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# Thermal manipulation of the embryo modifies the physiology and body composition of broiler chickens reared in floor pens without affecting breast meat processing quality<sup>1</sup>

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**ABSTRACT:** Selection in broiler chickens has increased muscle mass without similar development of the cardiovascular and respiratory systems, resulting in limited ability to sustain high ambient temperatures. The aim of this study was to determine the long-lasting effects of heat manipulation of the embryo on the physiology, body temperature (T<sub>b</sub>), growth rate and meat processing quality of broiler chickens reared in floor pens. Broiler chicken eggs were incubated in control conditions (37.8°C, 56% relative humidity; RH) or exposed to thermal manipulation (TM; 12 h/d, 39.5°C, 65% RH) from d 7 to 16 of embryogenesis. This study was planned in a pedigree design to identify possible heritable characters for further selection of broiler chickens to improve thermotolerance. Thermal manipulation did not affect hatchability but resulted in lower T<sub>b</sub> at hatching and until d 28 post-hatch, with associated changes in plasma thyroid hormone concentrations. At d 34, chickens were exposed to a moderate heat challenge (5 h, 32°C). Greater O<sub>2</sub> saturation and reduced CO<sub>2</sub> partial pressure were observed

( $P < 0.05$ ) in the venous blood of TM than in that of control chickens, suggesting long-term respiratory adaptation. At slaughter age, TM chickens were 1.4% lighter and exhibited 8% less relative abdominal fat pad than controls. Breast muscle yield was enhanced by TM, especially in females, but without significant change in breast meat characteristics (pH, color, drip loss). Plasma glucose/insulin balance was affected ( $P < 0.05$ ) by thermal treatments. The heat challenge increased the heterophil/lymphocyte ratio in controls ( $P < 0.05$ ) but not in TM birds, possibly reflecting a lower stress status in TM chickens. Interestingly, broiler chickens had moderate heritability estimates for the plasma triiodothyronine/thyroxine concentration ratio at d 28 and comb temperature during the heat challenge on d 34 ( $h^2 > 0.17$ ). In conclusion, TM of the embryo modified the physiology of broilers in the long term as a possible adaptation for heat tolerance, without affecting breast meat quality. This study highlights the value of 2 new heritable characters involved in thermoregulation for further broiler selection.

**Key words:** body composition, broiler chicken, heat stress, meat quality, respiratory physiology, thermal manipulation of embryos

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## INTRODUCTION

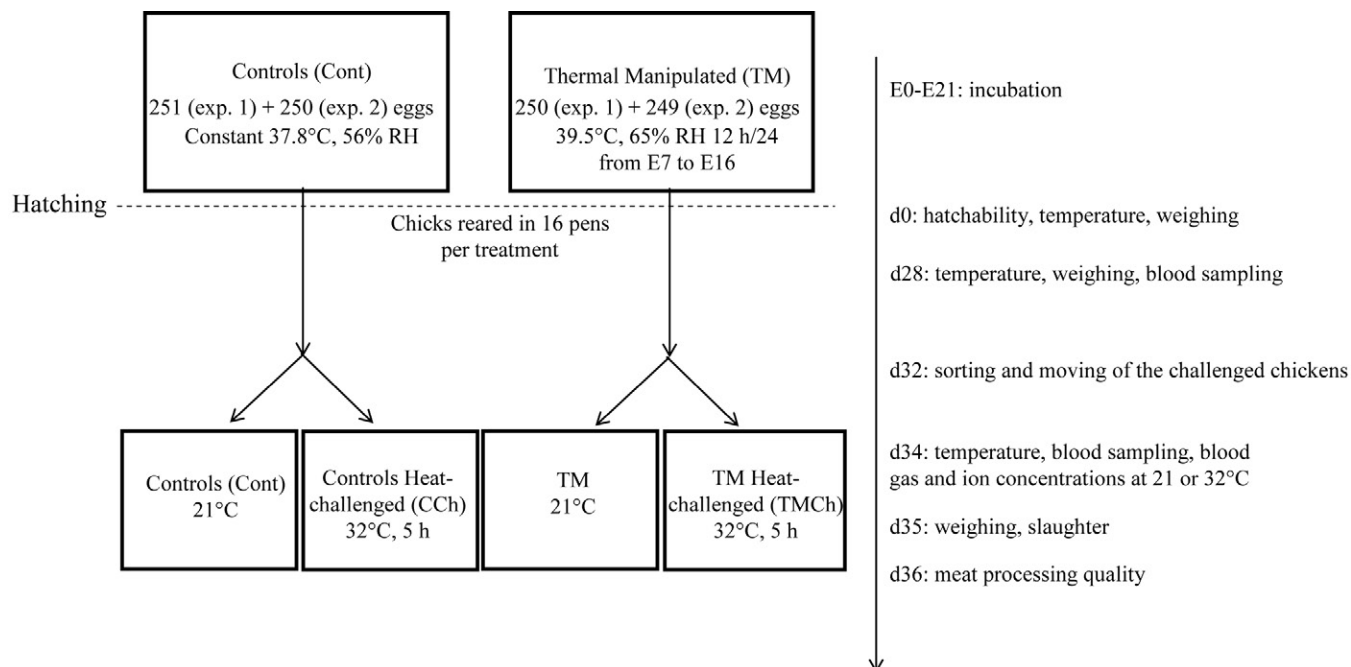
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Genetic selection has increased body and muscle mass of fast-growing chickens over the last 50 yr. However, their cardiovascular and respiratory systems have not developed as well (Havenstein et al., 2003a,b), resulting in an impaired ability to cope with high ambient temperatures. Heat exposure induces hyperthermia, modifies respiratory and ion physiology, feed consumption and growth (Yahav, 2009) and increases the heterophil/lymphocyte ratio marker of stress (Altan et al., 2000). High temperatures change



**Figure 1.** Flow chart of the experimental design including 2 successive experiments (Exp. 1 and 2). The abbreviation Cont corresponds to control chickens incubated in standard conditions (37.8°C and 56% relative humidity (RH)) and TM to chickens subjected to thermal manipulation during incubation (39.5°C and 65% RH 12 h/d from days of embryogenesis E7 to E16), both groups being kept at 21°C at d 34. Abbreviations CCh and TMCh correspond to control and TM chickens submitted to a heat challenge (32°C for 5h) at d 34, respectively. E = embryonic day.

the hormonal and metabolic status (Geraert et al., 1996; Piestun et al., 2008; Boussaid-Om Ezzine et al., 2010) and meat processing quality of birds (Debut et al., 2005) and increase muscle oxidative stress (Mujahid et al., 2007).

