Effect of low incubation temperature and low ambient temperature until 21 days of age on performance and body temperature in fast-growing chickens

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ABSTRACT Thermal manipulation during embryogenesis was previously reported to decrease the occurrence of ascites and to potentially improve cold tolerance of broilers. The objective of our study was to explore the effects of the interaction of cold incubation temperatures and cool ambient temperatures until 21 d of age on performance and body temperature. Ross 308 eggs were incubated either under control conditions I_0 (37.6°C) or with cyclic cold stimulations I_1 (6 h/d at 36.6°C from d 10 to 18 of incubation) or with 2 cold stimulations I_2 (30 min at 15°C) at d 18 and 19 of incubation. These treatments were followed by individual rearing and postnatal exposure to either standard rearing temperature T_0 (from 33°C at hatching to 21°C at d 21) or continuously lower temperature T_2 (from 28°C at hatching to 21°C at d 21) or exposure to cyclically lower temperature T_1 (with circadian temperature oscillations). Treatments I_1 and I_2 did not significantly alter hatchability compared to control incubation (with 94.8, 95.1, and 92.3%, respectively), or hatching BW and overall chick quality. Hatching body temperature (Tb) was 0.5 and 0.3°C higher in I_1 than in I_0 and I_2 groups, respectively ($P = 0.007$). A doubled occurrence of health problems was observed with T_2 condition, regardless of incubation or sex. At d 3, BW was 2% lower with treatment I_1 than with I_0 and I_2 and was 3% higher in T_1 and T_2 groups than in T_0, but these effects disappeared with age. Group T_2 presented a 5% higher feed intake than the control group T_0 between 3 and 21 d of age ($P = 0.025$). Feed conversion ratio (FCR) was affected by experimental conditions ($P < 0.001$), with low FCR values obtained with I_2 incubation in control or cyclically cold postnatal conditions. Maximal FCR values were observed in the continuously cold postnatal conditions, in males submitted to control incubation and in females submitted to I_1 incubation, revealing sex-dependent effects of the treatments on performance.

Key words: incubation, ambient temperature, thermotolerance, cold, broiler chicken

INTRODUCTION

In past decades, a large improvement in poultry production has been made due to genetic selection breeding fast-growing broilers strains (Havenstein et al., 2003; Zuidhof et al., 2014). These advances in chicken growth rate were not associated with a parallel development of the respiratory, metabolic, and locomotor functions. Fast-growing chickens are sensitive to inflammation,

disease, and changes in their environment (Cheema et al., 2003; Loyau et al., 2015), which probably results from reduced adaptive capacities. Indeed, broiler chickens have difficulties coping with changes in ambient temperatures. Renwick and Washburn (1982) demonstrated that brooding broiler chicks under cool temperatures (26.7°C vs. 32.2°C) increased mortality and decreased feed efficiency with chicks being particularly sensitive to low temperatures. Under low-temperature conditions, fast-growing chickens have limited capacity to satisfy their oxygen demands, which increases the occurrence of ascites (Olkowski et al., 2005; Druyan et al., 2007).

Mortalities due to ascites syndrome caused severe economic losses up to $1 billion in 1996 (Maxwell and Robertson, 1998). Chicks are not yet homeotherms until the end of the first wk after hatching, and the regulation of body temperature and feedback mechanisms are still immature (Tzschentke, 2007). Mortality rates

of chickens were estimated as tripled during the first wk of age compared to the remainder of the grow-out period (Heier et al., 2002; Yassin et al., 2009). Chicks cannot cope with low ambient temperatures without adequate shelter and heating (Collin et al., 2005; Mujahid and Furuse, 2009). Therefore, broiler production uses high amounts of energy for heating in the brooding period and has to compensate for the circadian thermal amplitude in the management of rearing temperature.

It was previously demonstrated that heat acclimation during embryogenesis is a technique that confers to broilers the adaptive capacity to cope with high ambient temperatures (Piestun et al., 2008; Loyau et al., 2015). This was achieved by changes in thermoregulatory and metabolic mechanisms without major alteration of performance (Piestun et al., 2008; Loyau et al., 2013, 2014a). Specific programs of cold acclimation during egg incubation also were investigated in chickens, improving their cold tolerance in the long term. Shinder et al. (2011) provided evidence that early cold conditioning (2 times 30 min at 15°C during d 18 and d 19 of incubation) improved the ability of broilers to maintain body temperature during later cold exposure. This treatment also increased the body weight of chickens reared at both standard and lower ambient temperatures. Furthermore, Yalçın et al. (2012) showed that cyclically cold thermal manipulation during specific phases of embryogenesis (from d 10 to d 18 of incubation) at 36.6°C and 58% relative humidity (**RH**) enabled broilers to cope with later cool ambient temperatures without altering growth, despite reducing hatchability. By applying the same treatment, Loyau et al. (2014a) also demonstrated that cold incubation induced modifications in antioxidant pathways, as shown by a higher hepatic catalase activity at hatch-

