

Effect of early cold exposure on the endocrine responses of broiler chickens and the incidence of ascites syndrome.

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Abstract

Two hundred and fifty day-old broiler chickens were housed in a high altitude farm and divided in two experimental groups (cold-exposed and control). At the age of 3 and 4 d, the cold-exposed chickens were exposed to 15°C for 3 h, while the control birds were reared under normal condition. During the period between 14 and 28 days, both groups of chickens were exposed to a low environmental temperature. For hormonal analyses, blood samples were taken at days three and four (before and after cold exposure), and followed by samples taken at seven, 14, 28 and 42 days. The mean plasma corticosterone level was significantly higher in cold chickens, who were first exposed to cold temperature at days three and four of age. However, this result was reversed at day 28 during the second cold challenge. Cold-exposed birds showed significantly higher T_3 levels compared to control chickens at day three after exposure to cold temperatures. During the second cold exposure (day 28), the T_3 level was significantly lower in cold chickens compared to control chickens. At days three (after cold exposure) and four (before cold exposure), the concentrations of T_4 were significantly lower in cold-exposed chickens compared to the control chickens. Such hormonal responses coincided with a marked reduction of ascites mortality in the early cold-exposed chickens compared with control chickens. In the present study, repeated cold exposure resulted in a moderate response; lower corticosterone and T_3 levels resulted from the second cold exposure than from the first one alone. It can be concluded that early cold exposure improves the thermotolerance of broiler chickens at a later age, which consequently leads to an adaptation that reduces the incidence of the ascites syndrome.

Introduction

Ascites syndrome appears in fast-growing broiler chickens, mostly during the winter, and it is a considerable cause of mortality (Maxwell and Robertson, 1998). This syndrome is multifactorial and caused by exogenous and/or endogenous factors (Decuyper *et al.*, 2000). A major cause of ascites is hypoxemia that can occur due to increased oxygen demands in chickens that are growing rapidly. Ambient temperature is related to the rate of metabolic activity and hence, to the amount of oxygen required by the birds (Kuhn *et al.*, 1984). The ability to cope with acute stress in general and thermal stress in particular is determined mainly by the extent of plasma corticosterone elevation and the capability to maintain body temperature, respectively (Felszeghy *et al.*, 2000). Corticosterone and thyroid hormones are important for regulating the metabolic rate during the post-hatch period (Decuyper *et al.*, 2000), and they play a

considerable role in the susceptibility of broiler chickens to ascites (Hassanzadeh *et al.*, 2004; De Smit *et al.*, 2006). This becomes even more apparent when low ambient temperatures are present (Scheele *et al.*, 1992).

The ontogeny of the physiology of control systems is most important for the adaptability of organisms throughout their life. The processes of adaptation to ambient temperature take place at three different levels: genetic, phenotypic and epigenetic (Nichelmann, 1992). Epigenetic adaptation is an adaptation to an expected environment. It is innate, but not genetically fixed, and is mainly caused by changes in gene expression (Tzschentke *et al.*, 2001). Chickens can be conditioned to thermal stress tolerance during the prenatal and early postnatal period by the epigenetic adaptation mechanism (Nichelmann *et al.*, 2001). During this period, the thermoregulatory feedback mechanism is not completely mature (Tzschentke, 1997), and thermal conditioning can induce a long-

lasting “memory” that imparts a better ability to cope with thermal stresses later in life (Yahav and Hurwitz, 1996; Decuypere *et al.*, 2001).

It has been found previously (Shinder *et al.*, 2002 and personal communication) that in broiler chickens, cold conditioning at an early age induces cold-stress tolerance at a later age and this has been correlated with a general reduction of mortality rate. Therefore, this study aimed to investigate the influence of early age cold conditioning on the endocrine responses of broiler chickens and to assess their ability to cope with a metabolic adaptation that may favor an increased incidence of ascites.

Materials and Methods

A total of 250 day-old broiler chickens (Ross 308) were obtained from a commercial hatchery, which was located at a high altitude. Chickens were housed and divided in two experimental groups (cold-exposed and control) at a high-altitude farm (2,100 m above sea level). Each group of chickens was distributed over five floor pens (25 chickens per pen). All birds had *ad libitum* access to commercial feed and drinking water. At the age of 3 and 4 d, the cold group chickens were exposed to 15°C for 3 h while, the control birds were reared under normal condition as described by Shinder *et al.* (2002). During the period of 14 to 28 d both groups of chickens were again challenged to low environmental temperatures. At day 14, the temperature was lowered 2°C per day until the temperature was 15°C (day 21); the temperature was maintained at this level until the end of day 28. Blood samples were taken on days three and four (before and after cold exposure) to analyze the levels of T_3 , T_4 , corticosterone and hematocrit, and these were followed by further blood tests at days seven, 14 (before the second cold challenge), 28 (during the second cold challenge) and 42 of age. Hematocrit was measured immediately, whereas the plasma was stored at -20°C until the T_3 , T_4 and corticosterone levels were analyzed (Decuypere *et al.*, 1983; Hassanzadeh *et al.*, 2004).

