Effects of Turning Duration During Incubation on Corticosterone and Thyroid Hormone Levels, Gas Pressures in Air Cell, Chick Quality, and Juvenile Growth

K. Tona, O. Onagbesan, B. De Ketelaere, E. Decuypere, and V. Bruggeman

Laboratory for Physiology and Immunology of Domestic Animals, Department of Animal Production, Faculty of Agricultural and Applied Biological Sciences, K. U. Leuven, Kasteelpark Arenberg 30, B-3001 Leuven, Belgium

ABSTRACT Two peaks of embryo mortality have been identified during incubation of chicken eggs (the first and the final phases of incubation), and both are linked to egg turning. Turning in the first week enables proper formation of extra-embryonic membrane and in the final week avoids embryo malpositioning. The hatchability of the eggs, however, may depend on physiological parameters (e.g., hormone levels or gas pressures) that turning may influence to ensure proper embryo development and survival. It is not clear how long turning should continue during this second phase to ensure these conditions, but it is general practice to turn eggs until 18 d. This study describes the effects of turning chicken eggs for different durations during the last days of incubation on embryo physiological parameters that may be linked to embryo survival, hatchability, and broiler posthatch performance. Cobb eggs were incubated for 21 d under standard incubation conditions but with varying turning durations. Eggs were turned until 12, 15, or 18 d and left in a horizontal position until transferred to hatcher baskets on the 19th day. Blood samples from embryos were analyzed for corticosterone at d 15, d 18, internal pipping (IP), and hatch. Triiodothyronine ($T_3$) and thyroxine ($T_4$) were measured at IP and at hatch. Partial pressures of CO$_2$ ($p$CO$_2$) and O$_2$ ($p$O$_2$) in the air cell were measured at d 18 and at IP stage. Hatchability of fertile eggs, incubation duration, weights of 1-d-old chicks, chick quality, and their growth potential determined as absolute weight and relative growth (RG) to d 7 were recorded. Corticosterone levels increased in all treatments with embryo age until hatch, but there were no significant differences among treatments. Eggs turned until 18 d had higher $p$CO$_2$ and lower $p$O$_2$ at IP than those turned for 12 or 15 d. $T_3$ and $T_4$ were higher at IP in eggs turned for 18 d compared with the other two groups in which $T_3$ and $T_4$ were not different. $T_3/T_4$ ratio in 1-d-old chicks was also lower in eggs turned until 18 d. Incubation duration, and weights of 1-d-old chicks were similar for all treatments groups. Hatchability and percentage of high quality chicks were lower for eggs turned for 15 d compared to the 2 other groups, which were not different. However, 7-d-old weights and RG decreased with increasing duration of egg turning. We concluded that although turning until 18 d benefited hatchability and chick quality, it depressed potential posthatch performance of the chick to 7 d of age.

(Key words: chick juvenile growth, chick quality, egg turning, physiological hormone)


INTRODUCTION

Egg turning is a natural phenomenon during incubation by hens. Artificial incubation requires mechanical turning to simulate such a condition. During artificial incubation, egg turning has been reported to reduce embryo malpositioning (Robertson, 1961), to prevent abnormal adhesion of the embryo or embryonic membranes to the shell membrane (New, 1957), and to encourage the complete and timely closure of the chorioallantois at the small end of the egg (Wilson, 1991). Turning is required to ensure proper utilization of albumen by the developing embryo within the normal incubation period (Randle and Romanoff, 1949). Knowledge of the physiological effects of turning on protein accumulation in amniotic fluid, growth rate of area vasculosa, and gas exchange (Deeming, 1989ab; Tazawa, 1980; Wilson, 1991; Pearson et al., 1996) has emphasized the importance of this aspect of incubation. Egg turning during incubation involves several parameters such as frequency, axis of setting and turning, turning angle, planes of rotation, and stage of incubation requiring turning (Wilson, 1991; Elibol et al.,

