Improvement of cold resistance and performance of broilers by acute cold exposure during late embryogenesis[1]

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ABSTRACT The aim of this study was to fine-tune previous acute cold exposure treatments of broiler embryos during late embryogenesis to improve lifelong cold resistance and performance. Six hundred Cobb hatching eggs were incubated under standard conditions and then exposed to 3 treatments: control; cold treatment in which embryos were exposed to 15°C for 30 min on d 18 and 19 of incubation (30×2); and cold treatment similar to 30×2 but with 60-min exposures (60×2). Egg shell temperature (T_{egg}) and heart rate (HR) were monitored pre- and posttreatment. Upon hatching, hatchability, body weight, and body temperature were recorded. From 14 to 35 d of age, three quarters of the chickens in each treatment were raised under ascites-inducing conditions (AIC) and the remaining birds were raised under standard brooding conditions (SBC). The T_{egg} and HR decreased significantly in response to increased exposure time on d 18 of incubation. On d 19 of incubation, before the second cold exposure, the 30×2 group showed greater T_{egg} and HR than the controls, and during the second exposure they maintained these parameters better than the 60×2 embryos. No treatment effect on hatchability was observed. At 35 d of age ascites incidence among 30×2 chickens under AIC was significantly less than that among the controls ($P < 0.01$), and body weight of these chickens under either SBC or AIC was significantly higher than that of the controls. Under SBC relative breast muscle weight was significantly higher in 60×2 chickens, whereas the relative heart weight was higher in both cold-treated groups than in the controls. It can be concluded that repeated short acute cold exposures during late embryogenesis significantly reduced ascites incidence and improved growth rate under either SBC or AIC. These results may be related to a prenatal epigenetic adaptation of the thermoregulatory and cardiovascular systems to low ambient temperature.

Key words: embryogenesis, cold exposure, egg shell temperature, broiler, ascites

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INTRODUCTION

During the perinatal period of chickens, a time window of opportunity occurs for imprinting of physiological control systems. This imprinting is probably related to neural imprinting at the microstructural level (i.e., in terms of synaptic plasticity) and also to environment-induced modifications of gene expression. Perinatal epigenetic temperature adaptation may be used as a tool for adapting poultry embryos or hatchlings to subsequent climatic conditions (Tzschentke and Plagemann, 2006; Tzschentke, 2007). In chickens and other precocial birds, epigenetic temperature adaptation can be induced by changes in incubation temperature toward the end of embryonic development (Minne and Decuypere, 1984; Tzschentke and Nichelmann, 1997; Collin et al., 2007; Piestun et al., 2009; Shinder et al., 2009; Tzschentke and Halle, 2009) and also by posthatch thermal conditioning (Yahav and Hurwitz, 1996; Shinder et al., 2002).

Ambient temperature ($\mathbf{T_a}$) is the most important environmental factor in this context (Horowitz, 2002). With domestic fowl (e.g., broilers, turkeys, and laying hens), for several decades the practice was to keep the incubation temperature relatively constant (French, 1997) to avoid possible deleterious effects on the development of the chicken embryo (Peterka et al., 1996; Krausova and Peterka, 2007). However, in nature the incubation temperature ranges from less than 20°C to more than 40°C (Webb, 1987) as a result of the necessity for parent birds to search for food or to avoid predators. Recent studies demonstrated the beneficial effects of increasing the incubation temperature to improve thermotolerance acquisition without impairing

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the development of the embryo or the performance of the fast-growing broiler (Yahav et al., 2004). However, most studies of incubation temperature reduction during embryogenesis have appeared to demonstrate negative effects. Long-term decreases in incubation temperature induced teratogenic effects and increased mortality and incubation time (Peterka et al., 1996; Tazawa et al., 2001; Mortola, 2006).

In contrast to the effects of long-term cold exposure, short-term cold exposure during the late phase of embryogenesis, when embryos switch from the ecto- to the endothermic phase, was found to induce greater thermogenesis ability in the hatched chicks (Minne and Decuypere, 1984; Nichelmann, 2004).

One of the main posthatch hazards faced by the fast-growing broiler is related to its inability to satisfy bodily oxygen demands under low ambient temperatures. Poor heart performance is a major factor in the pathogenesis of hypoxemia in fast-growing broilers (Olkowski, 2007); it was also found to be the main cause for development of the ascites syndrome, which leads to significant economic losses under cold conditions (Wideman and French, 1999; Druyan et al., 2007). A model of ascites-inducing conditions (**AIC**; Luger et al., 2001) was suggested to improve chickens' cold resistance throughout their life span, in light of previous findings that external factors such as an artificial increase of T_4 in chicken plasma significantly reduced the frequency of ascites incidence (Luger et al., 2002) or that administration of a β-adrenoceptor blocker (atenolol) with the diet (Hassanzadeh et al., 2002) numerically reduced ascites incidence in broiler chickens reared at high altitude. Cold conditioning (i.e., exposure to 15°C for 3 h at 3 and 4 d of age) was found also to significantly reduce broiler mortality under exposure to AIC, up to 49 d of age (Shinder et al., 2002). In previous studies it was shown that acute cold exposures during the last phase of embryogenesis considerably decreased ascites incidence in chickens reared under AIC (Shinder et al., 2009).