Several studies have evaluated the effects of thermal manipulation of the embryo (TM) on embryo physiology (Moraes et al., 2003, 2004), subsequent thermotolerance and growth performance (Yahav et al., 2004a,b; Collin et al., 2005), meat yield and processing quality (Collin et al., 2007). However, TM during mid [Embryonic day (E)8 to E10] or late (E16 to E18) embryogenesis was not found to improve thermotolerance in the long term (Collin et al., 2007; Tona et al., 2008). Thermal manipulation at 39.5°C and 65% relative humidity for 12 h/d from d 7 to 16 of embryogenesis was recently reported to improve heat tolerance without decreasing growth in caged broilers. This was associated with lower body temperature until slaughter age (Piestun et al., 2008, 2009a). However, no information is available on the consequences of such effective TM on the performance, meat quality and physiology of floor-reared chickens.

The aim of our study was therefore to test the effects of TM during embryogenesis and heat challenge at slaughter age on growth, feed efficiency, body temperature and composition, meat processing quality and physiological parameters in chickens reared in semi-commercial conditions. Using a pedigree design, we estimated the heritability of these traits to check which could be used in selection schemes.

## MATERIALS AND METHODS

All experiments were performed in accordance with the legislation governing the ethical treatment of birds and were approved by the Ethics Committee (Comité d'Ethique en Expérimentation Animale Val de Loire, Tours, France, N° 2010–12 and 2011–9).

### Experimental Design

Two successive batches of fertile Cobb 500 broiler eggs crossbred from 72 hens (38 to 45 wk of age) and 19 males from the 2 parental lines used to produce the Cobb 500 commercial birds at INRA were obtained in a pedigree design. For each experiment, around 500 eggs were divided into 2 groups (Control and TM) with similar BW and genetic origin (Fig. 1) and were incubated in 2 semi-commercial automatic incubators (type 360 E, SMA Coudelou, Rochecorbon, France). Control eggs (Cont) were maintained at 37.8°C and 56% relative humidity (RH) during the whole incubation period (Bruzual et al., 2000). Thermal manipulation was applied at 39.5°C and 65% RH for 12 h/24, from E7 to E16 (Piestun et al., 2008). All eggs were turned through 90° every hour.

At E7 of incubation, infertile eggs and early dead embryos were identified by candling. From E19 to E21 of incubation, eggs were exposed to hatching conditions (37.6°C at 70% RH). At hatching (E21),

after the feathers of the chicks had dried, each one was taken out of the incubator for immediate measurement of body temperature and BW. Body temperature (**T<sub>b</sub>**) was measured using a digital thermometer inserted into the distal colon (Tex, Pelimex, France) in 33 to 35 chicks per treatment.

Chicks of each treatment were transferred to a single poultry house divided into 16 pens (i.e., 8 pens per treatment equally distributed from d 0 to d 32). The temperature was gradually decreased from 33°C on d 0 to 21°C on d 25 and remained at 21°C. Water and standard feeds (21.7% CP, 2992 kcal/kg from d 0 to d 28, and 20% CP, 3100 kcal/kg from d 28 to d 35) were supplied ad libitum. On d 32, Control and TM birds were divided into 2 groups: a heat-challenged and a nonchallenged group. All birds were put in crates and sorted within pens considering their parental origin. The challenged group was transferred to a second thermally-controlled room. After a 2 d resting period after moving, heat-challenged chickens were exposed to 32°C for 5 h at d 34 whereas nonchallenged chickens remained under the standard conditions.

#### **Growth Performance and Thermotolerance Variables**

Body weights of all chickens were measured at d 28 and d 35, and feed consumption per pen was measured during both the starting and the finishing periods. Body temperature (**T<sub>b</sub>** measured in distal colon) and comb temperature (an indicator of heat dissipation) of male chickens were measured at d 28 and d 35 at 21°C and during the heat challenge (32°C) using an electronic thermometer (Testo 110, Testo, Germany) and an infrared thermometer (TES 1326S, TES, Taipei Taiwan), respectively. Measurements were performed only in males because of their greater sensitivity to heat than females (Piestun et al., 2008).

#### **Measurement of Blood Variables**

**Blood Gas and Electrolytes.** At d 34 total blood was collected on heparin from the brachial veins of male control and TM chickens exposed to 21°C or 32°C ( $n = 9$  to 11/treatment). Blood gases and electrolytes were immediately analyzed using a blood analysis system (True Point, IRMA, ITC Nexus DI, Edison, NJ). Partial pressure of carbon dioxide (**pCO<sub>2</sub>**), and oxygen (**pO<sub>2</sub>**), pH, hematocrit (**Hct**), sodium (**Na<sup>+</sup>**), potassium (**K<sup>+</sup>**), ionized calcium (**iCa**), bicarbonate (**HCO<sub>3</sub><sup>-</sup>**), total carbon dioxide (**TCO<sub>2</sub>**) concentrations, base excess in blood (**bEb**), base excess in extra-cellular fluid (**Becf**), oxygen saturation percentage (**O<sub>2</sub>Sat**), and total hemoglobin (**tHb**) were measured on total blood.

**Plasma Hormone and Metabolite Concentrations at d 28 and d 34.** Four mL of blood was collected on EDTA from all male broiler chickens at 28 d of age (152 to 170 per treatment) and from 9 to 11 male chickens/treatment at 34 d. Blood was centrifuged at 3000 × *g* for 10 min at 4°C to measure plasma concentrations of thyroid hormones, corticosterone, insulin, uric acid, triglycerides, and glucose. Plasma triiodothyronine (**T<sub>3</sub>**) and thyroxine (**T<sub>4</sub>**) concentrations were measured by RIA as described by Darras et al. (1996). The antisera for **T<sub>3</sub>** and **T<sub>4</sub>** were purchased from Byk-Belga (Brussel, Belgium). The intra-assay CV were 4.5% and 5.4% for **T<sub>3</sub>** and **T<sub>4</sub>**, respectively. Plasma corticosterone concentrations were measured using a commercially available double antibody RIA-kit (n° 07-120103, MP Biomedicals, NY, USA). Plasma insulin concentrations were determined by RIA with a guinea pig anti-porcine insulin antibody (Ab 27–6, generously provided by G. Rosselin, Saint-Antoine Hospital, Paris, France) using chicken insulin as the standard (Ruffier et al., 1998). All samples were diagnosed within the same assay to avoid inter-assay variations. Plasma uric acid, triglyceride and glucose concentrations were measured enzymatically using commercial colorimetric kits (Biomerieux, Marcy l'Etoile, France).

**Blood Cell Count.** Four mL of venous blood was sampled in EDTA at d 34 and was centrifuged at 300 × *g* for 15 min at 4°C. A smear of leucocytes and platelets was spread on a slide and immersed in May Grunwald reagent (VWR International, Fontenay-sous-bois, France) for 3 min and washed in May Grunwald reagent half-diluted with neutral water for 3 min. Slides were then immersed in a Giemsa solution (VWR International, Fontenay-sous-bois, France) for 30 min and rinsed with neutral water. Percentages of neutrophil, eosinophil, basophil, lymphocyte and monocyte cells were calculated after cell counting using an optic microscope (Axioplan 2, Zeis, France). The heterophil/lymphocyte ratio was then calculated as a reliable indicator of stress (Altan et al., 2000).

