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Adaptive response of chickens to hot environments induced by changing incubation temperature

Collin A.¹, Loyau T.¹, Bedrani L.¹, Berri C.¹, Métayer-Coustard S.¹, Praud C.¹, Duclos M.J.¹, Tesseraud S.¹, Rideau N.¹, Hennequet-Antier C.¹, Everaert N.², Mignon-Grasteau S.¹, Yahav S.³

¹ - INRA, UR83 Recherches Avicoles, F-37380 Nouzilly, France

² - Department of Biosystems, K.U.Leuven, Kasteelpark Arenberg 30, 3001 Leuven, Belgium

³ - Institute of Animal Science, The Volcani Center, Bet Dagan P.O. Box 6, 50250 Israel

Summary

Fast-growing chickens have gained muscle mass throughout selection programs without similar improvement in the cardiovascular and respiratory systems that are of major importance for thermoregulation. Broiler chickens therefore cope with hot spells by reducing feed consumption and growth during moderate and chronic heat exposure, whereas a dramatic increase in morbidity and mortality occurs during acute heat exposure. Research has recently focused on early thermal acclimation in order to alleviate heat stress in the long term. This technique is based on fine tuning of incubation conditions, taking into consideration the rate and duration of elevated temperature and relative humidity during the critical period of embryogenesis with the aim of stimulating long term thermotolerance. The consequences of such treatment on growth performance and meat quality were explored to ensure the potential application of this strategy. The physiological basis of the technique is the induction of epigenetic and /or metabolic mechanisms that regulate body temperature in the long term. Early thermal acclimation could favor poultry robustness in the face of environmental changes without

significantly affecting growth performance. We present a review of the main strategies of early heat exposure and the physiological concepts underlying these methods. The research axes to explain the mechanisms underlying the adaptive response are then explored, as well as the potential interest of embryo heat exposure for poultry production.

Introduction

Poultry production has increased in recent decades in countries that experience hot spells or chronic heat (Asia, North Africa, South America, etc...), and the genotypes used are often the same as those used in temperate areas in Europe and the United States. Exposure to moderate chronic heat induces a decline in performance: birds tend to decrease their heat production by limiting feed consumption, which increases the growth period to slaughter. High mortality and morbidity occurred in poultry houses in North Africa and in France during hot spells in 2003, resulting in huge economic losses (Amand *et al.*, 2004). Such heat waves lead to welfare problems, especially during the finishing period when poultry species are the most sensitive to heat (Tesseraud and Temim 1999). Acute heat exposure increases blood corticosterone concentrations (Yahav *et al.*, 2004b), modifies the immune response (Altan *et al.*, 2000), increases oxidative stress (Mujahid *et al.*, 2005), causes respiratory alkalosis induced by hyperventilation (Arad and Marder 1983), and is considered as stressful event in chickens (Debut *et al.*, 2005). Working in Venezuela, Lozano *et al.*, (2006) attributed 10% of the mortality during the finishing period to heat strokes. Genetic selection of broiler chickens has increased growth rate. However, some of the visceral organs such as the cardiovascular and respiratory systems are not enlarged in the same manner as muscle (Havenstein *et al.*, 2003). The efficiency of vital functions such as thermoregulation, soliciting the respiratory and cardiovascular systems for hyperventilation and heat loss (Leterrier *et al.*, 2009), is thus reduced. Different genetic strategies (cross-breeding with chickens selected for thermotolerant genotypes),

management (feed restriction during the hottest hours of the day, acclimation, etc...) or nutritional techniques (sequential feeding, etc...) can be applied to reduce the effects of heat exposure in avian species. One recently developed method aiming to increase thermotolerance in chickens without affecting performance is embryo heat exposure during embryogenesis. This should result in physiological and metabolic changes that will be of long lasting effect throughout the animal's life.