Abbreviation Key: IP = Internal pipping; $T_3$ = triiodothyronine; $T_4$ = thyroxine; $p$O$_2$ = $O_2$ partial pressure; $p$CO$_2$ = CO$_2$ partial pressure; RG = relative growth.
2002). Reports are conflicting as to when are the most critical periods for turning of chicken eggs during the final stages of incubation (Deeming, 1991; Wilson, 1991; Elibol et al., 2002). However, it is common practice to turn eggs until 18 d of incubation and generally agreed that adequate egg turning during incubation improves hatchability (Becker et al., 1969; Elibol et al., 2002). Robertson (1961) reported also that excessive high frequency turning leads to a depression of hatchability. Even though the physical consequences of not turning eggs have been studied widely, the physiological basis on which egg turning influences hatchability have not been fully elucidated. Neither have the consequences of inadequate turning of eggs on chick quality or their growth performance been studied. Increased embryo mortality or depressed hatchability may stem from changes in hormone levels involved in the hatching process, levels of metabolic rate of the embryo, or, indeed, O\textsubscript{2}/CO\textsubscript{2} exchanges at different periods of incubation consequent to inadequate turning of the eggs. Pearson et al. (1996) recently showed that lack of turning of chicken eggs during the last stages of embryonic development (d 12 to 19) impaired O\textsubscript{2} consumption through the chorioallantoic gas exchanger, resulting in less embryonic growth. Therefore, this study aimed to investigate the effects of turning until 12, 15, or 18 d of incubation on embryo corticosterone levels, gas partial pressures in the air cell, and thyroid hormones. These parameters will be related to the duration of incubation and hatchability of eggs, chick quality, and juvenile growth of chicks.

**MATERIALS AND METHODS**

**Experimental Design**

Two different experiments were conducted with Cobb broiler breeder eggs. Eggs were collected from breeders aged between 28 and 58 wk and stored for 7 d. The eggs were set for incubation in forced-draft incubators at a specific dry-bulb temperature of 37.6°C and a wet-bulb temperature of 29°C. Eggs were turned once per hour through an angle of 90°. Egg turning was stopped after 12, 15, or 18 d of incubation. Eggs from different breeder ages were divided equally among replications and turning treatments. When turning was stopped before d 18 of incubation, the eggs stayed in turning trays and were kept horizontal until transfer time. These turning durations were selected on the basis of 1) the presence of glucocorticoids in the blood of chick embryos between the 10th and 15th day and increase in blood plasma concentrations between the 15th and 19th day of incubation (Avrutina and Kisljuk, 1982; Avrutina et al., 1985); and 2) the fact that the hypothalamo-hypophyseal-thyroid axis is already functional at the 13th day of incubation (Freeman, 1974; Iqbal, 1989).

**Experiment 1**

Three replications of 150 eggs per turning duration (total of 1,350 eggs) were studied in order to measure thyroid hormones, corticosterone levels in blood, and gas pressures in the air cell. Eggs used for hormone levels and gas pressure measurement were randomly selected.

**Gas Partial Pressures.** Within each replication, 16 eggs with evidence of living embryos per turning duration were used to measure gas partial pressures (p\textsubscript{O\textsubscript{2}} and p\textsubscript{CO\textsubscript{2}}) in the air cell on d 18 of incubation. The same was measured at internal pipping (IP) by using samples of 12 eggs per replication per turning duration. These measurements were made directly in the air cells of the eggs by means of a blood gas analyzer to determine p\textsubscript{CO\textsubscript{2}} and p\textsubscript{O\textsubscript{2}}. This method of measurement of gas partial pressures in the air cell has previously been described by Dewil et al. (1996), Buys et al. (1998), and Tona et al. (2003b).

