type B 36I, Bretagne, France). The thermally treated eggs of each treatment were divided into 2 subgroups, each transferred into one of 2 experimental incubators (SMA Coudelou type 540E) during 3 hours (12:00–15:00), 6 hours (12:00–18:00), 12 hours (12:00–24:00) and 24 hours (continuously from E16 to E18) per day of thermal manipulation. Both incubators were kept at 39.5 ± 0.1 °C and $65 \pm 2.0\%$ rh. Immediately after the thermal treat-

ments were terminated, the eggs were transferred back to the semi-commercial incubator. The eggs in all incubators were turned through 270° every hour. At the 7th day of incubation, infertile and dead embryos were removed after candling. At the 19th day of incubation, the eggs were transferred to a hatching incubator kept at 37.8 °C and 70% rh.

During hatching, the number of chicks hatched was recorded every hour. After hatching and feather dryness (approximately 2 h post hatch), each chick was taken out of the incubator for immediate measurements that were conducted in the following order: T_b and BW. Body temperature was measured using a Digital Thermometer DM 852 (accuracy of 0.1 °C; manufactured by Ellab A/S, France), inserted in the distal colon at a constant depth, immediately after the chicks were gently handled individually.

During the hatching procedure and after the measurements were conducted, the chicks from each treatment were randomly divided into 2 groups: naïve (N) and challenge (C), and were situated in two temperature-controlled rooms. The chicks of both undivided rooms were raised under regular conditions (32 ± 1 °C). At the age of 3 days, the chicks from the challenged group were thermal-challenged (TC) at 41.0 °C for 6 hours, whereas the naïve chicks continued to be exposed to the regular conditions. During the last hour of TC exposure, T_b (from 20 individuals per embryonic treatment) was measured. Identical measurements were conducted with the naïve chicks. Eighteen hours after the heat challenge was terminated, BW and T_b of the challenged (Control (C), D1C to D4C) and naïve (Control (N), D1N to D4N) chicks (20 individuals per embryonic treatment) were monitored.

2.2. Statistical analysis

Data were subjected to analysis of variance (one way ANOVA) and to all pairs Tukey–Kramer-HSD test, by means of the JMP® software [16]. Hatchability was analyzed by the chi-square test. During and

Table I. Hatchability, body weight (BW) and body temperature (T_b) after hatching and feather dryness of broiler chicks exposed to different durations of thermal manipulation (TM) during embryogenesis: 3 hours per day (D1), 6 hours per day (D2), 12 hours per day (D3) or 24 hours per day (D4) of exposure at 39.5 °C between days 16 and 18 of embryogenesis.

Variables	Treatments					SEM
	Control	D1	D2	D3	D4	
No. of hatched eggs	158	153	158	170	161	----
Hatchability (%)	87.8 ^{ab}	82.7 ^b	87.8 ^{ab}	91.4 ^a	91.0 ^a	----
BW (g)	45.49	45.53	45.74	45.52	45.67	0.27
T_b (°C)	36.86 ^a	36.34 ^b	36.40 ^b	36.43 ^b	36.46 ^b	0.01

Within rows, values designated by different letters differ significantly ($P \leq 0.05$).

Table II. Body temperature (T_b) of naïve and thermal-challenged chicks at the age of 3 days. Treated embryos were submitted to 3 hours per day (D1), 6 hours per day (D2), 12 hours per day (D3) or 24 hours per day (D4) of exposure at 39.5 °C between days 16 and 18 of embryogenesis.

T_b (°C)	Treatments					SEM
	Control	D1	D2	D3	D4	
Naïve	41.00 ^a	40.77 ^c	40.85 ^{bc}	40.75 ^c	40.96 ^{ab}	0.04
Challenged	42.60 ^a	41.89 ^b	42.01 ^b	41.83 ^b	41.93 ^b	0.09

Within rows, variables designated by different letters, differ significantly ($P < 0.05$).

after the TC, data were analyzed for each room separately by one-way ANOVA to avoid confounds resulting from differences of conditions. Means were considered significantly different at $P \leq 0.05$.

3. RESULTS

The different durations of thermal manipulation during E16 to E18 did not affect the BW of the hatched chicks, but significantly affected hatchability, which was higher in the D3 and D4 treatments compared to the D1 treatment (Tab. I). It further affected the T_b of the heat-conditioned chicks, which was significantly lower in all treatments compared with the controls.

At the age of 3 days, the T_b of the D1N and D3N naïve chicks was significantly lower than that recorded in the control or

D4N treated chicks (Tab. II). During the challenge, all 4 heat-conditioned groups exhibited significantly lower T_b compared to the controls.

Eighteen hours post-challenge, the challenged chicks belonging to the D1C treatment maintained significantly lower T_b than that of D2C, D3C and D4C (Tab. III). In the naïve chicks a similar numerical trend was monitored with significance between D1N and D2N only. The BW of the naïve chicks continued to be similar between embryonic treatments, whereas that of the challenged group demonstrated a significantly higher BW of the D2C and D3C chicks compared to the controls (C) and D1C chicks (Tab. III).

4. DISCUSSION

A major concern in dealing with domestic animals is how to maintain or even improve

Table III. Body weight (BW) and body temperature (T_b) of naïve and thermal-challenged chicks at the age of 4 days (18 hours post challenge). Treated embryos were submitted to 3 hours per day (D1), 6 hours per day (D2), 12 hours per day (D3) or 24 hours per day (D4) of exposure at 39.5 °C between days 16 and 18 of embryogenesis.