Early heat acclimation in avian species

Previous studies (Yahav and McMurtry 2001; De Basilio *et al.*, 2001) showed that postnatal exposure of 3 or 5 day-old chicks (for 24h at 37.5-38 °C) enables chickens to control body temperature efficiently during exposure to heat challenge (at 34 or 42d), and can reduce the mortality rate by approximately 50%. Furthermore, the cloacal temperature of chicks exposed to 38 °C on day 5 remained significantly lower in tropical conditions until 40 days of age than that of controls (Venezuela; De Basilio and Picard 2002). The body temperatures of chickens during the growth period seem to be effective indicator of potential heat tolerance (De Basilio *et al.*, 2003): chickens that did not survive heat challenge at 34 days of age had higher body temperatures (0.6 °C) prior to heat challenge than those of the survivors. Postnatal heat exposure also improves later growth in broiler chickens (Yahav and Hurwitz, 1996; Halevy *et al.*, 2001) and has a positive impact on intestinal morphometry with higher and voluminous brush border cells (Uni *et al.*, 2001; Temim *et al.*, 2009). Finally, De Basilio *et al.*, (2001) studied the interaction between this method and the feed distribution mode, evaluating the effects of distributing energy-rich feed during day time and protein-rich diet during the night time while using high cyclic temperature conditions. The results showed that postnatal heat exposure combined with a sequential feeding program had beneficial effects on survival during heat challenge. This was also associated with slight positive effects on growth performance (+ 4%). Although postnatal acclimation seems to be an interesting method, it is still relatively difficult to apply on a large scale due to the difficulty of maintaining 38 °C for 24 hours in the poultry house, which depends on the type and efficacy of the heat source (De Basilio *et al.*, 2003). Thermal modifications during embryogenesis may therefore be easier for the poultry industry to apply.

Fine tuning of increasing incubation temperature during embryogenesis and the associated adaptive responses

Improving of the embryo's heat thermotolerance

was a subject of study for many years (Minne and Decuyper 1984; Iqbal *et al.*, 1990; Piestun *et al.*, 2008, 2009a; Yahav, 2009; Druyan *et al.*, 2012). However, the subject has been reconsidered during the last 10 years, especially in the Middle East (ARO The Volcani Center, Israel; Ege University, Turkey), Europe (INRA Nouzilly, France; Catholic University of Leuven, Belgium; Humboldt University of Berlin, Germany; Wageningen University, The Netherlands) and the United States (North Carolina University; USDA Maryland). The hypothesis on which these studies were based emphasizes the positive effects of elevating incubation temperature on the improvement of long lasting thermotolerance. Thermal changes during incubation have the advantage of mimicking natural incubation conditions in avian species (Piestun *et al.*, 2008). Several studies, testing different thermal conditions during embryogenesis to achieve better thermotolerance, also examined their consequences on other parameters. These studies evaluated the sensitive periods of embryogenesis during which the thermoregulatory threshold response could be manipulated, without affecting hatchability and/or performance. The main factors in the fine tuning of thermal changes during embryogenesis responsible for improvement of thermotolerance are the critical period of embryogenesis and the level and the duration of thermal changes (Yahav *et al.*, 2004a). Age of the breeders has also to be considered.

• Critical period during embryogenesis:

The period of development and maturation of thermoregulatory mechanisms, including the hypothalamus-pituitary -thyroid and -adrenal axes, has been proposed as the critical period for thermal manipulation to achieve efficient thermotolerance (Piestun *et al.*, 2008, 2009). Several authors have tested increases in incubation temperature before embryonic day 10 (E10; Yahav *et al.*, 2004a, Collin *et al.*, 2007, Piestun *et al.*, 2008), during the phase before the adrenal axis is established. Other authors have chosen the period following E13 (Moraes *et al.*, 2003, 2004, Yahav *et al.*, 2004a,b, Collin *et al.*, 2005), preceding the phase during which the production of thyroid hormones significantly increases. These studies, undertaken in broiler chickens, led to interesting results in the short term: they resulted in significant decreases in body temperature post-hatching and up to 28 days of age (Collin *et al.*, 2007). Oxygen consumption of embryos submitted to heat exposure between days E16 to E18 of embryogenesis has also been shown to be reduced as compared to control embryos (Tona *et al.*, 2008). However, cloacal temperatures were no longer different between treatments at 42

days post-hatching, and treated broilers were more sensitive to heat than controls when submitted to a heat challenge at 35 °C. Similarly, embryo heat exposure applied to layer eggs during late embryogenesis only affected body temperature until 8 days of age (Walstra *et al.*, 2010). The reason why the lower body temperature acquired by early heat exposure was not maintained was thought to be the result of interference with mechanisms regulating growth when the treatment was applied during late embryogenesis (Collin *et al.*, 2007). Recent results in Cobb broiler chickens from Piestun *et al.*, (2008, 2009a) indicate that the period between days E7 and E16 of embryogenesis, covering the overall setting of both regulatory axes, can lower body temperature in the long term. Heat tolerance was increased at 34 days of age, demonstrated by a 50% reduction in mortality during heat challenge.