ing. With both cold embryo manipulations, experimental results demonstrated fewer incidences of ascites and a reduction of mortality rates during later cold exposure during the growing phase (Shinder et al., 2011; Akşit et al., 2013). These techniques could be interesting in order to increase the robustness of chickens from their first d of life, allowing to decrease ambient temperature in farm buildings at the starting period, especially during the phase when temperature is currently decreased in poultry houses (i.e., until 21 d of age). Thus, the objective of our study was to investigate the interaction of low or standard incubation temperature and postnatal standard or low rearing ambient temperatures, either constant or fluctuating, on performance and body temperatures in fast-growing chickens during the first 3 wk of age.

MATERIALS AND METHODS

All experimental procedures were approved by the Ethics Committee for Animal Experimentation Val de Loire (CEEA Val de Loire, Tours, France, N° 2,014,111,809,444,741 (APAFIS#70).03).

Incubation Process

A batch of 1,188 hatching eggs from Ross 308 broiler breeders at 36 wk of age and 3 d of egg storage was obtained from a commercial hatchery with mean egg weight of 63.5 ± 4.0 g and randomly divided into 3 incubation groups. The first group, including 3 trays of 132 eggs, was placed in standard incubation conditions (I_0) at 37.6°C and 56% RH. The second group, including 3 trays of 132 eggs, was incubated in cyclically cold incubation (I_1) conditions with reductions by 1°C
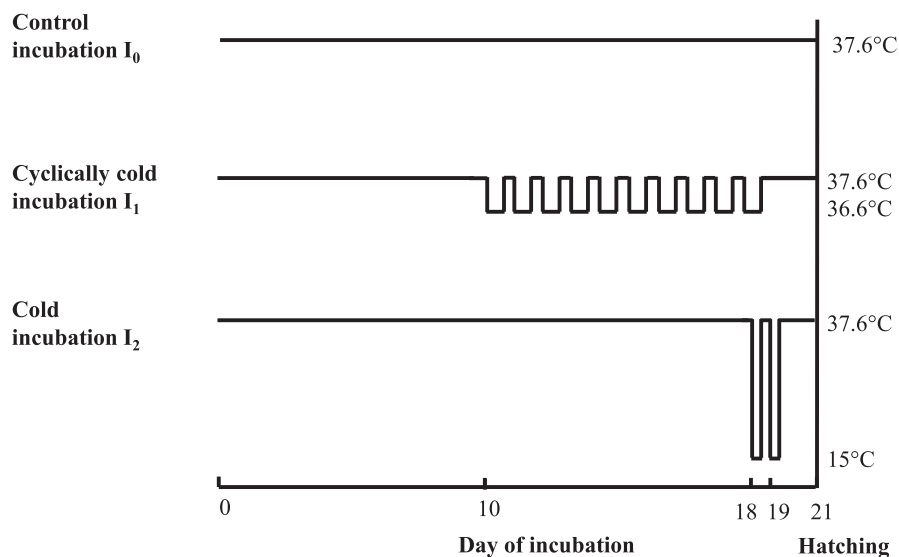

Figure 1. Incubation treatments. Incubation I_0: Control eggs were incubated until hatch at 37.6°C. Relative humidity was set at 56% until d 18 of embryogenesis. Incubation I_1: Eggs were incubated at 37.6°C and 56% RH, except from d 10 to d 18 of incubation when they were exposed to 6 h/d at 36.6°C, 60% RH. Incubation I_2: Eggs were incubated at 37.6°C and 56% RH and exposed for 30 min to 15°C at d 18 and 19 of incubation. At d 18, all eggs were transferred to the hatcher where relative humidity was set at 70% until d 21 of incubation.

during 6 h per d from d 10 to d 18 of incubation with RH of 60% (Figure 1), conditions being fixed at 37.6°C and 56% RH for the remainder of incubation. The cold incubation condition $(\mathbf{I_2})$ consisted of exposing 3 trays of 132 eggs to 15°C and 81% RH during 30 min on d 18 (transfer from the incubator to a cold room) and d 19 of incubation (transfer from the hatcher to a cold room), eggs being incubated at 37.6°C and 56% RH for the remainder of incubation. At d 18 of incubation, all eggs were moved to a common hatcher set at 37.6°C and 70% RH, with only the 30 min interruption at d 19 for the transfer of group I_2 to the cold room.