The aim of this study was to fine-tune the optimal conditions for repetitive acute cold exposures during the last phase of broiler embryogenesis. It was based on the hypothesis that such exposures would significantly improve cold resistance during the birds' life span.

MATERIALS AND METHODS

Experimental Design

All procedures were carried out in accordance with the accepted ethical and welfare standards of the Israeli Ethics Committee (IL 48/06). Six hundred fertile Cobb eggs, weighing 61 to 66 g, were obtained from a 34-wk-old flock. The eggs were divided into 3 treatments and incubated together in a Danki Medium-Size Incubator (Danki ApS, Kristiansgade, Denmark) under standard conditions of 37.8 ± 0.3°C and 56 ± 2.5% RH. At 19 d of incubation (**E19**) all eggs were transferred to a separate Danki Hatcher Type 3 (Danki ApS) at 37.2 ± 0.3°C and 60 ± 2.5% RH.

To conduct the cold treatments, the eggs, except for the controls, were transferred to a controlled-environment room, where special care was taken to keep the eggs not less than 10 cm apart during cold exposure, to minimize thermal radiative interaction, and to enhance uniformity of the cold treatment. The 3 treatments were as follows: control; 2 successive 30-min exposures to 15°C and 60% RH at 1200 h on day 18 of incubation (**E18**; 435 h) and E19 (459 h), respectively (**30×2**); and 2 successive 60-min exposures to 15°C and 60% RH at 1200 h on E18 (435 h) and E19 (459 h), respectively (**60×2**). The 30×2 and 60×2 exposures were conducted in the same controlled-environment room. At the end of the cold exposures the eggs were returned to standard hatchery incubation conditions

Chicks were removed as they hatched to minimize effects on subsequent chick quality and hatching. Their BW and body temperature ($\mathbf{T_b}$) were measured and their gender was determined. Only males were used in the posthatch experiment. Males from all treatments were placed in a controlled-environment room (35 ± 1°C, 60 ± 2.5% RH) and raised under standard brooding conditions (**SBC**), with T_a gradually reduced to 27 ± 1°C at 14 d of age. From 14 until 35 d of age 15 chickens from each treatment, 45 altogether, were reared under SBC and the remainders were exposed to AIC.

AIC

Ascites-inducing conditions were applied according to Luger et al. (2001). Briefly, 174 chicks (50, 63, and 61 from control and treatments 30×2 and 60×2, respectively) were exposed to AIC (i.e., 20 ± 1°C) from 14 to 21 d of age, followed by exposure to 15 ± 1°C until the end of the experiment. Water and feed were provided for ad libitum consumption.

Temperature Measurements

Before (in incubator) and during cold exposure of 30 and 60 min, eggshell temperature ($\mathbf{T_{egg}}$) of 8 eggs per treatment was measured every 5 min in a cold-controlled room with an infrared imager (Model PM545, FLIR Systems, Danderyd, Sweden). Egg length and width were measured to calculate eggshell surface area according to Van Brecht et al. (2005).

Body temperature of all hatched and 3-d-old chicks was measured with a digital thermometer (Model ST8030CB, Mesure Wuxi Technology, Taipei, Taiwan), which covered a range from 32.0 to 45.0°C with accuracy of ± 0.1°C and a 10-s response time.

Sensible Heat Loss from Eggs

Sensible heat loss (**SHL**) from the eggs was calculated according to Shinder et al. (2009). Briefly, cal-

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culations were based on the assumption that heat exchange occurs via the whole eggshell surface, the area of which was determined from the length and breadth of the egg, and the egg was designated with a characteristic dimension corresponding to the diameter of a sphere with the same surface area. Also, the very low air velocity (<0.3 m/s) to which the eggs were exposed necessitated the use of a theoretical heat transfer model based on free convection and radiation. The equivalent spherical egg model incorporated mean values of available or especially derived heat transfer coefficients to calculate the SHL.

Heart Rate

Heart rate (**HR**) of embryos from each treatment (n = 8) was measured before (in the incubator) and every 10 min during the cold exposure (in cold room) with a Buddy digital embryo heart rate monitor (Avitronics, Torquay, UK). To measure hear rate in the incubator, a tent covering the incubator was used. In the tent a similar temperature as in the incubator was used during the measurement.

Body, Breast Muscle, and Heart Weights

Individual BW was measured at hatching, on d 14 of age, when AIC exposure started, and on d 35 of age. On d 35 all the remaining birds were necropsied. Body, breast, and heart weights were recorded and used, with the bird BW, to calculate the relative breast and heart weights.