#### **Body Composition and Breast Muscle Traits**

At d 35, after 8 h of feed withdrawal, all chickens from each treatment were slaughtered at the INRA experimental processing plant. Birds were stunned in a water bath (125 Hz AC, 80 mA/bird, 5s) and killed by ventral neck incision. Carcasses were then processed as described by Berri et al. (2007). After a night in a cold chamber, the right breast Pectoralis (**P.**) major and P. minor muscles and abdominal fat (total of the fat tissue lining the abdominal cavity from the cloaca until around the gizzard and esophagus) were excised and weighed. Breast muscle yield (without bone and without skin) was measured as the ratio of P. major and P. minor muscle mass to total BW at d 35. The ultimate

pH (**pHu**) of the P. major muscle was measured by direct insertion of a pHmeter electrode in the muscle. Breast meat color was measured on the upper ventral side of the P. major muscle using a Miniscan Spectrocolorimeter. Color was measured by the CIELAB system where L\* represents lightness, a\* redness, and b\* yellowness of meat. Pectoralis major muscle drip loss was measured 5 d postmortem as described by Berri et al. (2007). Lipid oxidation in the breast muscle was measured by spectrophotometric thiobarbituric acid tests (**TBARS**) to estimate the degree of meat oxidation in both normal and pro-oxidant conditions. More precisely, 1 g of muscle was homogenized in 10 mL of 0.15 M KCl + 0.1 mM butylhydroxytoluene, and held in boiling water for 10 min with 1% (wt/vol) 2-thiobarbituric acid in 50 nM NaOH, and 2.8% (wt/vol) trichloroacetic acid (Lynch and Frei, 1993). After cooling at room temperature, the pink chromogen was extracted with n-butanol and its absorbance was measured at 535 nm. The TBARS were calculated using 1,1,3,3-tetramethoxypropane. The TBARS were also measured after oxidation with FeSO<sub>4</sub> (Kornbrust and Mavis, 1980): 1 g of P. major muscle was incubated in 1.1 mM FeSO<sub>4</sub> and 0.4 mM ascorbic acid for 1 h at 37°C followed by the addition of 8.5 mL of KCl 0.15 M and 0.1 mM butylhydroxytoluene.

### Statistical and Genetic Analysis

Zootechnical and physiological parameters were analyzed using the GLM procedure (SAS Inst. Inc., Cary, NC) with model [1] for traits recorded in males at 28 d, model [2] for traits recorded in males at 34 d, and with model [3] for traits recorded in males and females at 35 to 36 d, as follows:

$$y_{ijklmn} = \mu + T1_i + T2(T1)_{ij} + S_k + E_l + B_m + e_{ijklmn} \quad [1]$$

$$y_{iklmn} = \mu + T1_i + S_k + E_l + B_m + e_{iklmn} \quad [2]$$

$$y_{ijklmno} = \mu + T1_i + T2(T1)_{ij} + S_k + E_l + B_m + G_n + T1(G)_{in} + T2(T1, G)_{ijn} + e_{ijklmno} \quad [3]$$

where  $y_{i(j)klmn(o)}$  is the performance of animal n(o) at 34 d (equations [1] and [3]) or at 28 d (Eq. [2]),  $\mu$  the general mean,  $T1_i$  the fixed effect of incubation treatment ( $i = TM, Cont$ ),  $T2(T1)_{ij}$  the fixed effect of heat challenge  $j$  nested within incubation treatment  $i$  ( $j =$  heat challenged or not heat challenged),  $S_k$  the fixed effect of side in the room  $k$  ( $k =$  left or right),  $E_l$  the fixed effect of experiment  $l$  ( $l = 1, 2$ ),  $B_m$  the fixed effect of block  $m$  ( $m = 1$  to 4),  $G_n$  the fixed effect of gender  $n$ ,  $T1(G)_{in}$  the fixed effect of incubation treatment  $i$  nested within gender  $n$ ,  $T2(T1, G)_{ijn}$  the fixed effect of heat challenge  $j$  nested within

**Table 1.** Effects of thermal manipulation during incubation on zootechnical variables from hatching to d 28

Item	Treatment <sup>1</sup>		P-value
	Cont	TM	
No. of eggs	501	499	
Hatchability, % fertile eggs	86.13	83.19	0.2208
Hatching BW, g	48.1 ± 0.2	47.6 ± 0.2	0.4182
Feed conversion ratio d0-d 28, g/g	1.49 ± 0.02	1.47 ± 0.02	0.8412
BW d 28, g	1525 ± 14	1534 ± 14	0.4143

<sup>1</sup>Cont = chickens incubated in control conditions [37.8°C and 56% relative humidity (RH)] and TM = birds subjected to thermal manipulation during incubation (TM, 39.5°C and 65% RH 12h/d from E7 to E16). Data are presented as means ± SEM.

incubation treatment  $i$  and gender  $k$ , and  $e_{i(j)klmn(o)}$  the residual pertaining to animal  $n(o)$ .

Results are presented as least square means (**lsmeans**) of main effects. The  $T_4$ ,  $T_3/T_4$ , corticosterone and insulin concentration data were log-transformed before being statistically analyzed. Genetic parameters were estimated by REML with the same effects as in GLM and an additive genetic effect for animal with Variance Component Estimation 6 (**VCE6**; Groeneveld et al., 2010). The pedigree file included 795 birds. Due to the low number of birds for such an analysis, 3-trait analyses were performed, always including BW at d 35 to avoid bias due to selection in this genotype selected for rapid growth. All the possible 2-trait combinations of the other traits were also tested to obtain genetic correlations between traits. The estimates of heritability and their SE presented below are the mean of estimates (of heritability and of SE) of each analysis. Heritability estimates were consistent between analyses, whatever the trait combination used. In contrast, due to the very low heritability of the majority of recorded traits and to the low number of birds, genetic correlation estimates could not be considered as reliable in this experiment and are not presented below.

## RESULTS

### Effects of TM on Zootechnical Parameters from Hatching to d 28

Hatchability of control and TM chickens did not differ ( $P > 0.10$ ; Table 1). No significant difference ( $P > 0.10$ ) in BW was observed between treatments at hatching and at d 28. The feed conversion ratio was not affected ( $P > 0.10$ ) by incubation conditions during this period.