- **Level of increase in incubation temperature during embryogenesis:** Thermal treatment during embryogenesis has been based in many studies on increases to 39.5 °C to achieve improved thermotolerance (vs. 37.8 °C; Moraes *et al.*, 2004, Collin *et al.*, 2007, Piestun *et al.*, 2008). Increasing the incubation temperature to 41 °C appeared to be too drastic to maintain hatchability as high as that of the controls (Yahav *et al.*, 2004a). Relative humidity (RH) in the incubator has been simultaneously increased from 56% to 65% in order to limit egg dehydration during heat exposure at 39.5°C (Piestun *et al.*, 2008).

- **Duration of exposure to elevated temperature:** The treatment consisted of alternating phases of high temperature (39.5 °C) and RH (65%) and phases of regular temperature (37.8 °C) and RH (56%). The daily exposure period usually tested was 3 h/d to avoid deleterious effects on hatchability. However, in our previous study (Collin *et al.*, 2005), durations of 6, 12 and 24 h/d were also tested, with similar short-term effects on body temperature of chicks. Piestun *et al.*, (2008) showed that a 10 day treatment with daily exposure of 12 or 24h affected the long term heat tolerance. While 12h/d at 39.5 °C did not affect hatchability or performance post-hatching, continuous treatment at 39.5 °C between days E7 and E16 of embryogenesis significantly reduced hatchability and broiler performance post-hatching (Piestun *et al.*, 2009b).

- **Age of breeders:** Yalçin *et al.*, (2005) investigated the relationship between age of breeders and the effectiveness of the thermal treatment. In thermally-treated chickens (from day E8 to day E10 of embryogenesis), they reported a

greater reduction in body temperature when eggs were obtained from 28-week-old breeders than with eggs from 58-week-old breeders, during the recovery from a heat challenge at four weeks of age. In most of the recent studies dealing with thermal treatment of embryos, 34 to 44 week-old breeder hens were used (Yahav *et al.*, 2004a, Piestun *et al.*, 2008).

Taken together, the treatment combining 39.5 °C with 65% RH for 12 h/d between days E7 and E16 of embryogenesis seems to enhance the thermotolerance of broiler chickens without affecting hatchability.

Short-term effects of thermal treatment during embryogenesis on growth and meat production

Different conditions of heat exposure during embryogenesis were tested on the criteria of body temperature, hatchability and thermoresistance. However, a technique applicable in practice has to maintain broiler performance. Treatments consisting of 6 or 12 h/d heat exposure at 39.5 °C from day E16 to E18 of embryogenesis seemed to trigger the initial chick growth (Collin *et al.*, 2005). Yalçin *et al.*, (2008) also recorded a transitory increase in body weight in chicks that were heat-treated from day E10 to E18 of embryogenesis (6 h/d at 38.5 °C). Exposure of 3 h/d to 39.5 °C between days E16 and E18 of embryogenesis did not seem to decrease growth performance up to slaughter age. Moreover, Collin *et al.*, (2007) showed no change in feed conversion ratio or body weight at days 28 or 42 of age between heat-treated and control chickens. In the latter study, breast meat quality (ultimate pH measured 24h post-mortem, drip loss) was slightly affected by the heat-treatments. Piestun *et al.*, (2008, 2009a) recently validated optimal conditions for embryo heat treatment (39.5 °C and 65% RH for 12 h/d from E7 to E16 of embryogenesis) in broiler chickens (Cobb 500) reared in cages; These conditions reduced body temperature and increased survival during a heat challenge at 35 days, without affecting hatchability or growth. However, a recent study by our laboratory (Loyau *et al.*, 2011) showed slightly lower body weight (reduced by 1.4%) at day 34 in group-reared Cobb-500 chickens exposed to similar thermal treatment during embryogenesis. The slight effect on body weight may have been due to interactions between animals minimizing the treatment effect or inducing additional stress. It is also important to validate (or even adapt) these incubation conditions to other genotypes to ensure high hatchability and viability of chickens that were heat-treated during embryogenesis (Bedrani *et al.*, 2009).

Embryo thermal treatment may also have beneficial effects on body composition. Breast muscle yield at slaughter age has been reported to be significantly higher than in control chickens in some studies (Collin *et al.*, 2007, Piestun *et al.*, 2011). One hypothesis is that the heat-treatment changes the rate of myoblast proliferation (Piestun *et al.*, 2011), enhancing myofiber diameter or number in the Pectoralis major muscle. Stimulation of satellite cell DNA synthesis has also been described by Halevy *et al.*, (2001) after early heat exposure of chicks on Day 3 post-hatching (37 °C over 24 h). These authors reported a significantly higher breast yield in the treated chickens at slaughter age. A lower fat pad yield in chickens that were thermally treated during embryogenesis has also been observed both at regular and high temperatures (Loyau *et al.*, 2011; Piestun *et al.*, 2011).