Hatching

At the end of hatching, all unhatched eggs were opened for macroscopic analysis in order to classify them as "non-fertile" and those with dead embryos. Non-fertile eggs were not included in hatching rate determination. Chicks were wing-tagged, and 50 to 81 chicks were randomly taken in the 3 incubation groups to be macroscopically examined for determining their quality according to the scores proposed by Tona et al. (2003). Briefly, the chick quality was measured on 8 different criteria, such as activity, down and appearance, retracted yolk, eyes, legs, navel area, remaining membrane, and remaining yolk on the navel, that were added to be rated on a total score of 100 points, corresponding to the best chick quality score. Determination of the sex was then realized macroscopically by observation of the cloaca.

Rearing Period

One-hundred-ninety-two chicks from each incubation group were randomly assigned to 3 different rearing rooms. Thirty-two males and 32 females from I_0, I_1, and I_2 (Figure 2) were placed in the control temperature $(\mathbf{T_0})$ room where the ambient temperature was gradually decreased until 21 d of age from 33 to 21°C. In a second room, 32 males and 32 females from groups I_0, I_1, and I_2 were exposed to cyclical variations $\mathbf{T_1}$ of temperature from 33 to 28°C at d 0 to 21°C at d 21 in a gradual decrease. In a third identical room, 32 males and 32 females from I_0, I_1, and I_2 groups were submitted to cooler ambient temperature $\mathbf{T_2}$, decreasing gradually on d 21 from 28 to 21°C. The rearing temperature in each room was monitored by a temperature controller each min during the entire experimental period (Thermo Button, Proges Plus, Willems, France) and data were collected via the software Thermotrack PC for analyses (Figure 3). Chicks were kept under a light:dark regimen of 16:8 after 3 d of age. They were reared in groups of 4 chicks of the same sex per cage (59 cm length × 45 cm width × 59 cm height) from 0 to 3 d of age (at a maximal stocking density of 1,258 g/m2). Thereafter, only 12 chicks per sex and per group were selected to achieve the same group mean BW (±S.E.) to be reared individually in cages up to 21 d of age in each room (at a maximal stocking density of 3,982 g/m2). Standard feed and water were provided ad libitum.

Measurements

The body temperature of chickens was measured individually at d 0 (after opening the hatcher) and d 11 by inserting an electronic thermometer (PX-TH519, Tex, Pelimex, Ingwiller, France) in the cloaca to a depth of 1 cm. Body weight was measured at d 0, d 3, d 11, and d 21 and feed consumption was recorded per cage for the period d 0 to d 3 and thereafter for each chick at d

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Incubation conditions	396 eggs Control Incubation (I_0) 37.6°C, 56% RH	396 eggs Cyclically cold Incubation (I_1) from d 10 to 18 of incubation, 36.6°C, 60% RH 6 h/d	396 eggs Cold Incubation (I_2) at d 18 and 19 of incubation, 15°C, 81% RH 30 min/d
Postnatal conditions	**32 chicks/sex then 12/sex** Control Incubation Control temperature ($I_0 T_0$) 33°C d 0 → 21°C d 21	**32 chicks/sex then 12/sex** Cyclically cold incubation Control temperature ($I_1 T_0$) 33°C d 0 → 21°C d 21	**32 chicks/sex then 12/sex** Cold incubation Control temperature ($I_2 T_0$) 33°C d 0 → 21°C d 21
	32 chicks/sex then 12/sex Control Incubation Cyclically cold temperature ($I_0 T_1$) 28°C/33°C d 0 → 21°C d 21	**32 chicks/sex then 12/sex** Cyclically cold incubation Cyclically cold temperature ($I_1 T_1$) 28°C/33°C d 0 → 21°C d 21	**32 chicks/sex then 12/sex** Cold incubation Cyclically cold temperature ($I_2 T_1$) 28°C/33°C d 0 → 21°C d 21
	32 chicks/sex then 12/sex Control Incubation Cold temperature ($I_0 T_2$) 28°C d 0 → 21°C d 21	**32 chicks/sex then 12/sex** Cyclically cold incubation Cold temperature ($I_1 T_2$) 28°C d 0 → 21°C d 21	**32 chicks/sex then 12/sex** Cold incubation Cold temperature ($I_2 T_2$) 28°C d 0 → 21°C d 21

