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The effect of thermal manipulations during embryogenesis of broiler chicks (*Gallus domesticus*) on hatchability, body weight and thermoregulation after hatch ☆

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Abstract

The significant developments in the genetic selection of fast-growing broiler chickens cause difficulties for broilers in coping with extreme environmental conditions. This study was conducted to elucidate the effect of repetitive short-term increases in incubation temperature on hatchability and chick's body weight (BW) and thermoregulation immediately after hatch. Thermal manipulation (TM) had no effect on hatchability when the three trials were combined for statistical analysis, and had no effect on chick's BW. It caused a significant reduction in chicks T_b, and significant decline in plasma thyroid hormones concentration, but had no effect on plasma corticosterone concentration. It can be concluded that TM did not affect BW but had a positive effect on thermoregulation, most probably in reducing metabolic rate.

Keywords: Chick; Embryogenesis; Thermal manipulation; Thermoregulation; Thyroid hormones; Body temperature; *Gallus domesticus*; Bird

1. Introduction

Recent decades have seen significant developments in the genetic selection of fast-growing meat-type broiler chickens. However, this fast growth has coincided with inferior development of the visceral systems (Havenstein et al., 2003), causing significant difficulties for broiler chickens in coping with extreme environmental conditions, i.e., hot spells. To sustain thermal tolerance and to avoid the deleterious consequences of heat stress, two direct responses are elicited: the rapid thermal shock response and acclimation (Horowitz, 1998).

The rapid heat stress response was successfully modulated by early age thermal conditioning of post-natal chicks (Arjona et al., 1988, 1990; Yahav and Hurwitz, 1996; Yahav, 2000, 2002), which exploited the immaturity of the temperature regulation mechanism in young chicks during their first week of life (Dunnington and Siegel, 1984; Modrey and Nichelmann, 1992). However, post-hatch environmental temperature manipulation is complicated, whereas the use of such manipulations during incubation would be very likely to lead to a better thermal response of the embryo, followed by efficient thermal response of the growing chicken.

It was previously documented that exposing embryos to high or low temperatures during incubation improved

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their capacity to adapt to hot or cold environments, respectively, in the post-hatch phase (Decuypere, 1984; Minne and Decuypere, 1984; Janke et al., 2002).

The effects of the duration of alterations of the 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 (Holland et al., 1997), whereas a long-term increase affected the embryo morphology (Kaplan et al., 1978), increased incidences of malpositions and decreased hatchability (Romanoff, 1972; French, 1994).

Thyroid hormones are well known to affect development of chick embryos (McNabb and King, 1993). Up to half of the incubation duration, the thyroid gland appears to possess a limited capability to synthesize hormones (Thommes, 1987). However, after the hypothalamic-pituitary-thyroid axis has been linked at days 10.5–11.5 of incubation, the thyroid gland has begun to function. The concentrations of thyroid hormones increase significantly prior to hatching, in preparation for their major role in the final maturation of many tissues and their role in integrating physiological factors to achieve a successful hatch (Black, 1978; Mallon and Betz, 1982; Decuypere et al., 1992).

Another important effect of thermal manipulation (TM) is related to stress. Epple et al. (1997) suggested that the embryonic chicken is susceptible to stress. Increasing incubation temperature during the 16th day of incubation, while the hypothalamic-hypophysial-adrenal axis is being activated (Wise and Frye, 1975), may affect the stress response of the hatched chick.

The present study aimed to elucidate the effects of repetitive short-term increases in incubation temperature, prior to chick embryonic stage 45, on the hatching rate, body weight (BW) and thermoregulation of Cobb chicks.

2. Materials and methods

2.1. Experimental procedure

Three independent trials were conducted to elucidate the effects of TMs during embryogenesis on hatching rate, chick BW and thermoregulation immediately after hatch. Fertile Cobb eggs from flocks at their optimal period for egg production were used. In the first two trials, 200 eggs were weighed and statistically divided according to JMP® statistics (SAS Institute, 2000) into 10 groups of 10 eggs in each treatment. In the third trial 400 eggs were weighed and statistically divided into 20 groups of 10 eggs in each treatment.