**Thyroid Hormones.** Blood samplings were done by cardiac puncture. Samples were collected from 12 embryos per replication per turning duration at IP stage for triiodothyronine (T\textsubscript{3}) and thyroxine (T\textsubscript{4}) measurements. Blood samples were also taken from samples of 25 newly hatched chicks from each replication according to turning duration to determine T\textsubscript{3} and T\textsubscript{4} levels. T\textsubscript{3} and T\textsubscript{4} concentrations were measured in plasma samples by RIA as described previously (Huybrechts et al., 1989; Darras et al., 1992). Antisera and T\textsubscript{3} and T\textsubscript{4} standards were purchased. Intra-assay coefficients of variation were 4.5 and 5.4% for T\textsubscript{3} and T\textsubscript{4}, respectively. All samples were run in the same assay in order to avoid interassay variability.

**Corticosterone.** Blood samples were collected at the 15th and 18th d of incubation from 10 embryos per replication per turning duration for the measurement of corticosterone concentrations. Blood samples collected for determination of thyroid hormones were also used to determine corticosterone levels at IP and in newly hatched chicks. Corticosterone concentrations in plasma samples were measured using a commercially available double antibody RIA kit (Decuyper et al., 1983; Meeuwis et al., 1989). All samples were run in the same assay in order to avoid interassay variability.

**Experiment 2**

Ten replications of 150 eggs per turning duration (total of 4,500 eggs) were observed in order to study hatching parameters such as incubation duration, hatchability, chick quality, and chick juvenile growth. Eggs were individually labeled in order to identify the hatchlings with the eggs. On d 18 of incubation, eggs were candled, and those with evidence of living embryos were transferred from turning trays to hatching baskets.

**Hatching Parameters.** Between 464 and 510 h of incubation, the transferred eggs were checked individually every 2 h. During this period, the times of the occurrence of IP and emergence from individual eggs were recorded. These data were used to calculate the hatching time (duration between IP and emergence). The total incubation
duration was then calculated as the time between setting and emergence. After 510 h of incubation, the number of hatchedit chicks was recorded. Eggs that failed to hatch were broken for macroscopic analysis in order to distinguish infertile eggs from eggs containing dead embryos. These data were used to calculate hatchability in relation to the number of fertile eggs.

Chick Quality and Juvenile Growth. All of the hatched chicks were examined macroscopically in order to score for quality and to classify them as chicks of high or suboptimal quality, as described by Tona et al. (2003a). Within each replication, samples of 45 1-d-old chicks of good quality according to the turning duration were weighed individually at the end of incubation. The chicks were reared up to 7 d of age at 30 to 32°C on floor pens at a stocking density of 14 to 15 chicks per m². The photoperiod was 23L:1D, and a standard broiler starter diet (2,800 kcal metabolizable energy, 18% crude protein) and water were provided ad libitum. Because chick BW on d 7 is linearly related to the chicken BW at slaughter age (Decuypere et al., 1979; McLoughlin and Gous, 1999; Tona et al., 2003c), the chicks were weighed again individually at the end of incubation. The chicks were turned for 12 d. The T3/T4 was therefore significantly lower in the chicks from eggs turned for 18 d (P < 0.001) compared to levels in eggs turned for 12 or 15 d. However, T3/T4 was not affected by turning duration. In 1-d-old chicks, T3 levels were higher for eggs turned until 12 or 15 d compared with those turned for 12 d. The T3/T4 was therefore significantly lower in the chicks from eggs turned for 18 d (P < 0.001) than in those turned for only 12 or 15 d. Between IP and emergence, T4 and T3 levels decreased (P < 0.001) in eggs turned for 18 d, whereas only T3 increased in embryos from eggs turned for 12 d (P < 0.05). Consequently, a significant rise in T3/T4 between IP and emergence was observed only in the 12- and 15-d turning groups but not in the 18-d turning group.