Figure 2. Experimental design. Eggs were submitted to control incubation I_0 (37.6°C and 56% RH), cyclically cold incubation I_1, or cold incubation I_2, and thereafter exposed from d 1 to d 21 to control temperature T_0, cyclically cold temperature T_1, and cold temperature T_2.
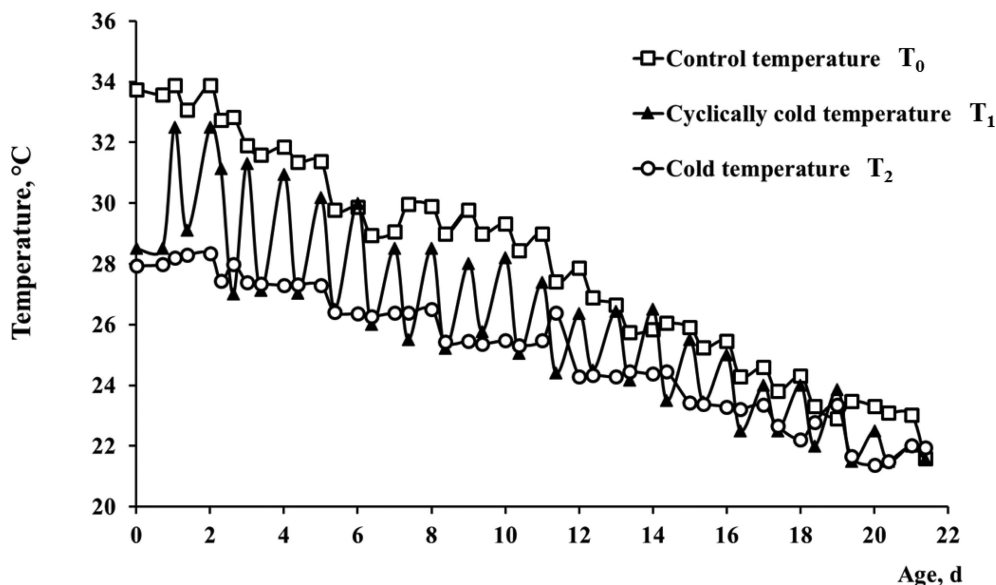
Figure 3. Rearing temperature recorded in the 3 breeding rooms. Chicks were exposed to control temperature T_0 from 33°C at d 0 to 21°C at d 21 or to cyclically cold temperature T_1 from 33/28°C at d 0 to 21°C at d 21 or to cold temperature T_2 from 28°C at d 0 to 21°C at d 21.

11 and d 21. Spontaneous mortality and the number of birds removed from the experiment because of health or welfare troubles were recorded daily.

Statistical Analysis

Data were collected and analyzed by statistic software Statview (version 5.0; SAS Institute, Cary, NC) in order to determine the effects of incubation group, postnatal condition, and their interaction on performance traits for each sex. The hatchability and mortality results were analyzed by using a Chi-square test. The responses to treatments and sex and their interactions concerning chick quality, body temperature, and weight were determined using analysis of variance (ANOVA) after having checked the normality of data distribution. The statistical significance of the difference was determined using a Student t test for the comparison of results from each group. Due to a significant heterogeneity of variance evaluated using Levene's test ($P < 0.001$) on data of FCR, values for this parameter were analyzed by nonparametric tests, including the Kruskal-Wallis test followed by the Mann-Whitney test. Chick quality scores also were analyzed using non-parametric statistic tests (Kruskall-Wallis, followed by Mann-Whitney test). Differences were considered significant when P-values were below 5% ($P < 0.05$), and values are presented ± standard error in the text or tables.

RESULTS

Performance

Hatchability Fertile hatchability rates were not different among incubation conditions with 92.3, 94.8, and 95.1% in I_0, I_1, and I_2 groups, respectively.

Chick Quality Scores No significant overall difference in chick quality was measured among incubation groups I_0, I_1, or I_2 at hatch (Table 1). The score of the navel area of I_1 chicks was significantly lower than that of I_0 chicks ($P = 0.046$), while the remaining membrane score was significantly degraded in I_2 chicks, as compared to I_0 controls ($P = 0.037$).

Body Temperature No significant interaction was observed between sex and incubation condition for body temperature at hatching (Table 2). Higher body temperatures were observed at hatching in females than in males ($P < 0.001$), and in I_1 than in I_0 and I_2 ($P = 0.007$). At 11 d of age, only the postnatal conditions had a significant effect on body temperature ($P = 0.045$) with higher Tb in the cyclically cold rearing temperature T_1 group (40.5 ± 0.1°C; n = 71) than in the constantly cold ambient temperature T_2 (40.3 ± 0.1°C; n = 68; $P = 0.015$). The group at standard temperature T_0 presented an intermediary Tb value (40.5 ± 0.1°C; n = 70), not significantly different from either other group ($P = 0.170$ and $P = 0.376$, respectively).

Body Weight Body weights at hatching were not different among incubation groups in either sex, but males presented significantly higher hatching BW than females ($P = 0.043$; Table 2). In males as in females, there was no significant effect of the interaction between incubation and postnatal conditions on BW, regardless of the age considered. At 3 d of age, BW was 2% lower in the cyclically cold incubation treatment I_1 than in I_0 and I_2 ($P = 0.039$; Table 3). It also was affected by postnatal condition with 3% higher BW in T_1 and T_2 groups than in T_0 ($P < 0.001$), but these effects disappeared with age. An expected, sex effect was significant on BW at 11 ($P = 0.007$) and 21 d of age ($P < 0.001$), without significant interaction with incubation or postnatal temperature conditions.