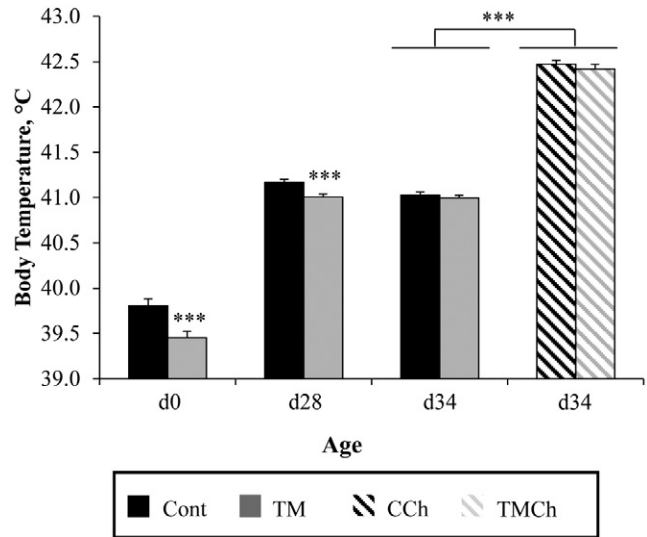
### Body Temperatures

At hatching, the Tb of TM chicks was significantly lower ( $P < 0.0001$ ; Fig. 2) than those of the controls

(39.4 and 39.8°C, respectively). Thermally-manipulated chickens maintained lower Tb than controls until d 28 ( $P < 0.0001$ ). At d 34, heat-challenged chickens developed significant hyperthermia compared with chickens kept at 21°C. However, there was no significant difference in Tb between TM and control chickens in either condition. Comb temperatures were not different between TM and control chickens at d 28 and d 34 at 21°C or at 32°C ( $P > 0.10$ ; data not shown).

### Blood Gas and Ion Concentrations at d 34

As heat modifies plasma gas and ion concentrations (Arad and Marder, 1983) and acid/base ratios, we measured these variables in all groups to identify possible adaptive mechanisms (Table 2). There was no significant long-term effect of incubation conditions during embryogenesis on  $\text{HCO}_3^-$ ,  $\text{TCO}_2$ ,  $\text{pO}_2$ , hematocrit,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{iCa}$ ,  $\text{Beb}$ , or  $\text{THb}$  in the blood of male chickens at d 34 ( $P > 0.10$ ). On the other hand,  $\text{O}_2$  blood saturation and  $\text{pCO}_2$  were greater and less in TM than in control chickens, respectively ( $P < 0.05$ ). There was also a tendency for pH to increase in the blood of TM compared with controls ( $P = 0.056$ ). The heat challenge within incubation conditions did not affect pH,  $\text{pO}_2$ ,  $\text{O}_2$  saturation, hematocrit,  $\text{Na}^+$ ,  $\text{K}^+$ , or  $\text{THb}$ . However, heat-challenged chickens exhibited significantly less  $\text{HCO}_3^-$ ,  $\text{pCO}_2$ ,  $\text{TCO}_2$ ,  $\text{Beb}$ , and  $\text{Beef}$  concentrations and  $\text{iCa}$  values than control chickens ( $P < 0.001$ ).



**Figure 2.** Body temperatures of male broiler chickens from hatching until d 34 of age according to incubation and heat challenge conditions. The abbreviation Cont corresponds to control chickens incubated in standard conditions [37.8°C and 56% relative humidity (RH)] and TM to chickens subjected to thermal manipulation during incubation (39.5°C and 65% RH 12 h/d from E7 to E16 of embryogenesis), both groups being kept at 21°C at d 34. Abbreviations CCh and TMCh correspond to control and TM chickens submitted to a heat challenge (32°C for 5 h) at d 34, respectively. \*\*\* $P < 0.0001$ .

### Plasma Hormone and Metabolite Concentrations

On d 28, we observed a significant decrease in plasma  $\text{T}_3$  concentrations ( $P < 0.05$ ) and  $\text{T}_3/\text{T}_4$  ratios ( $P < 0.01$ ) and a significant increase ( $P < 0.01$ ) in plasma  $\text{T}_4$  concentrations in TM broilers as compared with controls (Table 3). However, on d 34 incubation conditions had no effect on  $\text{T}_3$ ,  $\text{T}_4$ , and  $\text{T}_3/\text{T}_4$  concentrations. Heat

**Table 2.** Venous blood gas and ion concentrations in male broiler chickens at d 34

Blood parameter <sup>2</sup>	Treatment <sup>1</sup>				Incubation effect	Challenge (incubation) effect
	Cont (n = 10)	CCh (n = 10)	TM (n = 11)	TMCh (n = 9)		
pH, IU	7.31 ± 0.02	7.32 ± 0.02	7.35 ± 0.01	7.34 ± 0.01	0.0564	0.9862
$\text{HCO}_3^-$ , mM	29.0 ± 0.7a	25.4 ± 0.7b	28.5 ± 0.6a	24.9 ± 0.7b	0.4818	< 0.0001
$\text{pCO}_2$ , mm Hg	58.3 ± 2.3a	50.7 ± 2.2c	53.1 ± 2.1b	46.1 ± 2.3d	0.0354	0.0094
$\text{TCO}_2$ , mM	30.8 ± 0.7a	26.9 ± 0.7b	30.2 ± 0.7a	26.3 ± 0.7b	0.3878	< 0.0001
$\text{pO}_2$ , mm Hg	39.0 ± 1.1	51.9 ± 1.1	53.9 ± 1.1	46.9 ± 1.1	0.3317	0.1607
$\text{O}_2$ Sat, %	66.7 ± 4.3	75.0 ± 4.1	82.0 ± 3.9	78.6 ± 4.3	0.0278	0.3218
Hematocrit, IU	21.4 ± 1.2	23.0 ± 1.1	22.5 ± 1.0	20.3 ± 1.2	0.4681	0.2432
$\text{Na}^+$ , mM	147.9 ± 0.6	147.6 ± 0.6	147.7 ± 0.6	148.1 ± 0.6	0.7476	0.8311
$\text{K}^+$ , mM	6.32 ± 0.24	6.45 ± 0.23	6.02 ± 0.22	6.48 ± 0.24	0.5484	0.3455
$\text{iCa}$ , mM	1.57 ± 0.02a	1.52 ± 0.01b	1.60 ± 0.01a	1.51 ± 0.02b	0.3953	< 0.0001
$\text{Beb}$ , mM	1.92 ± 0.68a	-0.8 ± 0.64b	2.32 ± 0.61a	-0.57 ± 0.67b	0.3990	0.0005
$\text{Beef}$ , mM	2.59 ± 0.75a	-0.99 ± 0.71b	2.65 ± 0.68a	-1.00 ± 0.75b	0.9695	< 0.0001
$\text{THb}$ , g/dl	7.27 ± 0.40	7.82 ± 0.37	7.65 ± 0.36	6.89 ± 0.39	0.4638	0.2353

<sup>1</sup>Cont = chickens incubated in control conditions (37.8°C and 56% relative humidity (RH)) and CCh = control chickens submitted to a heat challenge (32°C for 5h) at d 34. TM = chickens who received thermal manipulation during incubation (39.5°C and 65% RH 12h/d from E7 to E16) and TMCh = TM chickens submitted to a heat challenge (32°C for 5h) at d 34. Variables were analyzed using the GLM procedure (SAS Inst. Inc., Cary, NC) with incubation as main effect and heat challenge within incubation condition as nested effect.