In each trial, the eggs were arranged in homological locations in two incubators. The incubators (Masalles, Spain, Type 65Hs), were identical and automatic. Incubation conditions from day 0 to day 21 were: 37.8°C and 56% rh (Bruzual et al., 2000) for the control group. In the thermally treated eggs during E16, E17 and E18, temperature was increased to 38.5°C and rh to 65% for 3 h (09:00–12:00) on each day. Immediately after the thermal treatments were terminated, incubation conditions were restored to the regular levels. Eggs in both incubators were turned through 270° every hour. Data loggers, Microlog of Fourier Systems, UIL Ltd. were placed in each incubator to monitor the conditions every 30 min.

At the 10th day of incubation, infertile and undeveloped eggs were removed after candling. At the 19th day of incubation, the eggs were transferred to hatching trays located in each incubator.

In each trial, hatched chicks were removed continuously and alternately from both incubators, and the number of chicks hatched was recorded every hour. In the last trial, the data on the first and the last quarters of the hatched chicks of each treatment and each gender were used to compare the BWs of early- and late-hatched birds. After hatching and feather drying (approximately 2 h post-hatch), each chick was taken out of the incubator for further measurements which were conducted in the following order: T_b; BW; gender identification; and blood sampling from the jugular vein of randomly chosen chicks. Females were recognized by a "comb-like" feather arrangement (alternating heights) of the lateral wing feathers.

2.2. Blood analysis

Radioimmunoassay of thyroxin (T_4) and triiodothyronine (T_3) was performed in plasma samples, using commercial Radioimmunoassay kits: Coat-A-Count, Canine, T_4 and T_3 kits—(Diagnostic products Corporation). The T_3 assay was characterized by intra- and inter-assay variation (cv) of 7.0% and 9.4%, respectively. The T_4 assay was characterized by intra- and inter-assay variation (cv) of 5.0% and 7.5%, respectively.

Plasma corticosterone concentration was measured with the Radioimmunoassay kit with the ImmuChem™ Double Antibody (ICN Biomedical, Inc., Diagnostics Division).

2.3. Statistical analysis

Data were subjected to analysis of variance (one way ANOVA) and to Student's t test, by means of the JMP® software (SAS institute, 2000). Hatchability was analyzed with the chi-square test. A combined analysis of hatchability of the three trials was made by Mann and Whitney test. Means were considered significantly different at $P \leqslant 0.05$.

3. Results

The hatching rate was significantly enhanced by the thermal treatment in trial 1 (92.3% vs. 86.9% in the treated and control embryos, respectively). In trial 2, a significant and opposite trend was observed: 89% of treated embryos were hatched compared with 95% of the control ones. In trial 3, there was no significant difference between the treatments: hatchability was 94.6% and 96.8% among the treated and control embryos, respectively. However, a combined analysis of hatchability of the three trials had demonstrated no effect of TM on this parameter ($P = 0.827$).

The BWs of chicks that were not differentiated according to gender did not differ between treatments (Trial 1; Table 1). In Trial 2 (Table 2), thermal treatment did not affect the BW of either gender, but the males were significantly heavier than the females. In the third trial (Table 3), there were no significant differences in BW between genders or between treatments. However, when the chicks of each gender or treatment were separated according to hatching time, the late-hatching females were found to be significantly heavier than the others.

In all three trials (Tables 1, 2 and 4), the T_b of chicks that had been thermally treated during embryogenesis

Table 1
Effects of thermal manipulation (TM) of embryos at 38.5°C and 65% rh for 3 h during E16, E17, E18 of incubation, on body weight (BW), body temperature (T_b) and plasma thyroid hormone concentrations at hatch

Variables	Control	TM
BW (g)	48.65±0.38	48.85±0.35
T_b (°C)	39.37±0.06[a]	38.94±0.05[b]
T_4 (ng/ml)	5.698±0.499[a]	4.412±0.371[b]
T_3 (ng/ml)	2.508±0.158[a]	2.027±0.139[b]

