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Thermal manipulations during broiler embryogenesis improves post-hatch performance under hot conditions ☆

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ABSTRACT

Previous studies on broilers conducted in our laboratory showed that intermittent thermal manipulation (TM) of 39.5 °C every 12 h during embryonic days E7–E16 improved thermoregulative parameters in the embryo and post-hatch, as well as the broilers' ability to cope with heat challenge at marketing age. The aim of this study was to investigate the effect of intermittent TM of 39.5 °C (65% RH) for 12 h/d (12 H), during E7–E16, on post-hatch performance and thermoregulation under both optimal and hot conditions. Chicks from the control and TM treatments were raised under "regular" conditions until 21 d of age. Then, each treatment was divided into 2 sub-treatments (growth conditions) – optimal conditions (25 °C) and hot conditions of 32 °C for 12 h/d – until 35 d of age. Under optimal conditions, no difference in growth rates was found between TM and control throughout the growth period, but TM had significantly larger relative breast muscle weight, and on days 13, 25, and 35 breast muscle in the TM treatment contained a higher percentage of large-diameter fibers than controls. Consequently, average fiber diameter was significantly larger in TM than in control. Relative weight of the abdominal fat pad was significantly lower in the TM treatment than in control, under both environmental conditions. Hot conditions negatively affected growth rates of broilers in both treatments. The TM chickens showed no body-weight advantage over controls, nevertheless, feed conversion between days 21 and 35 was lower in the TM chickens of both sexes than in controls. This could be attributed to lower body temperature and lower plasma thyroid hormone levels found in the TM chickens, indicating lower heat production rates. Greater muscle growth and lower relative weight of abdominal fat pad were found in the TM chickens than in controls, under hot conditions. It can be concluded that TM during embryonic development improves performance as well as thermoregulation.

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1. Introduction

Among the various factors affecting energy balance control in broilers, ambient temperature (T_a) is known to be the most critical (Lin et al., 2006). As genetic selection for body weight and breast muscle has progressed (Havenstein et al., 2003), chickens reared in hot climates have been exposed to endogenous heat loads that result from excessive metabolic heat production (Yahav, 2009), as well as high T_a, and these heat loads cause difficulties in coping with the environment. One strategy for coping with a hot climate is epigenetic temperature adaptation (Yahav, 2009), defined as non-genetic changes that occur within short critical developmental phases during pre- or early-postnatal ontogeny, and that affect gene expression and establish lifelong adaptation to an actual environment (Nichelmann and Tzschentke, 2002).

For many years, incubation temperatures were held relatively constant throughout incubation in order to eliminate possible negative effects of temperature changes on embryo development and hatchability, and chick quality (Krausova and Peterka, 2007). This contrasts with the incubation process in nature, where incubation temperatures fluctuate widely (Webb, 1987), and studies have demonstrated that carefully changing incubation temperature during critical time windows within embryogenesis might induce changes in the fowl's metabolic rate. Reduced incubation temperature for short time periods a few days before hatching resulted in increased embryonic heat production in chickens (Minne and Decuypere, 1984), ducks (Nichelmann et al., 1994; Tzschentke et al., 2001), and turkeys (Modrey, 1995). In most cases this effect was exhibited up to 10 d post-hatch (Loh et al., 2004), but it was characterized by enhanced

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ability of chickens to cope with cold challenge throughout their life span (Shinder et al., 2009). Recent studies demonstrated a long-lasting effect of intermittent thermal manipulation (TM) during embryogenesis, when it was applied for 12 h/d during a critical time window that coincided with the development and maturation of the thyroid axis, i.e., between embryonic (E) days E7 and E16. This TM was found to improve thermotolerance acquisition of broilers during acute post-hatch heat stress (Piestun et al., 2008a, 2008b). This improved thermotolerance was characterized by the following: (a) reduced thyroid gland activity, which lowered metabolic rate and associated heat production during embryogenesis and post-hatch; (b) significant post-hatch improvement of the vasomotor response of the peripheral blood system. These effects were not accompanied by a negative effect on performance (Piestun et al., 2009a).

Elevation of the incubation temperature has been reported to affect muscle growth: TM at various time windows, e.g., E16–E18 enhanced broilers' pectoral muscle-cell proliferation in the embryo and post-hatch, resulting in increased post-hatch muscle development and enhanced meat production (Piestun et al., 2009b); also, application of TM to the turkey embryo increased muscle fiber number and the weight of the semitendinosus muscle at 16 d post-hatch (Maltby et al., 2004). The present study, in continuation of previous studies (Piestun et al., 2008a, 2008b, 2009a, 2009b), aimed to investigate the effects of TM during the development and maturation of the thyroid axis in the embryo on broilers' performance, heat balance, and meat production throughout their growth under exposure to optimal or hot environmental conditions.

2. Materials and methods

2.1. Experimental designs

All the procedures in this study were carried out in accordance with the accepted ethical and welfare standards of the Israeli Ethics Committee (IL-005/05).