Table 1. Scores of the different parameters of quality in post-hatch chicks after incubation under different conditions.

Parameters[2]	Incubation group[1]			P-value
	I_0	I_1	I_2	
Activity (/6)	5.8 ± 0.2	5.5 ± 0.2	5.8 ± 0.1	0.466
Down and appearance (/10)	10.0 ± 0.0	9.8 ± 0.1	9.9 ± 0.1	0.387
Retracted yolk (/12)	10.7 ± 0.4	11.0 ± 0.3	10.7 ± 0.3	0.758
Eyes (/16)	16.0 ± 0.0	16.0 ± 0.0	16.0 ± 0.0	1.000
Legs (/16)	14.7 ± 0.5	15.7 ± 0.2	14.9 ± 0.3	0.053
Navel area (/12)	10.7 ± 0.2[a]	9.8 ± 0.2[b]	10.1 ± 0.2[a,b]	0.046
Remaining membrane (/12)	11.7 ± 0.2[a]	11.1 ± 0.3[a,b]	10.9 ± 0.2[b]	0.037
Remaining yolk (/16)	15.5 ± 0.2	14.6 ± 0.3	14.9 ± 0.2	0.080
Total quality score (/100)	95.1 ± 0.8	93.6 ± 0.6	93.1 ± 0.8	0.091

[1]Control incubation I_0: eggs were incubated until hatch at 37.6°C and 56% RH (n = 50); I_1: eggs were incubated from d 10 to d 18 of incubation, 6 h/d at 36.6°C (n = 77), 60% RH; I_2: eggs were exposed for 30 min to 15°C at d 18 and 19 of incubation (n = 81).

[2]For each parameter, values are given as mean ± standard error.

[a,b]Different letters correspond to significant differences ($P < 0.05$) between groups.

Chick quality was evaluated according to Tona et al. (2003).

Table 2. Body temperature and body weight of males and females at hatch depending on incubation group.

	Incubation group[1]			P-value	Sex		P-value	Incubation × Sex P-value
	I_0	I_1	I_2		Male	Female		
Body temperature (°C)[2]	39.2 ± 0.1[b]	39.7 ± 0.1[a]	39.4 ± 0.3[b]	0.007	39.2 ± 0.1[b]	39.7 ± 0.1[a]	0.001	0.053
Body weight (g)[3]	45.4 ± 0.2	45.1 ± 0.2	45.0 ± 0.2	0.477	45.4 ± 0.2[a]	44.9± 0.2[b]	0.043	0.911

[1]Control incubation I_0: eggs were incubated until hatch at 37.6°C and 56% RH; I_1: eggs were incubated from d 10 to d 18 of incubation, 6 h/d at 36.6°C, 60% RH; I_2: eggs were exposed for 30 min to 15°C at d 18 and 19 of incubation. For each parameter, values are given as mean ± standard error.

[2]I_0: n = 45; I_1: n = 45; I_2: n = 46; Males n = 47; Females: n = 62.

[3]I_0: n = 192; I_1: n = 192; I_2: n = 192; Males n = 272; Females: n = 304.

[a,b]Different letters correspond to significant differences ($P < 0.05$) between incubation groups or between sexes.

Feed Consumption From d 3 to d 21 There was no interaction between incubation condition and postnatal ambient temperature on feed consumption in either sex (Table 3). Besides a significant effect of sex ($P < 0.001$) with 6% higher feed consumption in males than in females, the T_2 group submitted to postnatal continuous cold exposure presented a 5% significantly higher feed consumption than the control group T_0 ($P = 0.025$), while T_1 group exhibited intermediary values.

Feed Conversion Ratio The low incubation temperature or low rearing temperature did not affect FCR from d 0 to d 3 and from d 3 to d 11 (data not shown). Between 3 and 21 d of age, FCR was significantly altered by experimental conditions ($P < 0.001$; Figure 4). Maximal FCR values were observed in the continuously cold postnatal conditions T_2, on the one hand in females submitted to I_1 incubation (1.58 g/g, Figure 4A) and on the other hand in males submitted to control incubation I_0 (1.49 g/g, Figure 4B). Low FCR values were obtained with I_2 incubation in control or cyclically cold postnatal conditions in both sexes: lower FCR values were recorded in the males incubated with I_2 or control incubation and reared in the control conditions (I_2T_0; $P = 0.002$ or I_0T_0; $P = 0.001$, respectively) and in the males incubated with I_1 or I_2 treatments reared under cyclically cold temperature (I_1T_1, $P = 0.004$ or I_2T_1, $P < 0.001$, respectively), than in the males with control incubation but constant cold rearing temperature

(I_0T_2). In females, feed efficiency was significantly improved by 4% with I_2 incubation as compared to I_1 incubation in cyclically cold postnatal conditions ($P = 0.011$). The other groups were intermediary.