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

Table 2
Effects of TM of embryos at 38.5°C and 65% rh for 3 h during E16, E17, E18 of incubation, on male and female chicks BW and body temperature (T_b) at hatch

Variables	Males		Females	
	Control	TM	Control	TM
BW (g)	55.2±0.57[a]	54.7±0.80[a]	52.8±0.82[b]	52.4±0.62[b]
T_b (°C)	39.43±0.08[a]	39.16±0.01[b]	39.54±0.09[a]	39.24±0.06[b]

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

Table 3
Effects of TM of embryos at 38.5°C and 65% rh for 3 h during E16, E17, E18 of incubation, on BW of early- and late-hatched males (EHM, LHM, respectively) and females (EHF, LHF, respectively)

Treatment	Body weight (g)					
	Males	Females	EHM	LHM	EHF	LHF
Control	51.0±0.39	49.8±0.41	50.7±0.49	51.2±0.49	48.8±0.57[b]	51.0±0.57[a]
TM	50.5±0.45	50.1±0.35	49.9±0.84	51.1±0.56	49.8±0.51	50.5±0.49

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

Table 4
Effects of TM of embryos at 38.5°C and 65% rh for 3 h during E16, E17, E18 of incubation, on body temperature (T_b) of early and late-hatched males (EHM, LHM, respectively) and females (EHF, LHF, respectively)

Treatment	T_b (°C)					
	Males	Females	EHM	LHM	EHF	LHF
Control	39.4±0.04[a]	39.5±0.06[a]	39.4±0.06[a]	39.4±0.05	39.4±0.07[a,*]	39.7±0.05[a,**]
TM	39.2±0.07[b]	39.1±0.05[b]	38.9±0.08[b,*]	39.4±0.09**	39.0±0.08[b,*]	39.3±0.08[b,**]

Within column, values designated by different letters differ significantly ($P \leqslant 0.05$).
Within rows, different number of stars, designate significant differences ($P \leqslant 0.05$) between early- and late-hatched males and females (EHM, LHM, EHF and LHF, respectively).

Table 5
Effects of TM of embryos at 38.5°C and 65% rh for 3 h during E16, E17, E18 of incubation, on plasma corticosterone and thyroid hormone concentrations of males and females after hatch

Variables	Males		Females	
	Control	TM	Control	TM
T_4 (ng/ml)	3.19±0.43	3.02±0.39	3.06±0.39	2.40±0.33
T_3 (ng/ml)	2.14±0.18[a]	1.54±0.14[b]	2.12±0.28	1.78±0.17
Corticosterone (ng/ml)	8.92±1.13	8.25±0.71	7.42±0.56	8.03±0.71

Within rows, values designated by different letters differ significantly ($P \leqslant 0.05$), $n = 15$.

was significantly lower than that of the control chicks. In Trial 3, in which chicks were divided according to early and late time of hatch, early hatched females exhibited a significantly lower T_b than the late-hatched chicks. Thermally treated males exhibited similar trend, but control males had a similar T_b, that was not affected by hatching time.

Plasma thyroid hormone concentrations were significantly lower in chicks that had been thermally treated during embryogenesis than in the control ones (Trial 1, Table 1). In Trial 3 (Table 5), only the plasma T_3 concentration of the male treated group was significantly lower. No significant differences in plasma corticosterone concentration were observed (Table 5).

4. Discussion

One of the major concerns in dealing with domestic animals is how to maintain or even improve performance when various manipulative treatments are being applied.

It was well documented previously that prolonged heat exposure during the first week of the chicks' life (Yahav and Hurwitz, 1996), or exposure to extremely high ambient temperatures (Yahav and McMurtry, 2001) adversely affected the performance of broiler chickens, although it improved their thermotolerance. On the other hand, application of a moderate thermal treatment not only increased thermotolerance, but it also significantly improved performance (Yahav, 2000; Halevy et al., 2001).