<sup>2</sup> $\text{HCO}_3^-$  = bicarbonate concentration,  $\text{pCO}_2$  = partial pressure of carbon dioxide,  $\text{TCO}_2$  = total carbon dioxide,  $\text{pO}_2$  = partial pressure of oxygen,  $\text{O}_2$  Sat = oxygen saturation;  $\text{Na}^+$  = sodium concentration,  $\text{K}^+$  = potassium concentration,  $\text{iCa}$  = ionized calcium concentration,  $\text{Beb}$  = base excess in blood,  $\text{Beef}$  = base excess in extracellular fluid and total hemoglobin (THb) of male broiler chickens at d 34. Data are presented as  $\text{lsmeans} \pm \text{SEM}$ . Different letters indicate significant differences ( $P < 0.05$ ) between treatments (a-d) when the challenge(incubation) effect alone or both incubation and challenge(incubation) effects were significant.

**Table 3.** Plasma concentrations of thyroid hormone [triiodothyronine ( $T_3$ ), thyroxine ( $T_4$ ) and  $T_3/T_4$  ratio), corticosterone, insulin and blood metabolites (uric acid, triglycerides, and glucose) in male broiler chickens subjected to different incubation conditions with or without heat challenge at d 34

Blood parameters	Day of sampling								Challenge (incubation) effect
	d 28			d 34					
	Treatment <sup>1</sup>		Incubation effect	Treatment <sup>1</sup>			Incubation effect	Challenge (incubation) effect	
Cont ( <i>n</i> = 170)	TM ( <i>n</i> = 155)	Cont ( <i>n</i> = 10)		CCh ( <i>n</i> = 10)	TM ( <i>n</i> = 11)	TMCh ( <i>n</i> = 9)			
<b>Hormones</b>									
$T_3$ , nmol/L	2.71 ± 0.05	2.55 ± 0.05	0.0339	2.56 ± 0.17b	1.59 ± 0.13a	2.75 ± 0.17b	1.68 ± 0.18a	0.3670	< 0.0001
$T_4$ , nmol/L	7.67 ± 0.28	8.63 ± 0.32	0.0042	8.56 ± 0.51	11.89 ± 1.29	8.55 ± 0.99	10.43 ± 1.19	0.3800	0.0763
$T_3/T_4$	0.34 ± 0.01	0.29 ± 0.01	0.0010	0.30 ± 0.02a	0.15 ± 0.02b	0.39 ± 0.07a	0.19 ± 0.04b	0.3234	< 0.0001
Corticosterone, nmol/L	34.5 ± 1.8	31.8 ± 1.8	0.0892	22.5 ± 3.4B	38.2 ± 3.4A	21.4 ± 3.3BC	31.5 ± 3.4ABC	0.4403	0.0187
Insulin, $\mu$ U/mL	97.8 ± 5.3	106.2 ± 5.5	0.2678	85.3 ± 13.7a	30.3 ± 13.7b	85.4 ± 13.1a	42.8 ± 14.5b	0.6515	0.0042
<b>Metabolites</b>									
Uric acid, mmol/L	404 ± 8	396 ± 9	0.5346	372 ± 24b	236 ± 24a	326 ± 23b	230 ± 25a	0.2773	< 0.0001
Triglycerides, mmol/L	1.62 ± 0.047	1.61 ± 0.04	0.8014	1.72 ± 0.12a	1.05 ± 0.12b	1.65 ± 0.11a	1.04 ± 0.13b	0.7310	< 0.0001
Glucose, mmol/L	15.81 ± 0.04	16.42 ± 0.04	0.0601	13.49 ± 0.32b	13.38 ± 0.17b	13.09 ± 0.24b	16.99 ± 0.24a	0.0063	< 0.0001

<sup>1</sup>Cont = chickens incubated in control conditions (37.8°C and 56% relative humidity (RH)) and CCh = control chickens submitted to a heat challenge (32°C for 5h) at d 34. TM = chickens who received thermal manipulation during incubation (TM, 39.5°C and 65% RH 12h/d from E7 to E16) and TMCh = TM chickens submitted to a heat challenge (32°C for 5h) at d 34. Data are presented as lsmeans ± SEM. Different letters indicate significant differences ( $P < 0.05$ ) between treatments (a,b) or ( $P < 0.1$ ) between treatments (A-C) when both incubation and challenge(incubation) effects or challenge(incubation) effect alone were significant.

challenge within incubation conditions decreased  $T_3$  ( $P < 0.0001$ ) and  $T_3/T_4$  concentrations ( $P < 0.0001$ ) in TM and control chicks, whereas  $T_4$  remained unchanged.

At d 28 and d 34 there was no significant effect of incubation conditions on plasma corticosterone concentrations. On d 34, the challenge increased corticosterone concentrations in both TM and control chickens, but to a slightly lesser extent in TM than in control chickens ( $P = 0.09$  between CCh and Cont values). Insulin concentrations were not modified at d 28 or d 34 whatever the incubation conditions, whereas they were decreased by the heat challenge within incubation conditions at d 34.

Incubation conditions did not affect ( $P > 0.10$ ) plasma uric acid concentrations at d 28 or d 34 although this variable was decreased by the heat challenge within incubation conditions at d 34 ( $P < 0.001$ ). There was no effect of TM on plasma triglyceride concentrations at d 28 or d 34 but this variable decreased in both heat-challenged groups at d 34 ( $P < 0.001$ ). At d 28, there was a trend for glycemia to be greater in the TM than in the control group ( $P = 0.06$ ). At d 34, TM chickens exposed to the heat challenge exhibited greater glucose concentrations than the other 3 groups.

### Blood Cell Count

The heterophil/lymphocyte (H/L) and basophil/total leucocyte ratios at d 34 are presented in Fig. 3. The H/L ratio was significantly increased by the heat challenge in control male broilers ( $P < 0.05$ ), whereas no effect of the heat challenge on this variable was observed in TM chickens ( $P > 0.10$ ). Thermal manipulation significantly

increased the basophil percentage at 21°C ( $P < 0.05$ ) but not during the heat challenge. Relative percentages of monocytes and eosinophils did not differ between groups ( $P > 0.10$ ; data not shown).

### Body Composition and Breast Meat Quality at Slaughter Age

A slight but significant effect of both incubation condition and heat challenge within incubation conditions was observed on BW at d 35 (Table 4): TM chickens exhibited a 1.4% lighter BW ( $P < 0.05$ ) than control chickens. However, this change in BW was not associated with any change in feed consumption between treatments during the heat challenge (data not shown). Breast yield was significantly increased by TM (especially in females,  $P < 0.05$ ), whereas the relative percentage of abdominal fat was decreased by TM ( $P < 0.05$ ). No major effect ( $P > 0.10$ ) of incubation conditions was measured on pHu, drip loss or the lipid peroxidation of meat measured by TBARS, either in standard or in pro-oxidant conditions. A slight effect of the heat challenge within incubation conditions on pHu ( $P < 0.05$ ) was observed, with greater values measured in heat-challenged chickens than in controls.