Variables	Treatments					SEM
	Control	D1	D2	D3	D4	
Naïve chicks						
BW (g)	89.4	86.8	89.7	91.7	86.9	2.17
T _b (°C)	41.01 ^{ab}	40.85 ^b	41.09 ^a	40.98 ^{ab}	41.02 ^{ab}	0.07
Challenged chicks						
BW (g)	87.2 ^b	87.8 ^b	93.9 ^a	93.7 ^a	89.2 ^{ab}	1.9
T _b (°C)	40.89 ^{ab}	40.73 ^b	40.98 ^a	41.00 ^a	41.03 ^a	0.05

Within rows, variables designated by different letters, differ significantly ($P \leq 0.05$).

performance when various manipulative treatments are being applied. It has previously been well documented that prolonged heat exposure during the 1st week of the chicks' life [23] or prolonged exposure to cold during this period (Yahav, unpublished data) adversely affects the performance of broiler chickens, although it improves their thermotolerance.

The duration for which the embryo is exposed to thermal manipulation can play a major role in the balance between performance parameters and the thermoregulatory parameters. In this study the duration of thermal manipulation affected hatchability. The highest hatchability was exhibited in chicks that experienced 12 and 24 hours of thermal manipulation. On the contrary, Thompson et al. [19] and Lay and Wilson [12] found no effect of increasing incubating temperature up to 40.6 °C at E16 for 24 h on hatching rate. The lack of consistency of our results with these studies may be related to differences in climatic conditions during the whole incubation period (differences in relative humidity, control temperature, and the background of the eggs prior to incubation). It is also to be noticed that the heat-conditioned embryos were always treated at the same time of the day (12:00), but their average age for each treatment was different (difference of 10.5 hours between D1

and D4), which might have had consequences on later hatching, growth or thermoregulatory response.

Although at hatching, no difference in BW was demonstrated as previously exhibited by Yahav et al. [26, 27], a significantly higher BW in the D2C and D3C compared to control and D1C chicks was exhibited at 4 days of age in challenged animals 18 hours post-challenge (Tab. III). This may have resulted from the fact that control and D1C chicks had a more intensive decline in weight gain during the TC, which somewhat contradicts with the difference in T_b between these two treatments during the challenge, and/or from growth acceleration in the D2C and D3C treated chicks. The thermal manipulation during E16 to E18 occurred during the development of foetal myoblasts and the main period for the development of muscle satellite cells [17], which contribute to the final muscle size and to its ability for hypertrophy. The thermal challenge at 3 days of age was chosen based on thermal conditioning conducted at the same age [24]. This age was found to be the optimal day for this treatment, because it initiates both the best improvement of thermotolerance acquisition and growth acceleration [25]. In this study, thermal challenge was conducted at the same age, however, for a shorter time (6 hours) using 41 °C, compared to thermal

conditioning which lasted 24 hours under 37.5 °C. It can be hypothesized therefore, that the combined treatments during embryogenesis and the age of 3 days might affect growth performance of the D2C and D3C chicks. It can be further speculated that only 3 hours of thermal manipulations during embryogenesis (Treatment D1) coupled with thermal challenge was not enough to induce this growth acceleration, whereas 24 hours of thermal manipulations during embryogenesis (D4C) did not stimulate or depress this process. However, this remains unsolved and further experiments have to be conducted to clarify the effect of duration of thermal manipulation on broiler chicken BW along their life span.

All different durations of thermal manipulation during embryogenesis caused a significant reduction in T_b of hatched chicks that ranged between 0.40 and 0.52 °C, meaning that duration as a parameter for thermal manipulation did not have a significant effect on T_b at hatch. A similar trend was monitored during the challenge at the age of 3 days and was similar to that monitored in chicks that experienced thermal manipulations at different time periods of embryogenesis and different temperatures [27]. The naïve chicks that experienced 3, 6 and 12 hours of thermal manipulation during embryogenesis had significantly lower T_b than the Control (N) at the age of 3 days. However, at the 4th day of age, Control chicks did not differ any more from the thermally-manipulated chicks, in the naïve as in the challenged group, with D1 chicks presenting the lowest numerical values for T_b .

Several hypotheses might explain the better acquisition of thermotolerance (i.e. lower T_b at day 3) of chicks thermally treated as embryos in comparison to control chicks: they might have developed enhanced thermolysis capacities and/or decreased thermogenesis capacities. Recent results [27] show lower T_3 concentrations in male chicks thermally treated as embryos than in control chicks, which could have an effect in reducing the intensity of energy metabolism and

thermogenesis in the treated birds. The mechanisms involved in the present changes in thermotolerance acquisition and their long-term effects still need to be further investigated.

It can be concluded that out of the four different durations of thermal manipulation, the best one to initiate improvement of thermotolerance acquisition is the one requiring 3 hours at 39.5 °C during E16 to E18, whereas the 6 and 12 hours may be the best ones to achieve high hatchability and to initiate growth. It seems that three whole days of incubation at 39.5 °C between E16 and E18 are longer than the optimal period for improving thermotolerance and/or growth. However, further research is needed to follow both responses during the whole life span of the chicks and to elucidate the underlying mechanisms.

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