Right Ventricle Weight:Total Ventricle Weight Ratio

Hearts were collected from all chickens (those that died during the trials and those necropsied at the end of the trials) and were dissected to record the weights of the right and left ventricles and septum (total ventricle). The right ventricle weight:total ventricle weight (**RV:TV**) ratio was calculated for each bird. Birds having RV:TV values above 0.3 were considered to have ventricular hypertrophy.

Ascites Diagnosis

Mortality was recorded throughout the AIC phase (i.e., from 14 d of age onwards). All dead birds were necropsied and examined to determine the cause of death. Birds with hydropericardium, heart dilation, and abdominal fluid accumulation were diagnosed as having died of ascites. At the end of the trial, all surviving birds were killed by cervical dislocation and necropsied, and those with abdominal ascitic fluid, hydropericardium, and ventricular hypertrophy were classified as ascitic.

Statistical Analysis

All results were subjected to one-way ANOVA with the treatment (control, 30×2, and 60×2) as fixed main effect. Student's *t*-test was used to test the separation of the means. These statistical analyses were conducted with JMP software (SAS Institute, 2005).

RESULTS

Prenatal Period

On E18, during the first 0.5 h of cold exposures (15°C), T_{egg} of eggs in treatments 30×2 and 60×2 decreased below that of control eggs (Figure 1A). In both treatments, after 30 min T_{egg} decreased from 38.3 ± 0.07°C to 26.9 ± 0.3 and 26.7 ± 0.4°C for treatments 30×2 and 60×2, respectively, and that in treatment 60×2 decreased further to 21.1 ± 0.3°C by the end of the 60-min cold exposure. On E19, before the second cold exposure and under identical optimal incubation conditions, T_{egg} differed significantly among the 3 treatments: in treatment 30×2 it was significantly higher than in the other treatments, whereas in treatment 60×2 it had not recovered to the control level but was significantly lower at 24 h after cold exposure (Figure 1B). The second cold exposure, on E19, also decreased T_{egg} after 30 min, but embryos from treatment 30×2 maintained a significantly higher T_{egg} than those in treatment 60×2 (29.0 ± 0.5 vs. 27.5 ± 0.3°C). In treatment 60×2 T_{egg} decreased further to 22.0 ± 0.5°C by the end of the 60-min cold exposure (Figure 1C). Sensible heat loss after 30 and 60 min of cold exposure was 645 ± 74 and 985 ± 138 cal, respectively.

Heart rate displayed a similar trend to that of T_{egg}: after 30 min of the first cold exposure on E18, HR decreased sharply from 252.4 ± 4.2 beats/min to 63.3 ± 2.6 and 68.3 ± 2.3 beats/min in treatments 30×2 and 60×2, respectively (Figure 2A), and after 60 min of cold exposure HR in treatment 60×2 decreased further to 34.6 ± 2.3 beats/min. On E19, before the second cold exposure, embryos of treatment 30×2 displayed significantly higher HR than those of control, but HR of the 60×2 embryos did not differ significantly from that of either control or 30×2 embryos (Figure 2B). During the second cold exposure, on E19, HR of both cold-treated groups decreased significantly below that of control (Figure 2C), but that of 30×2 embryos was significantly higher than that of 60×2 embryos (128.5 ± 4.5 vs. 114.4 ± 4.1 beats/min). By the end of the 60-min cold exposure HR of the embryos of treatment 60×2 decreased further to 56.0 ± 2.3 beats/min.

Postnatal Period

No effect was found of cold exposure on hatchability, which was similar in all 3 treatments (Table 1). At hatching no difference was found between treatments

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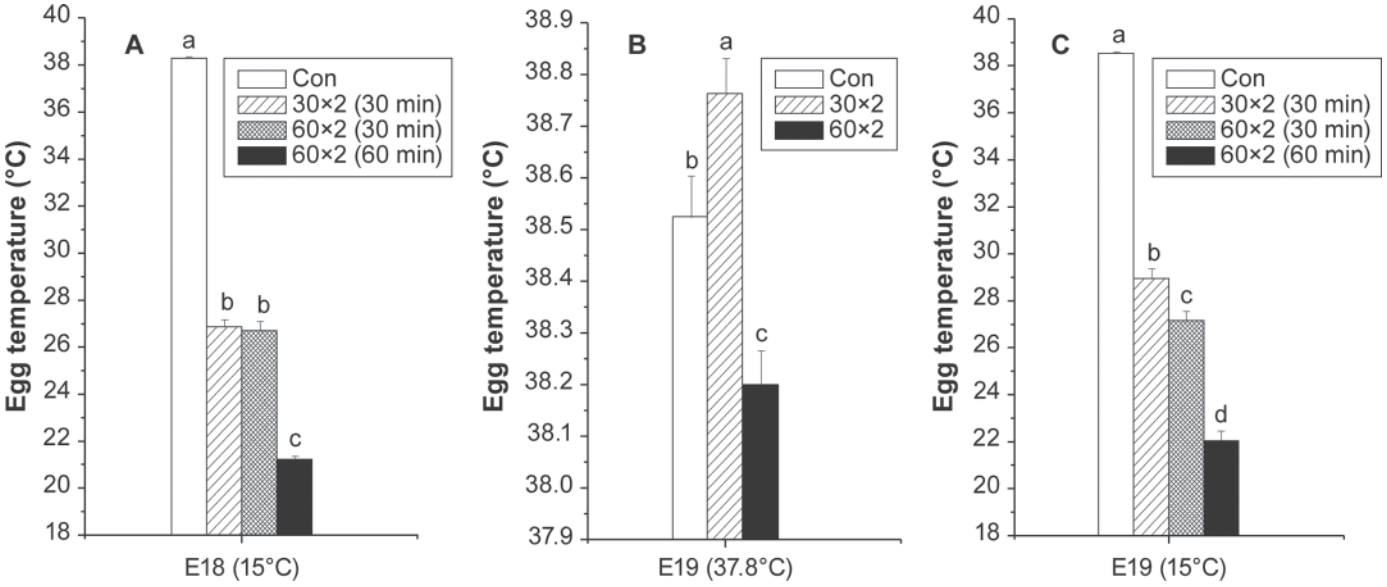