**Statistical Analysis**

The effects of turning duration and developmental stage on gas pressure, corticosterone level, and thyroid hormone concentrations were analyzed using a two-way, fixed effects ANOVA model (Neter et al., 1996). The model was

\[ Y_{ijk} = \mu + \alpha_i + \tau_j + (\alpha \tau)_{ij} + e_{ijk} \]

where \( Y_{ijk} \) = gas partial pressure, corticosterone level, and thyroid hormone concentrations of egg from turning duration \( i \) with developmental stage \( j; \mu = \) overall mean, \( \alpha_i = \) main effect of turning duration \( i; \tau_j = \) the main effect of developmental stage \( j; (\alpha \tau) = \) interaction between turning duration and developmental stage; and \( e_{ijk} \) = random error term for the gas pressure, corticosterone level, and thyroid hormone concentrations of egg \( k \).

Generalized linear regression was used to analyze the effects of turning duration on 1-d-old and 7-d-old chick weights. Logistic regression was used to analyze the effect of turning duration on hatchability and proportion of chicks of higher quality.

**RESULTS**

**Effect of Turning Duration on Corticosterone Levels**

Corticosterone concentrations in the blood of developing embryos during incubation according to turning duration are shown in Figure 1. Overall, the levels of corticosterone increased from d 15 until hatch (P < 0.001), irrespective of turning duration. At all developmental stages (from d 15 of incubation to hatch), plasma corticosterone levels were similar among turning treatments.

**Effect of Turning Duration on Thyroid Hormone Levels**

Blood plasma levels of T3 and T4 at IP stage and in newly hatched (1-d-old) chicks are shown in Table 1. At IP stage, T3 and T4 levels were higher in embryos of eggs turned for 18 d compared to levels in eggs turned for 12 or 15 d. However, T3/T4 was not affected by turning duration. In 1-d-old chicks, T3 levels were higher for eggs turned until 12 or 15 d compared with those turned for 12 d. The T3/T4 was therefore significantly lower in the chicks from eggs turned for 18 d (P < 0.001) than in those turned for only 12 or 15 d. Between IP and emergence, T4 and T3 levels decreased (P < 0.001) in eggs turned for 18 d, whereas only T3 increased in embryos from eggs turned for 12 d (P < 0.05). Consequently, a significant rise in T3/T4 between IP and emergence was observed only in the 12- and 15-d turning groups but not in the 18-d turning group.

**Effect of Turning Duration on Gas Partial Pressure**

Figure 2 shows the pCO₂ and pO₂ in air cells of eggs at d 18 of incubation and at IP stage. The overall trend showed that pCO₂ increased (P < 0.05) and pO₂ (P < 0.001) decreased between d 18 and IP. At d 18 of incubation, turning duration did not affect gas partial pressure. At IP stage, pCO₂ was higher (P < 0.05) in eggs turned until 18 d compared with those turned until 12 or 15 d, which showed no difference. Conversely, pO₂ was lower (P <
TABLE 1. Triiodothyronine (T₃), thyroxine (T₄), and T₃/T₄ according to turning duration

<table>
<thead>
<tr>
<th>Turning duration (d)</th>
<th>Embryos at internal pipping</th>
<th>Newly hatched chicks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₃</td>
<td>T₄</td>
</tr>
<tr>
<td>12</td>
<td>3.119⁺±0.244</td>
<td>7.262⁺±0.540</td>
</tr>
<tr>
<td>15</td>
<td>3.171ᵇ±0.249</td>
<td>7.841ᵇ⁺±0.712</td>
</tr>
<tr>
<td>18</td>
<td>4.355ᵇ⁺±0.280</td>
<td>10.348ᵇ⁺±0.761</td>
</tr>
</tbody>
</table>

ᵃᵇWithin rows, values sharing no common letter are different.
⁺Difference between internal pipping stage and newly hatched chick (P < 0.05).

0.001) in the air cell of eggs turned for 18 d of incubation compared to those turned for 12 or 15 d.