In the present study, a moderate thermal treatment was used (38.5°C; 65% rh) to minimize any deleterious effect of the treatment on hatchability and BW of the chicks after hatch. Indeed out of the three trials, one demonstrated significant increase in hatching rate, the second exhibited a significant reduction in hatching rate, and in the third no differences in hatchability between treatments were observed. However, a combined statistic analysis had shown no effect of the treatment on hatchability. The differences may result therefore from a small number of eggs per treatment.

As for chick's BW immediately after hatch, in the present study no significant effect of the thermal treatment during incubation was observed in any of the three trials. The significant differences observed in trial 2 were related to the genders. Previous results on BW differences between males and females after hatching were conflicting. Some authors (Godfrey and Jaap, 1952; Khan et al., 1975; Whiting and Pesti, 1983) found male chicks to be heavier than females, but others found no gender differences in BW of day-old chicks (Marks, 1986; Burke, 1992; Reis et al., 1997). In Trial 3, the significant differences in BW between the early and late-hatching control females must also be considered in light of conflicting results in the literature (Hager and Beane, 1983; Reis et al., 1997).

In the present study, the goal of TM during E16–E18 was to elucidate the possibility of changing the embryo's threshold for response to a hot environment, before the main activities related to thyroid hormones and preparation for hatching increased significantly (Decuypere et al., 1992), and such changes were observed in chicks during their first week of life. One of the mechanisms that induced thermotolerance involved the modulation of heat production, as a result of a significant and sustained reduction in plasma T_3 levels (Yahav and Hurwitz, 1996; Yahav and McMurtry, 2001). The finding that chicks could reduce plasma T_3 concentration further during thermal challenge suggested that there was a reduction in metabolic rate that led to improved thermotolerance acquisition (Yahav, 2000).

In the present study, the plasma concentration of thyroid hormones (T_4 and T_3) was lower to significantly lower in thermally treated than in control embryos, immediately after hatch. The reduction in plasma T_4 concentration suggested that there was a decline in the thyroid gland activity, whereas the decline in T_3 could be attributed to reduced peripheral deiodination activity. These results, together with the finding of a significant decline in body temperature of the chicks which had been thermally treated during embryogenesis, suggest that there was a reduction in the metabolic rate of the chicks, and may support the hypothesis of a change in

the threshold of the response of the animals to heat. However, this must be further studied.

Chicks that were thermally conditioned with low (Shinder et al., 2002) or elevated ambient temperatures (Yahav, unpublished data) during the first week of life subsequently exhibited reduced levels of the hormone corticosterone, which suggests a possible change in the "set point" of response to stress. However, in the present study, although the incubation temperature was increased during the period of hypothalamic-hypophyseal-adrenal activation, there was no effect of the treatment on the post-hatch plasma corticosterone concentration, which suggests that the treatment had no effect on the stress response of 1-day-old chicks.

It can be concluded that thermal treatments during E16–E18 of the chick's embryogenesis, did not affect hatchability and BW of the 1-day-old chick. The findings reinforced the evidence for a positive effect on thermoregulation, with reductions in T_b and in the plasma thyroid hormone concentrations, which most probably resulted in a reduced metabolic rate. It can also be speculated that this treatment did not alter the stress response of the hatched chicks.

5. Summary

TM (altering incubation temperature and relative humidity to 38.5°C and 65%, respectively, for 3 h in each of the 16th, 17th and 18th days of incubation) during broilers embryogenesis had no effect on the hatchability of the combined trials, and no effect on BW of the hatched chicks. However, in all experiments, T_b of chicks exposed to TM during incubation was significantly lower than that of the control ones. Both thyroid hormones were lower to significantly lower in the treated chicks, whereas there was no effect on plasma corticosterone concentration. It can be concluded that TM during the 16–18 days of incubation, did not affect BW of the 1-day-old chicks, but had a controversial effect on hatchability. However, it had a positive effect on thermoregulation, by causing a reduction in T_b and in plasma thyroid hormone concentrations, which most probably resulted in a reduced metabolic rate. It can be further speculated that the thermal treatment did not alter the stress response of hatched chicks.

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