Figure 1. Eggshell temperature A) during cold exposure on E18, B) in incubator (37.8°C) on E19 before cold exposure, and C) during cold exposure on E19. Con (control): constant 37.8°C; 30×2: 15°C for 30 min on d 18 (E18) and 19 (E19) of incubation; 60×2: 15°C for 60 min on E18 and E19. 30×2 (30 min): treatment 30×2 after 30 min of cold exposure; 60×2 (30 min): treatment 60×2 after 30 min of cold exposure; 60×2 (60 min): treatment 60×2 after 60 min of cold exposure. Values marked with different letters differ significantly (mean ± SE; n = 8; $P < 0.05$).

in BW of male chicks, but T_b of the 60×2 embryos was significantly lower than that of the control and 30×2 embryos. On d 3, however, T_b of the 30×2 embryos was significantly higher than that of the control embryos (Table 1).

Chick mortality from hatching through 14 d of age did not exceed 2.5%. Under AIC, ascites mortality and total incidence (mortality plus morbidity) in treatment 30×2 were significantly lower than in both control and 60×2 treatments, with no differences between the last 2 treatments (Table 2). The ascites mortality and incidence rates were significant at $P < 0.05$ and $P < 0.01$, respectively.

The BW of 14-d-old chicks was significantly higher in treatment 30×2 than in the other treatments (Figure 3A). On d 35, under both SBC and AIC, BW of healthy (nonascitic) chicks in treatment 30×2 was significantly higher than that of those in the other treatments (Figure 3B).

Relative breast muscle weight of birds reared under SBC was significantly higher in treatment 60×2 than in either of the other treatments (Figure 4). Figure 5 pres-