Effect of Turning on Production Parameters

The effects of turning duration on incubation duration, hatchability, chick quality, and chick weights are presented in Table 2. Turning of eggs for 12, 15, or 18 d during incubation had no effect on incubation duration when all eggs were left to hatch until the end of 21 d. However, when incubation duration was calculated at 50% hatch, incubation duration was shorter for eggs turned for 18 d (484.27 h) compared with eggs turned for 15 d (485.44 h) (P < 0.03). Hatching time was shorter for eggs turned for 12 d compared with those turned for 15 or 18 d (P < 0.05). Eggs turned for 15 d during incubation had the lowest hatchability (P < 0.05), whereas those turned for 18 d had the highest hatchability. The proportion of chicks of high quality [maximum score of 100 in the scoring system as described by Tona et al. (2003a)] was highest in chicks from eggs turned for 18 d and lowest for those from eggs turned for 15 d (P < 0.002). Seven-day broiler weights decreased significantly with increasing turning duration. Expressed as RG rate, chicks from eggs turned until 12 d grew faster than those from eggs turned until 15 or 18 d (P < 0.001).

Discussion

The results from this study provide information that the duration of chicken egg turning during incubation may cause changes in some physiological parameters that may be associated with embryo development and hatchability. The quality of chicks as well as their growth performance to 7 d of age were also affected.

Turning of eggs until 12, 15, or 18 d of incubation had no effect on the levels of plasma corticosterone in the developing embryo or newly hatched chicks. The increasing levels of corticosterone with advancing age of embryos found in this study are consistent with those reported previously and may be due to the increasing functionality of the hypothalamo-pituitary-adrenal axis (Wise and Fry, 1973; Decuypere et al., 1989). However, the lack of effect of turning duration suggests that corticosterone may not be involved in the mechanism by which turning, at the stages we studied, may affect hatching and production parameters (e.g., duration of 50% hatch, hatchability, chick quality, and 7 d-old chick weights) that were measured. In a separate experiment in which we injected adrenocorticotrophic hormone into embryos to determine the responsiveness of the adrenal axis to egg turning, turning duration had no effect on plasma corticosterone levels (data not shown). Plasma corticosterone increased to similar levels within 1 h of injection and decreased to similar levels within 150 min.
The differential effects of egg turning duration on pCO₂ and pO₂ were clearly manifest at IP even though this was not observed at 18 d. Egg turning beyond 15 d up to 18 d significantly increased pCO₂ and decreased pO₂ levels. These changes in gas pressure are likely to be a source of stress to the chicks. The continuation of turning beyond 15 d, increased T₃ and T₄ levels significantly in the embryo, which might be a sign of increased metabolic activity. This supposed increase in metabolism may also be causally related to the higher CO₂ and lower O₂ in the air cell of eggs turned until 18 d.

Irrespective of turning duration, total incubation duration was similar among eggs but the duration at which 50% hatch occurred was different. Hatchability of eggs was significantly higher in eggs turned for 18 d than in those turned for 15 d but not significantly different from those turned for 12 d. The higher hatchability in eggs turned for 18 d, compared to turning for 15 d, might have been aided by the higher hypercapnia and T₃ levels in the plasma at IP. High CO₂ level at IP is a potent stimulus for hatching (Vischedijk, 1968; Tullett, 1990). Similarly, T₃ has been shown to increase during incubation up to IP (Decuyper et al., 1979; Tona et al., 2003b) and that higher T₃ improves hatchability (Tona et al., 2003b). It was, however, surprising that, in the current study, despite higher pCO₂ and T₃ at IP, incubation duration and hatchability in eggs turned for 18 d were significantly shorter, when compared with eggs turned for 12 d. Tona et al. (2003b) showed in a comparison between eggs stored for a long period and those stored for a short period that higher pCO₂ and T₃ shortened incubation duration and increased hatchability simultaneously in eggs stored for a short duration. The lack of a significant difference between turning until 12 or 18 d on both hatchability and incubation duration despite clear differences in physiological parameters suggests that other intrinsic factors may be at play in the control of hatchability and incubation duration of eggs. Although the eggs used in this study were stored for similar durations, they were from breeders of different ages. Wilson (1991) found that lower quality eggs, like those laid by older breeders or eggs stored for longer time, were more sensitive to decreased turning compared to good quality eggs.