Mortality and Morbidity A total mortality rate of 3.9% was recorded, corresponding to 1.8% of non-eating chicks, 1.2% of cardiac death, and 0.9% of death due to ascites. An occurrence of health and/or welfare troubles at a rate of 11.4% was observed in the present experiment, corresponding to several cases of leg disorders leading to the removal of chickens for ethical reasons. The effect of incubation conditions was not significant within postnatal conditions. Most of the health troubles were observed between 3 and 11 d of age, and were significantly higher in the continuously cool postnatal conditions T_2 than in the T_0 and T_1 conditions (24 chicken vs. 9 and 13, with $P = 0.003$ and $P = 0.021$, respectively).

DISCUSSION

In this study investigating the effects of incubation temperature and postnatal temperatures during the first 3 wk of age in broiler chickens, neither of the 2 cold incubation groups affected hatchability in our experimental conditions. By contrast with previous findings of Yalçin et al. (2012) showing a degradation in

Table 3. Performance of chickens depending on incubation and rearing temperature conditions. Body weights of males and females were recorded at d 3 and d 21. Feed consumption (FC) was calculated between d 3 and d 21.

Traits	Incubation group (I)[1]				Postnatal group (P)[2]				Sex (S)			I×P	I×S	P×S	I×P×S
	I_0	I_1	I_2	I effect	T_0	T_1	T_2	P effect	Male	Female	S effect				
BW at d 3 (g)	84.4 ± 0.5[a]	82.6 ± 0.6[b]	84.2 ± 0.6[a]	0.039	82.1 ± 0.6[b]	84.9 ± 0.6[a]	84.2 ± 0.5[a]	<0.001	84.1 ± 0.4	83.4 ± 0.4	0.287	0.731	0.272	0.614	0.091
BW at d 11 (g)	357 ± 4	346 ± 4	358 ± 4	0.066	352 ± 4	357 ± 4	352 ± 4	0.523	360 ± 3[a]	347 ± 3[b]	0.007	0.880	0.597	0.153	0.839
BW at d 21 (g)	1069 ± 11	1038 ± 15	1064 ± 14	0.298	1053 ± 12	1077 ± 12	1040 ± 17	0.284	1108 ± 10[a]	1021 ± 8[b]	<0.001	0.750	0.937	0.968	0.065
FC d 3 to 21 (g)	1377 ± 14	1358 ± 14	1372 ± 15	0.453	1338 ± 13[b]	1373 ± 14[a,b]	1399 ± 15[a]	0.025	1414 ± 11[a]	1327 ± 10[b]	<0.001	0.760	0.970	0.872	0.443

[1]Control incubation I_0: eggs were incubated until hatch at 37.6°C and 56% RH; I_1: eggs were incubated from d 10 to d 18 of incubation, 6 h/d at 36.6°C, 60% RH; I_2: eggs were exposed for 30 min to 15°C at d 18 and 19 of incubation.

[2]Control temperature T_0 from 33°C at d 0 to 21°C at d 21 or cyclically cold temperature T_1 from 33°C/28°C at d 0 to 21°C at 21 or cold temperature T_2 from 28°C at d 0 to 21°C at d 21.

[a,b]Different letters correspond to significant differences ($P < 0.05$) between groups. For each parameter, values are presented as mean ± standard error. For BW at d 3, n = 189 to 192 chicks per incubation or postnatal group; for BW at d 11, n = 68 to 73 chicks per incubation or postnatal group; for data of FC and BW at d 21, n = 64 to 67 chicks per incubation or postnatal group.

hatchability by 5 points with I_1 treatment as compared to control incubation, hatchability was not different and above 92% of fertile eggs in both I_0 and I_1 groups. As expected from the study of Shinder et al. (2011), hatchability also was maintained high with the I_2 group, including 2 acute stimulations during late embryogenesis.

From our results, high quality scores of chicks over 92/100 evaluated from quality scores of Tona et al. (2003) were observed in all incubation groups, despite cold incubation treatments slightly affecting chick quality due to variations in some criteria. The cyclic cold stimulation I_1 degraded the score of navel closure, however, not leading to significantly higher mortality rates post hatch in this group. The quality score related to the remaining membrane was lower in I_2 group than with control incubation. Both observations could be due to a greater chick immaturity at hatching in I_1 and I_2 groups. Moreover, the thermoregulation and development of hatching birds were altered by the incubation treatments. Indeed, body temperature was significantly higher in I_1 than in I_0 and I_2 groups at hatch ($P = 0.007$); these effects were not observed later on. This observation was not reported previously with the same incubation program I_1 (Yalçin et al., 2012; Aksit et al., 2013; Loyau et al., 2014a). However the significantly greater body temperature observed in I1 than in I0 group at hatching is in agreement with the lower body temperature recorded in hatching chicks that were heat-exposed during embryogenesis (Piestun et al., 2008; Loyau et al., 2013), suggesting changes in the thermoregulatory control of metabolism induced by incubation temperature (2014b, 2014b, 2016; Loyau et al., 2015). Consistent with Shinder et al. (2011), cold incubation treatment I_2 later during incubation did not affect hatching temperature as compared to control incubation. Females also exhibited higher body temperatures than males ($P = 0.001$), suggesting differences in thermoregulatory mechanisms between sexes. This result could be explained by differences in times of hatch between sexes, as previously observed by Tzschentke and Halle (2009).