Mechanisms involved in embryo heat-acclimation

Thermoregulatory mechanisms underlying early thermal treatment have mostly been studied after hatching. There are probably several types. On the one hand, the neuro-endocrine response could affect an animal's heat loss (thermolysis) at the peripheral level (for example by redistributing blood vessels or flow, etc...; Yahav *et al.*, 2005). On the other hand, heat production (thermogenesis) and stress responses could be affected by early thermal exposure (Yahav and Plavnik, 1999). The proposed perinatal thermal treatment is intended to act through modifications of threshold responses to changes in ambient temperature during embryogenesis or the postnatal period. During these periods the neuro-endocrine thermoregulatory system is still able to adapt, especially through the thermosensitivity of neurons from the preoptic area of the anterior hypothalamus (Tzschentke, 2007). This could contribute to the long term modification of the individual reference temperature and consequently of the thermoregulatory threshold response of the animal (heat flows, behavior, etc...), thus inducing a quicker reaction to later changes in ambient temperature, by activating thermolysis and limiting thermogenesis (Collin *et al.*, 2011). This hypothesis is supported by the decrease in the plasma concentrations of the thyroid hormone T3 (triiodothyronine), which is well known to regulate heat production in birds and in mammals, as reported in several studies dealing with perinatal heat acclimation (Yahav and McMurtry 2001, Yahav *et al.*, 2004b, Piestun *et al.*, 2008). In a recent study of Piestun *et al.*, (2009a), O₂ consumption by embryos submitted to thermal treatment during the late phase of embryogenesis was also lower than

that of control eggs. In addition, the corticosterone peak induced by a heat challenge in the finishing period is lower in chickens that were thermally treated as chicks than in their control counterparts (Yahav *et al.*, 2007). This model showed that the expression of the avian uncoupling protein UCP3, potentially involved in heat production and in the limitation of oxidative stress, was strongly inhibited (Taouis *et al.*, 2002). The most effective conditions for exposing embryos to heat treatment are also those covering the period of the establishing of the thyroid and adrenal axes. Research is still going on to identify the target genes and enzymes potentially affected by these treatments and responsible for a decrease in thermogenesis in heat-treated chickens. One aspect of these mechanisms is probably explained by epigenetic modifications, e.g. possibly transmitted modifications of gene expression and/or phenotypes without any change in the DNA nucleotidic sequence. The postnatal or embryo heat exposure could modify the chromatin structure in the long term and /or the methylation of parts of the DNA regulating genes involved in thermoregulation. Studies by Yossifoff *et al.*, (2008), Kisliouk and Meiri (2009) and Kisliouk *et al.*, (2010) have shown that regulation of the hypothalamic expression of transcription factor BDNF (Brain-Derived Neurotrophic Factor), involved in postnatal heat-treatment, is regulated by such mechanisms. However, the potential transmission of this characteristic to future generations through the breeding of chickens has not yet been described in the literature.

The capacity of embryos to establish regulatory mechanisms lowering internal temperature that last until slaughter age may depend on their parental origin or their genotype, and the possible interference of breeder nutrition with these mechanisms remains to be explored. Current research is focused on determining the genetic effects on the physiological responses of animals to embryo heat acclimation and to subsequent heat challenge (Loyau *et al.*, 2011).

Conclusion

The techniques of embryo thermal treatment could be beneficial to avian production. They require fine tuning of temperature and relative humidity in the incubator, and adaptation of this tuning to the genotypes used. If the techniques of early heat treatment or early cold treatment, also under study, are further proven to be an advantage for large scale rearing of chickens without affecting

growth performance and/or product quality too much, they could enhance poultry robustness and welfare. Such techniques have to date been optimized only in broiler chickens, in terms of the later response of chickens to heat challenge at slaughter age. However, their behavioral responses and the effects of embryo thermal treatments on other genotypes such as layer lines remain to be investigated, especially during long periods of heat exposure. In theory, early thermal acclimation offers the possibility of reducing heating or climatization costs in poultry houses. These potential reductions have to be evaluated through an economic analysis, especially taking into account farming systems. Finally, embryo thermal exposure represents an excellent model for analyzing the mechanisms involved in thermoregulation and their interactions with growth, body composition and bird welfare.

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