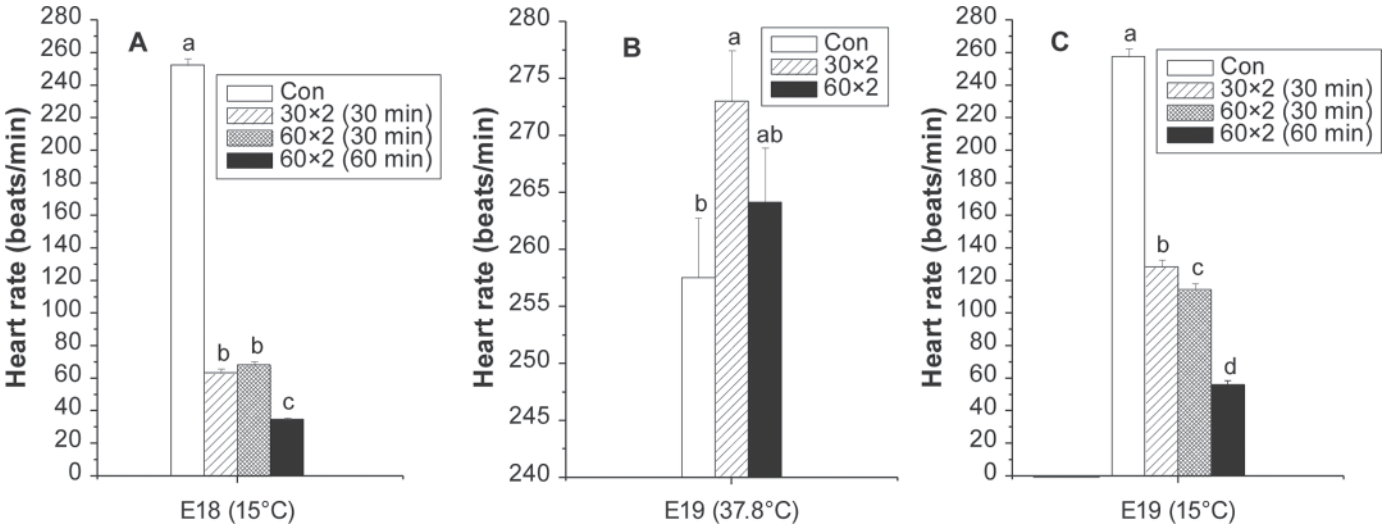

Figure 2. Embryo heart rate A) during cold exposure on E18, B) in incubator (37.8°C) on E19 before cold exposure, and C) during cold exposure on E19. Con (control): constant 37.8°C; 30×2: 15°C for 30 min on d 18 (E18) and 19 (E19) of incubation; 60×2: 15°C for 60 min on E18 and E19. 30×2 (30 min): treatment 30×2 after 30 min of cold exposure; 60×2 (30 min): treatment 60×2 after 30 min of cold exposure; 60×2 (60 min): treatment 60×2 after 60 min of cold exposure. Values marked with different letters differ significantly (mean ± SE; n = 8; $P < 0.05$).

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Table 1. Hatchability, BW at hatching, and body temperature (T_b) of hatched and 3-d-old control chicks (no prenatal treatment) and chicks prenatally exposed to 15°C for 30 min (30×2) and 60 min (60×2) on d 18 and 19 of incubation

	Treatment		
Variable	Control	30×2	60×2
Hatchability (%)	94.7	96.5	94.1
BW, males (g)	45.86 ± 0.24	46.21 ± 0.24	45.67 ± 0.22
T_b (°C)			
Hatched	41.07 ± 0.05[a]	41.17 ± 0.06[a]	40.57 ± 0.05[b]
3 d old	40.86 ± 0.03[b]	41.10 ± 0.05[a]	40.99 ± 0.06[ab]

[a,b]Mean ± SE values within a row with different superscripts differ significantly ($P \leq 0.05$) between treatments.

ents the BW of 14-d-old chickens under AIC that were identified as either healthy or ascitic on d 35; those in the control treatment that developed ascites were significantly ($P < 0.001$) heavier than healthy controls of the same age. Furthermore, prenatally treated chickens, from both treatments 30×2 and 60×2, that were healthy on d 35 were considerably heavier than healthy controls on d 14.

The relative heart weights and RV:TV ratio of 35-d-old chickens from both SBC and AIC are given in Figure 6. In general, relative heart weights of birds kept under AIC were significantly greater than those of birds kept under SBC. No differences were found among treatments for birds under AIC, whereas for those under SBC hearts of control birds were significantly smaller than those of birds under either 30×2 or 60×2 (Figure 6A). The RV:TV ratio of ascitic birds was higher than 0.3, irrespective of treatment, but significantly higher in ascitic control birds. Among bird reared under SBC, the RV:TV ratio of 60×2 birds was significantly higher than that of controls; no difference was found between birds from the 2 prenatally cold-treated groups (Figure 6B).

DISCUSSION

Recently acquired knowledge of the imprinting of physiological control systems, such as the thermoregulatory system, during the perinatal period (Tzschentke and Plagemann, 2006) revealed the possibility of inducing long-term temperature adaptation of an organism to acute changes in environmental conditions (Yahav and Hurwitz, 1996; Shinder et al., 2002, 2009). However, it was also well established that low incubation temperatures could lead to delayed hatching (Kühn et al., 1982) or even death of embryos (Peterka et al., 1996).

In a previous study (Shinder et al., 2009) the period from E18 through E19 was identified as the critical period for inducing cold tolerance. The use of exposure to a very low temperature (15°C) necessitated a very short exposure time of 30 or 60 min to avoid deleterious effect(s) on the embryo, especially because this period constitutes a critical phase that includes the start of preparations for internal pipping.

The ability to withstand exposure to AIC (Luger et al., 2001; Druyan et al., 2007, 2009) was used as a tool for quantitative estimation of cold resistance. In previous studies, prenatally cold-manipulated chickens reared under AIC exhibited a reduced incidence of ascites, but this effect was not statistically significant (Shinder et al., 2009). One of the main aims of the present study was to fine-tune the prenatal cold conditions to enhance the chickens' ability to withstand AIC.

A pivotal modification to the cold-exposure procedure that was introduced in the present study involved increasing the separation between the eggs during the cold exposure, thereby minimizing thermal radiative interactions. This modification was introduced to improve uniformity of T_{egg} and of SHL from the egg. As a result the difference between maximum and minimum T_{egg} after 30 min of cold exposure on E18 was about half of that found in the previous study (1.6 and 3.0°C, respectively). Consequently, T_{egg} during cold exposure was lower than in the previous study because of the higher total SHL after 30 and 60 min of cold exposure: 645 and 985 cal, respectively, compared with the previous 512 and 718 cal, respectively (Shinder et al., 2009). It seems that in the present study enhanced uniformity of the eggs' response to cold exposure was achieved, which enabled optimization of the treatment with respect to inducing acquisition of ascites resistance, which should have been detected in chickens reared under AIC. In-

Table 2. Number of chickens in treatments and mortality and ascites incidences of control chicks (no prenatal treatment) and chicks prenatally exposed to 15°C for 30 min (30×2) and 60 min (60×2) on d 18 and 19 of incubation and reared under ascites-inducing conditions from 14 to 35 d of age

	Treatment		
Variable	Control	30×2	60×2
Chickens (n)	50	63	61
Mortality (%)	52[a]	33[b]	56[a]
Ascites (%)	70[a]	44[1]	64[a]

[a,b]Mean values within rows with different superscripts differ significantly ($P \leq 0.05$) between treatments.