Incubation treatment I_1 consisting of repeated cold stimulations at 36.6°C (Yalçin et al., 2012) negatively but transiently affected BW at d 3. The absence of significant effect of incubation treatments on BW at 21 d is consistent with the results of Akşit et al. (2013) showing no effect of lower incubation temperature on body weights in mixed-sex groups of chickens, whereas Shinder et al. (2011) had reported positive effects of I_2 incubation on body weights at d 14 and 35 post hatch in males. Both postnatal cold conditions T_1 and T_2 induced higher BW than control conditions at 3 d of age, inconsistent with previous results showing unchanged to lower BW in chickens exposed to cold ambient temperature at early age (Collin et al., 2003; Shinder et al., 2011). In accordance with the present positive effect on growth, Aksit et al. (2013) showed greater BW of mixed-sex chickens from a young breeder flock exposed to postnatal cold. However, this effect was only tran-
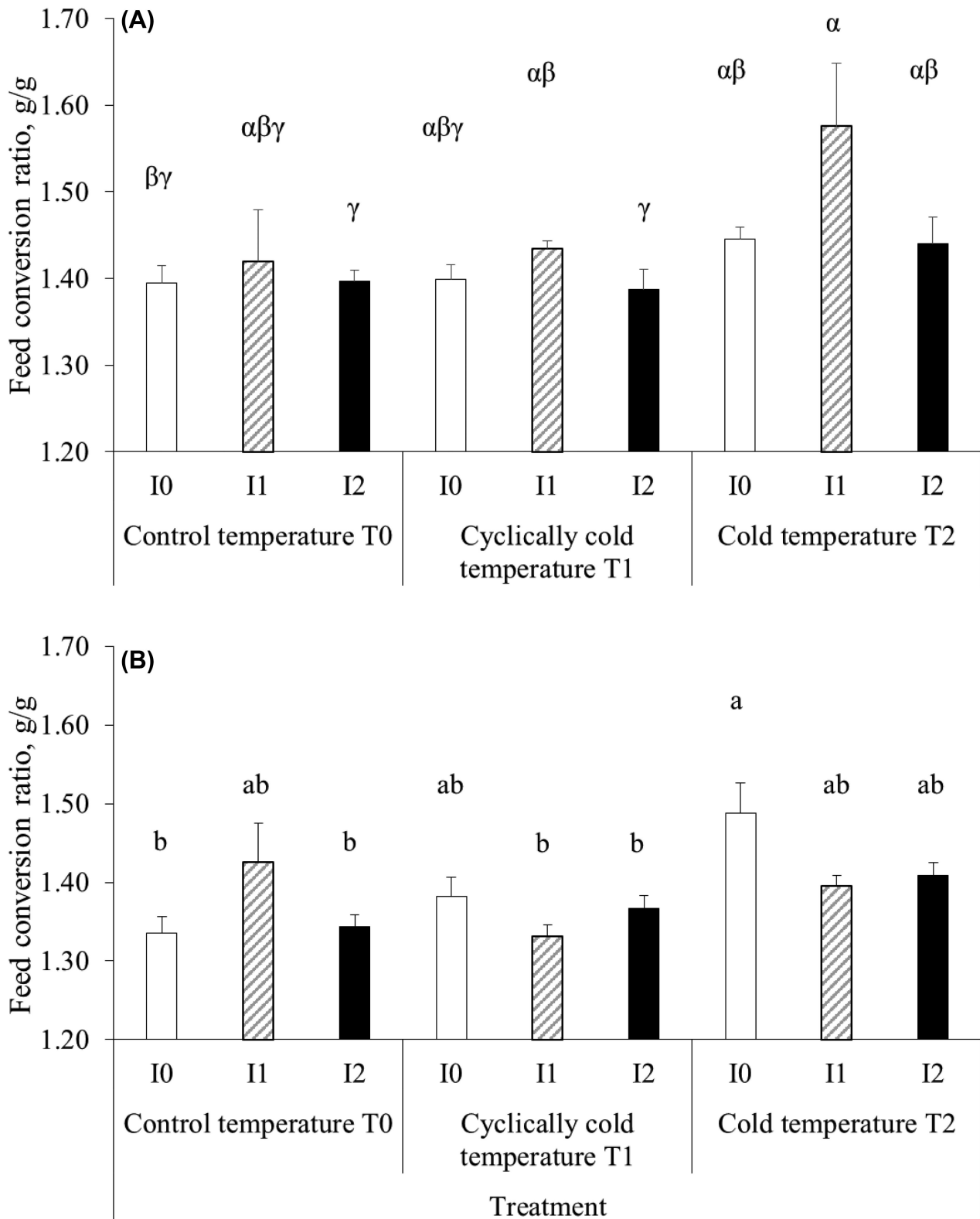
Figure 4. Feed conversion ratio of chickens between d 3 and d 21. Control eggs were incubated at 37.6°C, 56% RH (I_0), I_1 eggs were submitted to incubation at 36.6°C and 60% RH for 6 h/d from d 10 to d 18 of incubation, and I_2 were submitted to 30 min at 15°C at d 18 and 19 of incubation. Chickens were reared in cages at control temperature T_0 from 33°C at d 0 to 21°C at d 21 or at cyclically cold temperature T_1 from 33/28°C at d 0 to 21°C at d 21 or at cold temperature T_2 from 28°C at d 0 to 21°C at d 21. 4A) Feed conversion ratio calculated in females. Different letters (α, β, γ) correspond to significant differences ($P < 0.05$) between groups. 4B) Feed conversion ratio calculated in males. Different letters (a, b) correspond to significant differences ($P < 0.05$) between groups.