Body weights, feed intake and the feed conversion ratio (FCR) were recorded in all chickens every two weeks. Chickens were examined daily for lesions that suggested ascites, and the mortality rate was also recorded daily. At the end of the experiment, 50 chickens from each group were selected randomly and slaughtered. The heart was dissected and the atria, major vessels and fat were removed. The right ventricle/total ventricle (RV/TV) ratios were determined and classified as reported previously (Julian, 1987; Hassanzadeh *et al.*, 2000). Statistical analysis was performed using the "General linear model procedure" (SAS, 2002). Values are expressed as means \pm SEM. If a significant overall effect ($p < 0.05$) was found, the treatment means were compared with the use of the Scheffe test.

Table 1: Weekly mortality and the ratios of RV/TV in control and cold-exposed chickens that were slaughtered at the end of the experiment.

Groups	Non-ascites mortality	Ascites mortality					RV/TV ¹	
		Wk 3	Wk 4	Wk 5	Wk 6	Total	≥ 25 to ≥ 29	≥ 29
Control	4	-	2	3	11	16	19/50 ²	18/50
Cold-exposed	7	-	-	2	6	8	13/50	14/50

¹RV/TV= Right ventricular/ total ventricular weight ratios

²Number of right ventricular hypertrophy / 50 chickens

Results

Ascites mortality and RV/TV ratio

Weekly mortality due to right ventricle hypertrophy (RVH) and ascites, and the RV/TV ratios of surviving chickens that were slaughtered at six weeks of age, are presented in Table 1. Over the six-week growing period, 35 of the 250 birds died. The total number of birds that died due to RVH and ascites was 24 (9.6%) out of the 250 chickens. The rate of ascites mortality was numerically higher in the control group compared with the cold-exposed chickens (16 versus eight birds). The other 11 dead birds, in which four birds belonged to the control group versus seven birds in the cold-exposed group, had no lesions of ascites and died due to other causes that included of neonatal infection, arthritis and sudden death syndrome. At the end of experiment, the number of surviving birds that showed a RV/TV ratio of over 0.25 was obviously higher in chickens in the control group compared with those in the cold-exposed group.

Hormonal and hematocrit measurements

The mean plasma corticosterone level was significantly higher ($p < 0.0001$) in cold-exposed chickens compared to the control chickens, after they were first exposed to cold temperatures at both days three and four of age. However, this result was reversed by day 28 during the second cold challenge (Figure 1). Cold-exposed birds showed significantly higher ($p < 0.0001$) T_3 levels compared to the control chickens at day three after the first exposure to cold temperature. During the second cold exposure (day 28), the level of T_3 was significantly lower ($p < 0.0001$) in cold chickens than in control chickens (Figure 2).

At day three after the first cold exposure and day four before the first cold exposure, T_4 concentrations were significantly lower ($p < 0.0001$) in the cold-exposed chickens compared to control chickens, but no significant difference was found during the second cold exposure at the later ages (Figure 3). During the first six weeks of age, no significant difference was observed between the hematocrit levels of two group chickens (Figure 4).

Table 2: Mean body weight, feed intake and feed conversion ratio in control and cold-exposed chickens (values are means \pm SEM).

Parameters / groups	Control	Cold-exposed	p-value
Body weight (g/chicken)			
Day 1	40 \pm 0.8	39 \pm 0.3	Ns ¹
Day 14	309 \pm 05	314 \pm 06	Ns
Day 28	1034 \pm 14	1054 \pm 13	Ns
Day 42	1958 \pm 27	2075 \pm 48	0.01
Feed intake (g/chicken)			
Day 1-14	306 \pm 13	305 \pm 5	Ns
Day 14-28	1287 \pm 63	1252 \pm 48	Ns
Day 28-42	2203 \pm 26	2328 \pm 66	Ns
Day 1-42	3796 \pm 55	3884 \pm 108	Ns
Feed conversion ratio			
Day 1-14	1.13 \pm 0.04	1.11 \pm 0.02	Ns
Day 14-28	1.78 \pm 0.1	1.69 \pm 0.05	Ns
Day 28-42	2.30 \pm 0.06	2.30 \pm 0.12	Ns
Day 1-42	1.98 \pm 0.04	1.90 \pm 0.06	Ns