[1]Differs significantly ($P \leq 0.01$) from control.

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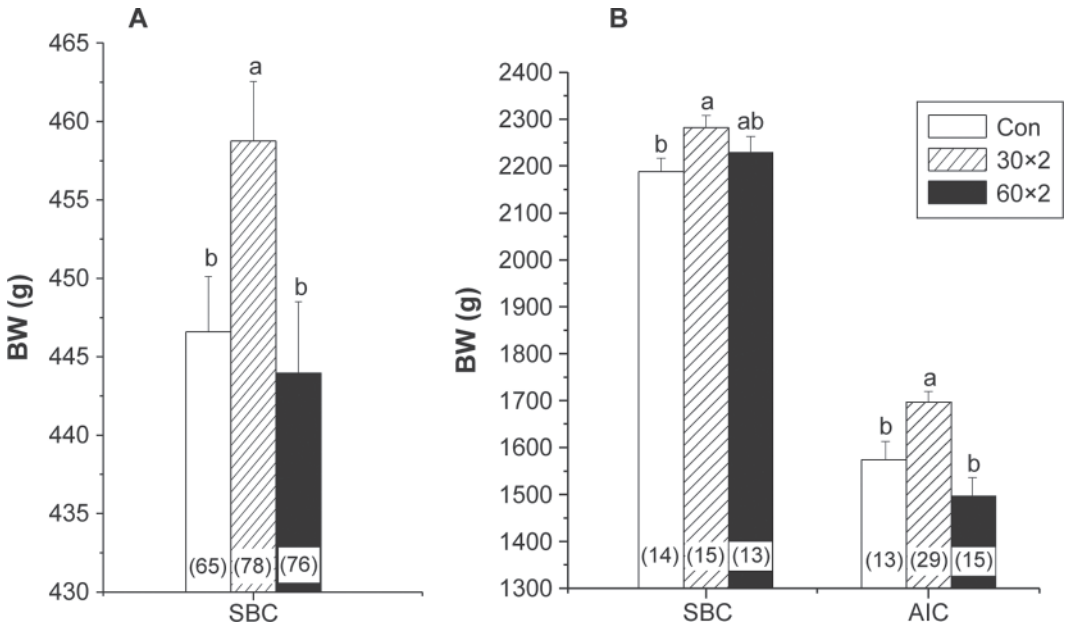

Figure 3. Body weight of control and prenatally cold-exposed chickens at A) 14 d of age and B) 35 d of age after rearing under either standard brooding conditions (SBC) or ascites-inducing conditions (AIC). Con (control): no prenatal cold exposure; 30×2: 15°C for 30 min on d 18 (E18) and 19 (E19) of incubation; 60×2: 15°C for 60 min on E18 and E19. Values marked with different letters differ significantly ($P < 0.05$). n-values are given in parentheses.

deed, the 2 prenatal exposures to cold (15°C) for 30 min, on E18 and E19, elicited significant ($P < 0.05$) reduction in mortality compared with the control, associated with significantly ($P < 0.01$) reduced ascites incidence. It could be hypothesized that decreasing T_{egg} to about 27°C, coupled with an associated total SHL of about 650 cal, was effective in imparting acquisition of long-lasting cold thermotolerance. The 60×2 treatment also achieved an increase of breast muscle but no advantage in cold resistance compared with control, which suggests that under the conditions of the present experiment the intensity of cold exposure in this treatment exceeded the scope of effective manipulation.