It is interesting that 1-d-old chick weights were similar among all treatments. This finding suggests that although the supposed higher metabolic rate indicated by higher pCO₂ and thyroid hormone levels may be associated with turning until 18 d, it did not affect the development of the embryos. It is also interesting that the number of high quality chicks was comparable for eggs turned for 12 or 15 d, but higher for those turned for 18 d. In a previous report (Tona et al., 2003b), we showed that high quality 1-d-old chicks had higher T₃ and T₄ levels at IP, as found in eggs turned for 18 d, than suboptimal quality chicks. Because the eggs used in these experiments were stored and were from breeders of different ages, turning until 18 d might have been a beneficial method for improving chick quality.

Romanoff (1935, 1936) noted that incubation conditions are important not only for hatchability but also for postnatal growth. Tona et al. (2003a,b) showed recently that chicks from breeders of different ages or storage duration that have similarly good quality scores may have different posthatch performance. Again, in this study, chicks of similar high quality, but now as a function of turning duration, had significantly different BW and RG at 7 d of age. Seven-day-old BW and RG decreased with increasing turning duration. Although it is tempting to relate this decreased RG posthatch with the lower T₃/T₄ ratio found in these chicks at hatch, confirming earlier work in which chicks of similar quality also showed lower growth rate combined with a decreased thyroid hormone ratio, the mechanistic explanation still remains unclear. Also, because yolk utilization in unturned eggs is significantly slower than in turned eggs (Deeming, 1989a), it is possible that excessive turning may lead to too rapid utilization of the yolk and therefore result in lower juvenile performance.

This study indicated that when eggs were turned from the beginning of incubation and the sensitive period for turning was largely covered, the time to end turning (when eggs are transferred to the hatcher) also had a significant effect on hatchability and chick quality. More importantly it once again showed that hatchability and chick quality were not the only or ultimate criteria for maximal posthatch performance.

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### TABLE 2. Production parameters according to turning duration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T12¹</th>
<th>T15¹</th>
<th>T18¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation duration at 50% hatch (h)</td>
<td>484.68 ± 0.42b</td>
<td>485.44 ± 0.43b</td>
<td>484.27 ± 0.36b</td>
</tr>
<tr>
<td>Hatching time (h)</td>
<td>21.22 ± 0.59b</td>
<td>23.70 ± 0.67a</td>
<td>23.28 ± 0.58a</td>
</tr>
<tr>
<td>Hatchability (%)</td>
<td>88.73 ± 1.00b</td>
<td>86.42 ± 1.52b</td>
<td>90.43 ± 1.09a</td>
</tr>
<tr>
<td>Chicks of higher quality (%)</td>
<td>88.18 ± 2.46b</td>
<td>87.31 ± 1.42b</td>
<td>91.81 ± 1.40a</td>
</tr>
<tr>
<td>Day-old chick weights (g)</td>
<td>48.82 ± 0.23</td>
<td>49.08 ± 0.23</td>
<td>49.17 ± 0.21</td>
</tr>
<tr>
<td>Seven-day-old chick weights (g)</td>
<td>147.83 ± 0.95b</td>
<td>142.60 ± 1.01b</td>
<td>137.90 ± 1.04b</td>
</tr>
<tr>
<td>Relative weight gains (%)</td>
<td>201.85 ± 2.06</td>
<td>185.02 ± 2.18b</td>
<td>177.56 ± 2.27</td>
</tr>
</tbody>
</table>

¹Within columns, values sharing no common letter were different (P < 0.05).
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