### Genetic Traits of Zootechnical Parameters

The genetic heritability of performance traits, temperatures, meat quality, body composition, blood hormones and metabolites are presented in Table 5. As expected, moderate to high heritability ( $> 0.2$ ) was observed for BW, meat quality, breast yield and relative abdominal fat con-



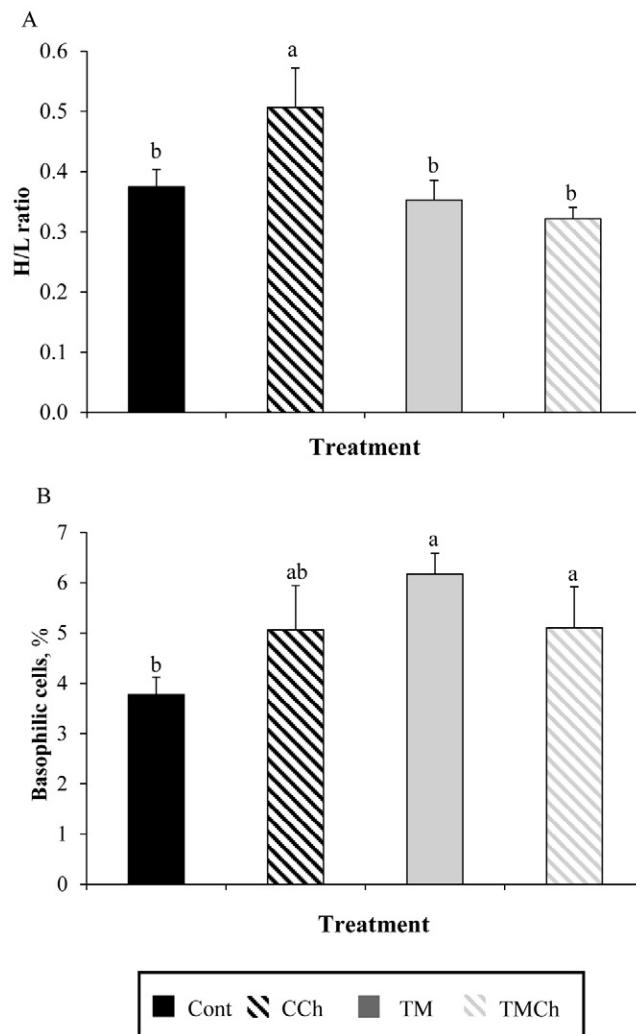
tent at d 35. Interestingly, we also observed moderate but significant heritability estimates for plasma  $T_3/T_4$  ratio at d 28, and comb temperature at d 34 during the heat challenge although, due to the low number of birds studied, the SE of estimates were large. However, low heritability values ( $<0.15$ ) were estimated for body and comb temperatures at 21°C, drip loss and plasma metabolite, insulin,  $T_3$  and  $T_4$  concentrations at 21°C.

## DISCUSSION

This study explored the effects of elevating temperature and relative humidity during incubation on zootechnical and physiological parameters in chickens reared in semi-commercial conditions in floor pens from d 0 to d 35 post-hatch. It was previously hypothesized that fluctuating temperatures during incubation mimicking natural incubation conditions could increase the adaptive capacities of chickens (Decuyper, 1984; Minne and Decuyper, 1984; Iqbal et al., 1989). Exposing embryos to high or low temperatures during incubation may therefore improve their ability to cope with cold (Nichelmann et al., 1994; Tzschentke and Basta, 2002; Shinder et al., 2011) or hot environments (Janke et al., 2002; Yahav et al., 2004a,b; Collin et al., 2007). In particular, increasing the incubation temperature during the setting up of both the hypothalamus-pituitary-thyroid axis (thermoregulation) and the adrenal axis (stress response) from E7 to E16 at 39.5°C and 65% RH for 12 h/24 was demonstrated to be an effective and long-lasting means of acquiring thermotolerance in Cobb 500 broilers reared in cages (Piestun et al., 2008, 2009a).

Consistent with these results, Tb, which is considered to be an effective indicator of later heat tolerance (De Basilio et al., 2003), was significantly lower at hatching and up to d 28 in TM male chickens reared in floor pens in our experiment. In a previous study, Piestun et al. (2008) demonstrated the association between lower Tb and acquisition of thermotolerance to heat challenge (35°C for 5 h). In the experiment presented here, one-half of each group of chickens was exposed to a moderate heat challenge at d 34 (32°C for 5 h in another room) to study the subsequent physiological (but not sub-lethal) effects. No significant difference in body temperatures between TM and controls was observed in our conditions. This may have been due to the lower challenge temperature (32 vs. 35°C). Crowding of chickens observed during handling for temperature measurements in floor pens can also have induced additional stress and hence minimized the effects of the treatment compared with chickens reared in cages in previous studies (Piestun et al., 2008).

Thermal manipulation during embryogenesis may modify the long-term thermoregulatory threshold responses to changes in ambient temperature (Tzschentke, 2007) and stressful events. The potential mechanisms involved



**Figure 3.** A) Heterophil/Lymphocyte (H/L) ratio resulting from blood cell count. B) Percentage of basophilic cells (in relation to total number of leucocytes). One hundred leucocytes were counted for each chicken. Cont ( $n = 10$ ) and CCh ( $n = 8$ ) correspond to control chickens incubated in standard conditions (37.8°C and 56% RH) but either kept at 21°C or submitted to a heat challenge (32°C for 5h) at d 34, respectively. TM ( $n = 12$ ) and TMCh ( $n = 7$ ) correspond to chickens subjected to thermal manipulation during incubation (39.5°C and 65% RH 12h/d from E7 to E16 of embryogenesis), and either kept at 21°C or submitted to a heat challenge (32°C for 5h) at d 34, respectively. a, b: different letters indicate significant differences between treatments ( $P < 0.05$ ).

may be long-term increased sensible heat loss capacity at a peripheral level (Druyan et al., 2012) and/or decreased heat production (Piestun et al., 2008; Collin et al., 2011), or stress level. Exploration of the consequences of embryo heat manipulation on physiology may therefore improve our understanding of the mechanisms involved in the acquisition of long-term thermotolerance. In our conditions, TM chickens exhibited decreased  $T_3$  and  $T_3/T_4$  ratios and greater  $T_4$  plasma concentrations than controls at d 28, when the birds were reared at 21°C. Thyroid hormones are known to regulate heat production in mammals and in avian species (Collin et al., 2009). Piestun et al. (2009a, 2011) demonstrated decreased plasma  $T_4$  and greater  $T_3$  concentrations in TM than in control chickens. It was suggested

**Table 4.** Body composition and breast meat quality at slaughter age (d 35) depending on incubation conditions, heat challenge at d 34 within incubation condition and sex