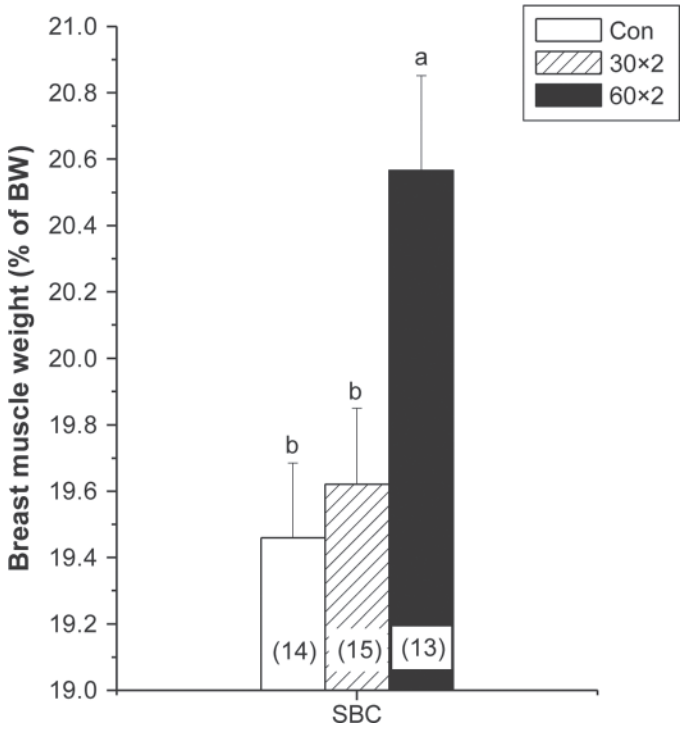

Figure 4. Breast muscle weight of healthy 35-d-old chickens reared in standard brooding conditions. Con (control): no prenatal cold exposure; 30×2: 15°C for 30 min on d 18 (E18) and 19 (E19) of incubation; 60×2: 15°C for 60 min on E18 and E19. Values marked with different letters differ significantly ($P < 0.05$). n-values are given in parentheses.

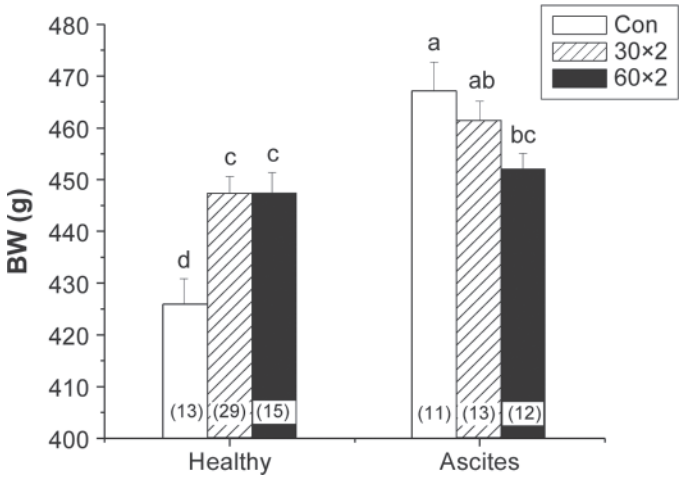

Figure 5. Body weight of 14-d-old control and prenatally cold-exposed chickens that were reared under ascites-inducing conditions until d 35 and found as healthy or with ascites syndrome. Con (control): no prenatal cold exposure; 30×2: 15°C for 30 min on d 18 (E18) and 19 (E19) of incubation; 60×2: 15°C for 60 min on E18 and E19. Values marked with different letters differ significantly ($P < 0.05$). n-values are given in parentheses.

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As was expected, the decrease in T_{egg} during cold exposure was accompanied by a decrease in embryo HR. This was consistent with earlier findings that abrupt exposure to lower environmental temperature during the final days of incubation resulted in reductions in HR and T_{egg}, which coincided with a reduced ability to thermoregulate and to conduct metabolic responses (Tazawa and Nakagawa, 1985; Tazawa et al., 2001).

The embryos in treatment 30×2 exhibited enhanced metabolic capacity, manifested in increased T_{egg} and HR on E19, before the second cold exposure. These responses may have resulted from the first cold exposure, on E18. After hatching, this tendency was manifested as a numerical alteration in T_b at hatch and on d 3 posthatch; a similar response to prenatal cooling has been reported by Tazawa et al. (2001). It has been well documented that in *Gallus gallus* endothermic responses could be detected from approximately E18 onwards, which suggested that the metabolic rate increased as a result of relatively lower incubation temperatures (Tazawa et al., 2001; Nichelmann, 2004). Although embryos have only relatively limited capability to regulate their T_b during this last period of embryogenesis, these findings might indicate a change in temperature setpoint imprinting that facilitates subsequent acceleration of the metabolic rate. It had been established that thermal manipulations during embryogenesis of chicks affected their posthatching metabolic rate, as indicated by changes in their T_b (Yahav et al., 2004; Collin et al., 2007; Piestun et al., 2008; Shinder et al., 2009). It could be concluded, therefore, that repeated short exposures to cold during the last phase of embryogenesis affected the metabolic rate, as indicated by the T_b changes observed in the present study.