sient in our study, whereas they observed a longer effect on growth (until 28 d of age), the difference being likely due to the shorter and lower ambient temperature they applied.

The highest FCR were observed in T_2 continuous cold postnatal conditions, in females of I_1 group and in males incubated with control incubation conditions, suggesting lower ability of these groups to use nutrients for growth as compared to their respective controls incubated and reared under standard conditions. This effect is likely due to additional heat production to counterbalance the effects of cold in an attempt to maintain a normal body temperature (i.e., thermoregulation), as observed in a previous study dealing with cold rearing temperatures (Collin et al., 2003). It was associated with 5% increased feed consumption in constant cold T_2 conditions, as compared to control postnatal temperature ($P = 0.025$), in an attempt to increase diet-induced thermogenesis for cold acclimation. Such decreases in feed efficiency were not observed under cyclically cold ambient temperatures, suggesting resting phases when temperatures were up again. Interestingly, I_2 incubation treatment led to minimal FCR values, however, not significantly differing from the control incubation groups under control or cyclically cooler rearing ambient temperatures for both sexes. The latter result suggests that cold acute stimulation during late embryogenesis, limiting the occurrence of ascites according to Shinder et al. (2011), would not degrade feed efficiency when chickens are submitted to temperature variations in poultry houses from hatching to 21 d of age.

The mortalities and morbidity measured in the present experiment were mainly due to lameness and cardiac death and were observed mostly between 3 and 11 d of age. No significant difference was observed in mortality rates and removal from the experiment resulting from legs problems between incubation groups, somehow inconsistent with previous results from Yalçin et al. (2007), indicating that exposition to low temperature during incubation could induce leg disorders. However, the constantly cool condition T_2 applied post hach negatively affected the health of the chicks, probably promoting the survival of the fittest, in accordance with Zhang et al. (2014), pointing out the increased inflammation associated with lipid-metabolism disorders in the livers of chickens exposed to cold stress. Previous data showed that at hatching, chicks have very low storage of minerals (Yair et al., 2015). Furthermore, Angel (2007) reported that the high BW of fast-growing broilers puts lot of pressure on leg bones, which causes a deviation of the corridor and a sprained gastrocnemius. Regarding the number of leg troubles recorded in the present experiment, the postnatal condition T_2 was probably too severe for the chickens reared in cages and more susceptible to leg problems than floor-reared chickens (Haye and Simons, 1978) to support growth and optimal muscle-skeleton development. At constantly low temperatures, chicks probably reduced their movements because they allocated available en-ergy to heat production to maintain major functions and homeothermy. To the contrary, the intermittent cyclical cold temperature T_1 allowed chicks to recover after the cold period (of around 8 h) of thermoregulatory requirements to bring temperature to equilibrium.

In conclusion, unlike the cyclical exposure to low temperature that barely affected the performance of chicks reared in cages, constant exposure to lower temperature during the first 3 wk post hatch induced some unfavorable sex-specific responses in terms of performance. Regarding the combined effects of incubation and rearing programs observed in the feed efficiency in males and females reared in cages, the underlying physiological and metabolic mechanisms remain to be explored, as well as their effects on floor-reared chicken performance and health status. How these treatments will influence economic (e.g., production cost), social (e.g., animal welfare), and environmental (e.g., non-renewable energy consumption for heating) criteria regarding broiler production is also necessary to investigate before potential applications of alternative incubation programs in poultry industry.

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