Treatment <sup>1</sup>	Females				Males				Statistical effects		
	Cont	CCh	TM	TMCh	Cont	CCh	TM	TMCh	Incubation	Challenge (incubation)	Sex
Body composition and breast meat quality <sup>2</sup>											
BW d35, g	2,064 ± 24c	2,038 ± 26cd	2,049 ± 25cd	2,007 ± 27d	2,330 ± 25a	2,310 ± 27ab	2,308 ± 25ab	2,259 ± 27b	0.0286	0.0373	< 0.0001
Breast yield, %BW	20.3 ± 0.2	20.6 ± 0.2	21.2 ± 0.2	20.9 ± 0.2	20.5 ± 0.2	20.3 ± 0.2	20.4 ± 0.2	20.5 ± 0.2	0.0144	0.9482	0.0142
Abdominal fat percentage, %BW	2.43 ± 0.11	2.21 ± 0.12	2.01 ± 0.12	2.06 ± 0.12	1.75 ± 0.12	1.76 ± 0.12	1.69 ± 0.12	1.75 ± 0.12	0.0241	0.5128	< 0.0001
pHu, IU	5.85 ± 0.01b	5.88 ± 0.01bcd	5.87 ± 0.02bcd	5.87 ± 0.02bc	5.89 ± 0.02ac	5.92 ± 0.02a	5.90 ± 0.02ac	5.91 ± 0.02ad	0.9018	0.0484	< 0.0001
Drip loss, % of breast muscle weight	3.67 ± 0.45	2.45 ± 0.48	2.21 ± 0.51	2.41 ± 0.50	1.99 ± 0.50	2.07 ± 0.48	2.07 ± 0.48	2.25 ± 0.50	0.2708	0.2337	0.1331
Lipid peroxidation of meat <sup>3</sup>											
LPM, mg MDA/kg	0.47 ± 0.04	0.42 ± 0.06	0.45 ± 0.03	0.46 ± 0.05	0.47 ± 0.04	0.45 ± 0.04	0.55 ± 0.06	0.50 ± 0.05	0.6889	0.3458	0.6843
Induced LPM, mg MDA/kg	4.95 ± 0.40	4.89 ± 0.46	5.25 ± 0.30	5.10 ± 0.30	4.33 ± 0.30	5.28 ± 0.25	4.57 ± 0.40	5.06 ± 0.19	0.2113	0.3578	0.3224

<sup>1</sup>Cont = chickens incubated in control conditions (37.8°C and 56% relative humidity (RH)) and CCh = control birds submitted to a heat challenge (32°C for 5h) at d 34. TM = chickens who received thermal manipulation during incubation (39.5°C and 65% RH 12h/d from E7 to E16) and TMCh = TM chickens submitted to a heat challenge (32°C for 5h) at d 34. Parameters were analyzed using the GLM procedure (SAS Inst. Inc., Cary NC) with incubation effect, heat challenge within incubation conditions (nested effect), sex, incubation within sex and heat challenge within incubation and sex as main effects.

<sup>2</sup>BW: BW at slaughter age (d35). Data are expressed as least square means (lsmeans) ± SEM. Different letters indicate significant differences ( $P < 0.05$ ) between treatments (a-d) when challenge(incubation) effect alone or both incubation and challenge(incubation) effects were significant.

<sup>3</sup>LPM: Lipid peroxidation of breast meat. LPM was measured in normal or induced oxidation conditions. Data are presented as least squares means ± SEM.

that TM resulted in a reduction in thyroid gland activity (Piestun et al., 2009a) or in hepatic or peripheral deiodination of  $T_4$  into  $T_3$  reported to be influenced by changes in ambient temperatures (Collin et al., 2003; Darras et al., 2006). Thermal manipulation of embryos could thus subsequently depress heat production in the long term. This would also be consistent with reduced  $O_2$  consumption reported in embryos during (Tona et al., 2008) and after TM (Piestun et al., 2009a) in previous studies. In our conditions, such effects on plasma thyroid hormone concentrations were not maintained until d 34 in males at 21°C. At 34d,  $T_3$  concentrations and  $T_3/T_4$  ratios were decreased by the heat challenge, consistent with previous findings reported by Piestun et al. (2008), but with no further interaction with TM as reported by these authors.

The stress level in chickens was also assessed by measuring plasma corticosterone concentrations and blood cell counts. Plasma corticosterone concentration has been used as a measurement of environmental stress in birds (Gross and Siegel, 1980; Beuving, 1989). The corticosterone peak induced by a heat challenge was shown to be reduced in chickens that were heat-conditioned and reared in cages compared with their control counterparts (Piestun et al., 2008). In our conditions, corticosterone concentrations increased significantly with the heat challenge, but to a slightly greater extent in control than in TM chickens, which is in accordance with the results of Piestun et al. (2008). Consistent with this, another marker of the stress response in birds (i.e., the H/L ratio; Gross and Siegel, 1983) was affected differently by the heat challenge in TM and control

chickens. The H/L ratio has been shown to be increased during various heat challenge conditions (Aksit et al., 2006; Zulkifli et al., 2009; Prieto and Campo, 2010) and enhanced during the heat challenge in controls but not in TM broilers at d 35. These findings suggest that the stress response in TM broilers was limited during the heat challenge compared with the control birds, and was a possible sign of improved adaptive capacities. In our study, the basophil percentage was increased by TM treatment. Modification of the basophil percentage in poultry has been reported during extreme stress conditions (Maxwell et al., 1992), feed restriction (Maxwell et al., 1990; Zuidhof et al., 1995) and acute heat exposure (Altan et al., 2000). Basophilia in humans is sometimes associated with chronic inflammation and a hypothyroid state, (Thonnard-Neumann, 1961; Athens, 1993). Whether the greater basophil percentage observed in TM chickens is related to long-term changes in the thyroid axis or inflammatory status remains to be elucidated.

These blood modifications were accompanied by changes in the ion and gas content in venous blood and there was a tendency for the pH of venous blood to be increased by TM; these conditions also decreased the partial pressure of  $CO_2$  and increased  $O_2$  saturation. This may indicate reduced metabolic intensity and/or a modification of respiratory physiology in the long term after TM. Such manipulation may be related to a slight state of metabolic alkalosis as already suggested in the short term with other conditions of thermal manipulation of embryos (Yalçin et al., 2008; Willemsen et al., 2011). In the absence of changes in these parameters when chick-

**Table 5.** Estimated heritability for growth parameters, body temperatures, variables of meat processing quality, body composition, and plasma hormone and metabolite concentrations