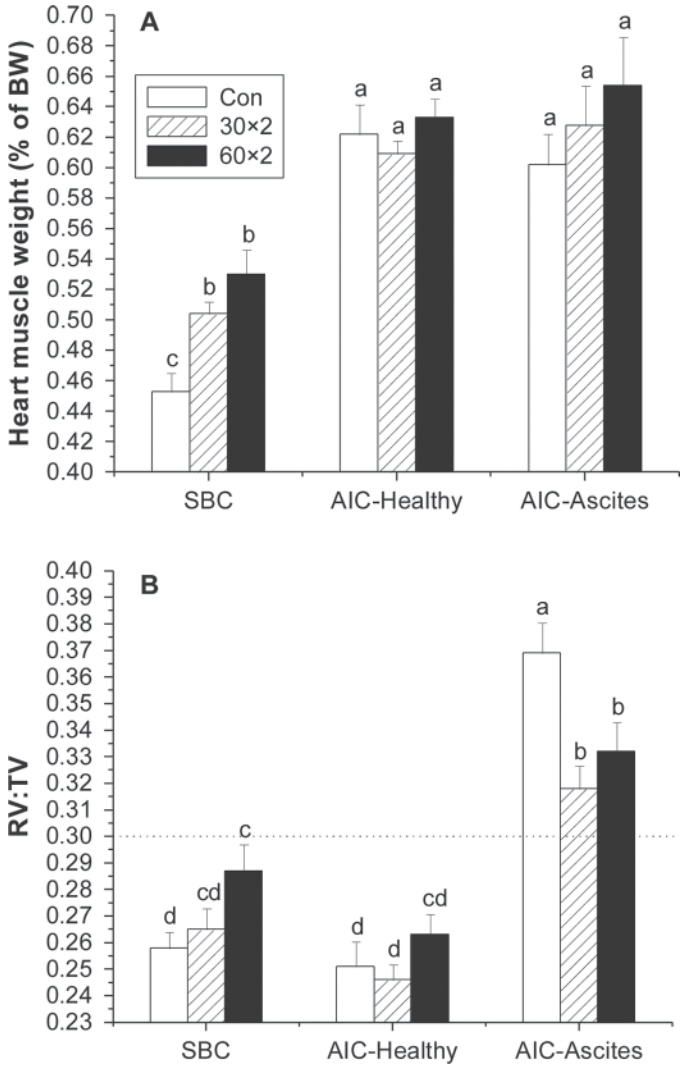

Figure 6. A) Heart muscle weight and B) right ventricle:total ventricle ratio (RV:TV) of 35-d-old control and prenatally cold-exposed chickens that were reared under standard brooding conditions (SBC), reared under ascites-inducing conditions (AIC) and remained healthy, or reared under AIC and found with ascites syndrome. Con (control): no prenatal cold exposure; 30×2: 15°C for 30 min on d 18 (E18) and 19 (E19) of incubation; 60×2: 15°C for 60 min on E18 and E19. RV:TV ratio higher than 0.3, indicated by the dotted line in B, was classified as ascites syndrome. Values marked with different letters differ significantly ($P < 0.05$).

The increases in T_{egg} and HR on E19, before the second cold exposure and as a result of the first cold exposure, could be attributed to imprinting of thermotolerance in the biochemical memory (Tzschentke and Plagemann, 2006), whereas the second cold exposure on E19 might have served as a training response for biochemical memory or as a result of further maturation of the embryo on E19.

It was well known that chickens that were heavier at an early age (i.e., from the age of 7 d) usually maintained their progressive weight advantage until marketing age (Willemsen et al., 2008). Progressive growth rate was associated with susceptibility to ascites (Olkowski, 2007) but not at the same rate (Gonzales et al., 1998). It was confirmed in the present study that control chickens that were heavier at 14 d of age displayed higher susceptibility to ascites. On the other hand, Druyan et al. (2007) found examples of broilers that exhibited high growth rates but did not develop ascites syndrome despite their high oxygen demand, probably because they were genetically resistant. Under the present experimental conditions, both the growth potential and acquired enhanced ability to withstand AIC were maintained only in chickens from treatment 30×2. It could be considered, therefore, that the 30×2 treatment was able to impart to (at least some) chickens an adaptation related to cold (ascites) resistance, which at the same time enabled them to maintain their fast-growth potential.

Hypertrophy of the heart was observed in chickens exposed to low T_a (Yahav et al., 1997; Shinder et al., 2002) and also in those that had undergone prenatal cold conditioning (Shinder et al., 2009). In all cases the heart hypertrophy was related to increased oxygen demands for maintenance (Wideman et al., 2007) causing an increase in heart rate that led to hypertrophy. Healthy chickens that were exposed to AIC exhibited

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heart hypertrophy although their RV and RV:TV ratio were not altered, whereas ascitic chickens exhibited a significantly enlarged RV:TV ratio (Luger et al., 2001, 2003) that caused insufficient cardiac output from the left ventricle and led, in turn, to ascites (Olkowski, 2007). In the present study, hypertrophy of the heart was developed under SBC also, as a result of prenatal cold exposure. Under AIC the expected heart hypertrophy, associated with an increased RV:TV ratio, was found in ascitic chickens, but the incidence was significantly lower in chickens that previously had experienced cold conditioning. It can be deduced that prenatal cold exposure influenced cardiovascular function and thereby enhanced the ability to cope with harsh environmental conditions, but this still has to be elucidated.

In conclusion, the prenatal 30×2 treatment significantly reduced the ascites development rate by 26%. The increased weight gain of the treated chickens, under SBC as well as under AIC, was consistent with a model that relates the effect to an increase in the metabolic rate. It may further be suggested that cold conditioning during the last phase of embryogenesis contributed to the inhibition of ascites syndrome development by increasing the chickens' cold tolerance. As a result, the performance in different conditions was improved without negative effects on poultry farming parameters. The applicability of these findings to commercial hatcheries should be investigated further.

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