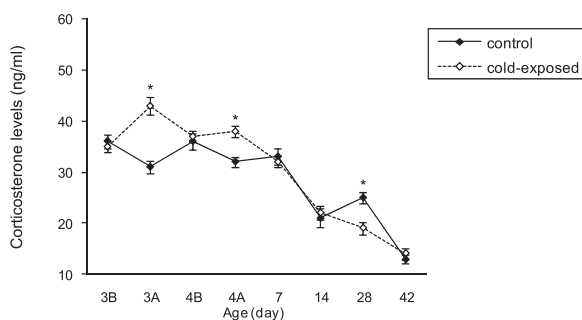
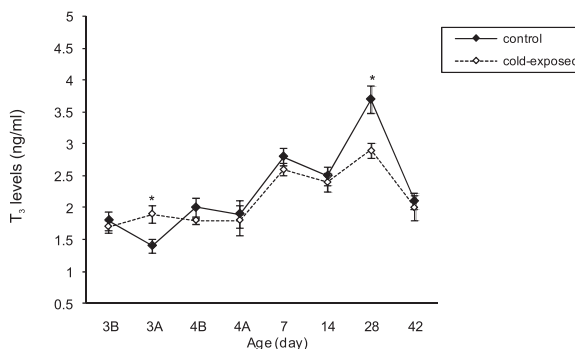
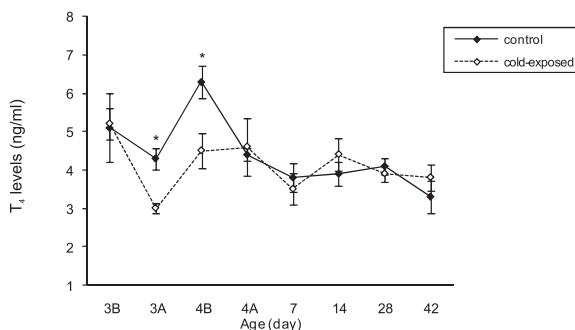
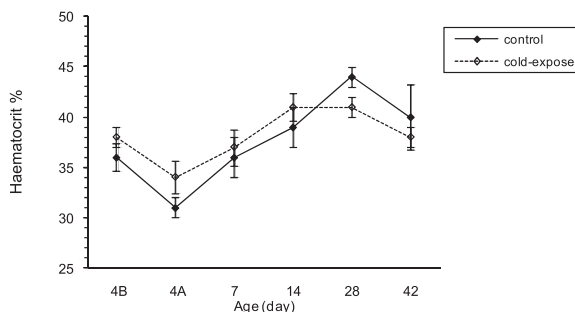
¹ Not significant

Growth performance

The mean body weight, feed intake and feed conversion ratio of the two groups of chickens are summarized in Table 2. There was no significant difference between the body weights of the two groups of chickens until day 42 of age. At this age, cold-exposed chickens had a significantly higher ($p < 0.01$) mean body weight than the control chickens. The feed intake and FCR in the two groups of chickens did not differ significantly throughout the entire experimental period.

Discussion

The structural or endocrine changes that are often associated with susceptibility to ascites in chickens may be influenced in the early stages of development, and perhaps even during embryogenesis (Decuyper *et al.*, 1991; Dewil *et al.*, 1996). Current observations by Hassanzadeh *et al.* (2004, 2008) indicate a concomitant alteration of the activity of thyroid and corticosterone hormones, and earlier hatching in high-altitude incubated embryos. As a metabolic adaptation to

Figure 1: Plasma levels of corticosterone in control and cold-exposed chickens. 3B = Day 3 before cold exposure; 3A = Day 3 after cold exposure; 4B = Day 4 before cold exposure; 4A = Day 4 after cold exposure. Asterisks indicate significant differences between the ages of measurement ($p < 0.05$, $n = 10$)**Figure 2:** Plasma T_3 levels in control and cold-exposed chickens. 3B = Day 3 before cold exposure; 3A = Day 3 after cold exposure; 4B = Day 4 before cold exposure; 4A = Day 4 after cold exposure. Asterisks indicate significant differences between ages of measurement ($p < 0.05$, $n = 10$).**Figure 3:** Plasma T_4 levels in control and cold-exposed chickens. 3B = Day 3 before cold exposure; 3A = Day 3 after cold exposure; 4B = Day 4 before cold exposure; 4A = Day 4 after cold exposure. Asterisks indicated significant differences between ages of measurement ($p < 0.05$, $n = 10$)**Figure 4:** Hematocrit values in control and cold-exposed chickens. 3B = Day 3 before cold exposure; 3A = Day 3 after cold exposure; 4B = Day 4 before cold exposure; 4A = Day 4 after cold exposure.

environmental hypoxia, these alterations lead to improved structural changes in the cardiopulmonary systems of broiler chickens. These authors hypothesized that genotype and environmental interactions may play an important role in the developmental changes of broiler chickens, which finally result in a reduced incidence of ascites.