A batch of 600 'Cobb' strain broiler (*Gallus domesticus*) eggs with an average weight of 55.5 ± 2.5 g were obtained from one breeder flock aged 31 week. The eggs were randomly divided into 2 incubation treatments: treatment 1 (Control)—eggs were incubated at 37.8 °C and 56% RH throughout the incubation period; treatment 2 (12 H)—eggs were subjected to TM of 39.5 °C and 65% RH for 12 h/d from 180 to 408 h of incubation (corresponding to E7 till E16 inclusive while the first day of incubation designated as E0).

The eggs were incubated in 2 Type 65Hs automatic incubators (Masalles, Barcelona, Spain). Eggs from both treatments were randomly divided between the 2 incubators (except during TM treatment, which took place in one incubator only) to avoid possible incubator effects. From E0 through E7 all eggs, in both incubators, were incubated under regular conditions of 37.8 °C, 56% RH and turned once per hour (Bruzual et al., 2000). On E7 eggs were candled, infertile eggs and those containing early-dead embryos were removed, and eggs of the TM treatment were transferred to the TM incubator under TM conditions for 12 h/d from E7 through E16. Both incubators contained equal numbers of eggs of both treatments. From E17 onwards, all eggs were maintained at 37.8 °C and 56% RH. Relative humidity in the TM treatment was elevated to 65% to prevent excessive water loss from the eggs that might result from their previously elevated incubation temperature. The RH elevation caused a slight reduction in the partial pressure of oxygen (pO_2) by 7.1 mm Hg.

Hatching took place in one hatchery. The number of chicks hatched from both treatments was recorded every hour. Dry-feather chicks, at approximately 2 h after hatching, were taken out of the incubator for immediate measurements of BW and body temperature (T_b) – with a digital thermometer (Super Speed Digital Thermometer; Procare Measure Technology Co., San Chung City, Taipei, Taiwan) with ± 0.1 °C accuracy – and sex determination. Blood was drawn from the jugular vein of 10 randomly chosen individuals from each treatment, for hormonal analysis.

After completion of the initial measurements, all chicks were placed in cages (in the 1st week, 5 chicks from the same sex per cage, thereafter 2 chicks per cage up to 21 days of age) measuring 40 cm × 28 cm × 45 cm in length, width, and height, respectively, with 2-cm-mesh-size wire floors. The cages were situated in 4 computerized controlled-environment rooms with temperature constant to ± 1.0 °C, RH to ± 2.5%, and air velocity to ± 0.25 m s^{-1}, under continuous fluorescent illumination. In each room there were 5 cages of males and 5 cages of females from each treatment. Chicks were raised under Cobb Broiler Management Guide conditions until 21 d of age. At 21 d of age, all chickens from each treatment × sex were shuffled. 40 chickens from each treatment × sex were randomly taken and allocated to each of 2 sub-treatments (growth conditions): naïve—chicks were raised under the optimal temperature of 25.0 ± 1.0 °C; hot conditions—chicks were raised at 32 ± 1.0 °C and ~65% RH for 12 h, and then under optimal conditions (according to the breeder company management guide) for 12 h. Thus, there were 30 chickens for each combination of treatments (control and TM) × sex (male and females) × sub-treatments (naïve and hot).

All chickens were raised up to 35 d of age (1 chicken per cage). Water and feed in mash form were supplied for ad libitum consumption. The diet was designed according to US National Research Council (1994) recommendations. At weekly intervals, T_b, BW, and feed consumption (FC) were recorded for individuals and groups (defined as treatment × sex × sub-treatment), and the feed conversion ratio (FCR) (kg feed/kg BW) was calculated for each group. Blood was drawn from the brachial vein ($n=10$) for hormonal analysis.

At 35 d of age chicks were weighed and killed. Breast muscle, heart, and abdominal fat-pad tissues were removed and weighed, and their relative weights were calculated.

2.2. Muscle sampling for histology and myofiber diameter analysis

Muscle samples – 1.2 × 0.5 × 0.5 cm in size – collected from 10 naïve male chickens from each treatment, hatched within the same time window and raised under optimal conditions, were taken from the superficial regions of the proximal left pectoralis major of each chicken on days 13, 25, and 35, as described previously (Halevy et al., 2004; Piestun et al., 2009b).

Myofiber diameter was determined by analyzing the myofiber lesser-diameter values (Dubowitz, 1985) according to Halevy et al. (2004). Briefly, muscle samples were fixed in 4% paraformaldehyde and embedded in paraffin, and 5-μm muscle sections were cut. Sections were stained with hematoxylin–eosin, and at least 10 arbitrary fields in 2 or 3 serial sections of each muscle sample were photographed. In each muscle sample, the fiber lesser-diameter was measured for individual myofibers with CELL^B software (Olympus, Hamburg, Germany).

2.3. Plasma thyroid hormones analysis

Radioimmunoassays of total thyroxin (T_4) and total triiodothyronine (T_3) were performed on plasma samples, with commercial radioimmunoassay kits (Coat-A-Count Canine T_4: Diagnostic Products Corporation, Los Angeles, CA, USA; RIA-gnost T_3: CIS Bio International, France). The intra-assay and inter-assay

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