Item	Heritability ( $h^2$ )
Growth performance	
BW d 28	0.30 ± 0.09
BW d 35	0.40 ± 0.09
Temperature	
Body temperature d 28	0.06 ± 0.05
Comb temperature d 28	0.02 ± 0.02
Body temperature d 34 at 21°C	0.02 ± 0.03
Body temperature d 34 during heat challenge	0.03 ± 0.07
Comb temperature d 34 at 21°C	0.12 ± 0.08
Comb temperature d 34 during heat challenge	0.33 ± 0.19
Breast meat quality	
Lightness	0.23 ± 0.07
Redness	0.14 ± 0.06
Yellowness	0.27 ± 0.08
Ultimate pH	0.40 ± 0.08
Body composition	
Drip loss	0.02 ± 0.03
Breast yield	0.29 ± 0.09
Abdominal fat percentage	0.21 ± 0.06
Plasma hormone concentrations	
Thyroxine $T_4$ d 28	0.06 ± 0.07
Triiodothyronine $T_3$ d 28	0.06 ± 0.08
$T_3/T_4$ d 28	0.17 ± 0.09
Corticosterone d 28	0.09 ± 0.08
Insulin d 28	0.01 ± 0.02
Plasma metabolite concentrations	
Triglycerides d 28	0.02 ± 0.08
Uric acid d 28	0.13 ± 0.08
Glucose d 28	0.01 ± 0.03

ens were subsequently submitted to the heat challenge, it remains to be determined whether these modifications represent an improvement in the adaptive capacities of TM chickens or not. Besides these original results regarding the long-term consequences of TM on blood gas and ion concentrations, anticipated effects of heat challenge were also observed on  $HCO_3^-$ ,  $PCO_2$ ,  $TCO_2$ ,  $iCa$ ,  $Bee_f$  and  $Beb$  concentrations, reflecting modifications in respiratory physiology probably related to hyperventilation. Hyperventilation is known to cause a severe loss of carbon dioxide from the lungs and results in a dramatic loss of blood bicarbonate (Smith and Teeter, 1987; Balnave and Gorman, 1993). Thus, decreases in  $Bee_f$  and  $Beb$  may reflect a modification of the acid-base balance due to heat stress (Borges et al., 2003).

The TM conditions of 39.5°C from E7 to E16 of embryogenesis for 12 h/d have already been proved not to affect hatchability or growth of chickens (Piestun et al., 2008). In agreement with these studies, TM did not affect hatchability or BW from hatching to d 28 in our

experimental conditions. However, at d 35 BW were slightly lower in TM and heat-challenged chickens than in control birds. This decrease was not associated with any significant change in the feed conversion ratio during both the finishing and the overall periods. Such a variation in BW was not reported in the study of Piestun et al. (2008) in d 35 broilers reared in cages. However, BW were about 2.5 and 3.4% greater in the TM and control groups, respectively, in our study than in the study by Piestun et al. (2008). The differences could be due to different experimental conditions (e.g., control temperature levels), or to interactions between chickens reared in floor pens, potentially increasing the stress levels in the finishing period. We also measured indicators of meat processing quality and body composition. These parameters were not significantly affected by embryo TM at slaughter age (d 35): ultimate pH, color, lipid peroxidation, and drip loss of breast meat were not modified by TM. However, these parameters were not dramatically affected by the heat challenge in our conditions. In previous studies, heat exposure was reported to increase lipid peroxidation (Altan et al., 2003; Lin et al., 2006), mitochondrial oxidative stress (Mujahid et al., 2007), and possibly to increase muscle membrane damage and affect meat quality (Debut et al., 2005; Sandercock et al., 2006), due to more drastic experimental conditions.

In our experiments, breast muscle yields were very high compared with other studies dealing with animals from the same commercial line, and this parameter was increased by TM only in females. Thermal treatment of embryos in other experimental conditions was previously reported to have beneficial effects on body composition in both sexes (Collin et al., 2007; Piestun et al., 2011, 2013). The latter authors suggested that the heat treatment of embryos changed the rate of myoblast proliferation, enhancing myofiber diameter or numbers in the P. major muscle. In further experiments, it would be valuable to verify whether an increase in the proliferation activity of satellite cells, the quiescent precursors of myogenic cells in the postnatal muscle, occurred or not in TM females, as previously observed in case of early postnatal (Halevy et al., 2001) or late embryo (Piestun et al., 2009b) thermal manipulations. We also observed a significant decline in the relative abdominal fat pad content with TM, which is a favorable trait for broiler production. This confirms the results of Piestun et al. (2011) showing reduced adiposity in TM broilers reared in cages. Adipose tissue begins to form in the embryo at around E12 (Speake et al., 1998). The TM from E7 to E16 of embryogenesis may therefore have affected tissue development, thus reducing the fat pad in the long term, as suggested by Hammond et al. (2007).

These changes in body composition at d 34 were associated with modifications in the glucose/insulin balance at d 28 as measured in TM male chickens. Indeed, glycemia in these birds tended to be greater in the fed state at d 28 (16.42 vs. 15.81 mmol/L) as compared with their control counterparts, despite a lack of difference in plasma insulin concentrations. This result is consistent with previous findings showing an association between high abdominal fatness and low glycemia in domestic birds (Chartrin et al., 2006; Nadaf et al., 2009; Rideau and Métayer-Coustard, 2012). The glycemic response to the decrease in insulin resulting from the heat challenge may also follow different kinetics in TM and in control chickens. No modification in plasma uric acid concentrations resulting from AA oxidation or in plasma triglyceride concentrations were observed at 28 d post-hatch after thermal manipulation. Changes in such metabolite concentrations were previously reported after TM by Yalçın et al. (2008), but in other experimental TM conditions.

In the context of global warming and breeding of broilers in hot climates, it might be valuable to select broiler chickens taking into account both thermotolerance and broiler performance criteria. The present study was conceived with a pedigree design to evaluate the heritability of the different parameters measured. Consistent with previous studies focusing on broiler performance and meat processing quality (Le Bihan-Duval et al., 2001; 2008), moderate to high heritability was revealed for BW, breast yield, abdominal fat pad percentage, lightness, yellowness and ultimate pH of the breast muscle meat. We also recorded, to our knowledge for the first time in broilers, moderate heritability for comb temperature on d 34 during the heat challenge and for the  $T_3/T_4$  plasma concentration ratio at 21°C on d 28. The comb is involved in heat dissipation (Yahav et al., 2005) and broilers with high comb temperature may be more able to dissipate heat, and thyroid hormones are well-known regulators of heat production. These heritability estimates suggest that for these 2 traits variability between individuals is partly under genetic control, and may be selected to improve thermotolerance in broilers. These preliminary results should be confirmed on a larger number of birds, which in addition would allow the estimation of genetic correlations between traits, and to check whether their selection might reduce performance or not. However, in our conditions body temperature was not a heritable parameter, probably because measuring it in floor pens increased the crowding of birds along the pen walls during the finishing period. In subsequent studies, advantage should be taken of the development of infrared technology which makes it possible to obtain more precise temperature values with less handling of birds (Giloh et al., 2012).

In conclusion, we studied for the first time the growth performance and physiology of thermally manipulated broilers from E7 to E16 at 39.5°C and 65% RH reared

in semi-commercial conditions. We showed that broiler temperatures were lower than those of controls until d 28, whereas growth at this age and hatchability were not affected. Body weight was slightly reduced at slaughter age in thermally manipulated chickens but, interestingly, their abdominal fat content was reduced and their meat processing quality was not affected. Thermal manipulation during embryogenesis had long-lasting effects on physiology, which may potentially modulate gene expression and metabolism in peripheral tissues, and ultimately heat production of TM chickens. The mechanisms underlying such long-lasting effects remain to be further elucidated, especially in relation to glucose/insulin and thyroid-regulated metabolism.

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