Aulie, (1977) reported that cold conditioning

applied in faster growth chickens at an early age improves the thermoregulatory capacity of chickens at later ages. Shinder *et al.* (2007) concluded that repeated cold exposures to chicks to 15°C (at both three and four days of age) increased their ability to maintain body temperature and enhanced thermotolerance during the second cold challenge at a later age. These authors demonstrated that, concomitant with the cold-stress tolerance, the number of dead birds decreased in the early cold-exposed group of chickens compared with the ones grown at normal temperatures.

In the present study, the ascites mortality and the ratio of RV/TV decreased in the group of cold-exposed chickens compared to the control chickens when exposed to a second cold challenge at a high altitude. This was accompanied with a moderate response, such as the lower corticosterone and T_3 levels that resulted from the second cold exposure than from the first one. These results suggest an enhancement in the capacity of the conditioned chickens to cope with repeated acute cold exposures, which leads to an improvement in the tolerance of broiler chickens at a later age, as considered by Shinder *et al.* (2002, 2007). In our study, exposing chickens to short-term cold conditioning led to initial increased of corticosterone and T_3 levels at an early age, while the results were reversed at day 28 during the second cold challenge. This could be suggesting that a shift to a new stress-related threshold may play a significant role in the ability of the chickens to cope with new metabolic stressors, such as a second episode of cold temperature and/or hypoxia due to high altitude. The late alterations in stress-related neuroendocrine and thermo-adaptive hormones might be the result of thermal conditioning at an early age. Such alterations mainly involve the adjustment of the hypothalamo-pituitary-adrenal axis, as argued by Felszeghy *et al.*, (2000). It could be hypothesized that developmental changes induced by environmental conditions, such as low ambient temperatures, may also play a role in the interactions of endogenous and environmental factors. This could benefit broiler chickens by leading to a decreased susceptibility to ascites by a similar epigenetic adaptation to that which was previously suggested in chicks hatched at high altitudes (Hassanzadeh *et al.*, 2004, 2005, 2008) or hatched in an environment with a high concentration of carbon dioxide (Buys *et al.*, 1998; Hassanzadeh *et al.*, 2002; De Smit *et al.*, 2006).

However, the significantly lower T_4 concentration observed at day four before cold exposure in cold-exposed chickens compared to control chickens is not fully understood, but the significant reduction of T_4 and increase of T_3 levels after a cold challenge at day three of age could be related to an increased rate of T_4 conversion into T_3 . The behavior of the corticosterone levels was similar to those of the levels of T_3 at days three and 28 of age. This is probably caused by the cold

stress response through the synergism association between thyroid and corticosterone hormones (Decuyper *et al.*, 1983; Meeuwis *et al.*, 1989).

In the present study, the hematocrit values of the two groups of chickens did not significantly differ during the six weeks of study, which is in agreement with previous reports in which there has not always been an association between ascites syndrome and the levels of hematocrit (Shlosberg *et al.*, 1998; Hassanzadeh *et al.*, 2000). However, the average hematocrit values of both groups of chickens were higher than the normal values. Such high hematocrit levels could be related to the fact that the chickens were reared at high altitude, which might explain the significant effect of the cold thermal stress on hematocrit values.

Except for the significant difference between the final body weights of the two groups of chickens, cold exposure at an early age had no significant effect either on feed intake or on FCR throughout the entire experimental period in our study. These results correlate with the report of Yardimci *et al.* (2006). A similar result on the improvement of final body weight in cold-conditioned chickens compared with control chickens was previously reported by Shinder *et al.*, (2002). However, the pattern between their finding and our results was not similar. In our study, the differences in the final growth curves between the cold-conditioned and control birds could be related to a higher incidence of ascites and RVH in the control group compared with the cold-exposed chickens, as ascites causes a significant deterioration in the growth performance of broiler chickens (Julian, 1993; Hassanzadeh *et al.*, 2002, 2003).

In conclusion, our data indicate that cold exposure at an early age results in an enhancement in the cold tolerance of broiler chickens in later life. This could be related to the change in the endogenous functions of chickens, such as the levels of plasma corticosterone and thyroid hormones. The change of these important parameters is favorable to epigenetic adaptations that might be beneficial to the metabolic rate or the structural size of the cardiopulmonary systems in broiler chickens. These combined effects could lead to a lower degree of susceptibility of birds to pulmonary hypertension. Further experiments need to be performed to determine the optimal temperature and the best timing for early cold exposure to produce these beneficial effects.

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