Short cold exposures during incubation and postnatal cold temperature affect performance, breast meat quality, and welfare parameters in broiler chickens

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Meat quality in cold-acclimated chickens

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ABSTRACT

Cold stimulations during egg incubation were reported to limit the occurrence of ascites in broilers submitted to cold temperature after 14 d of age. However, data is lacking on the impacts of such strategy in case of cold temperature condition at chick starting. This study aimed to evaluate the effects of incubation and post-hatch cold challenge on performance, breast muscle integrity and meat processing quality in broiler chickens.

Ross 308 eggs were incubated under control temperature (**I_0,** 37.6°C) or submitted to 15°C during 30 min on d 18 and 19 of incubation (**I_1**). Chicks from each group were reared in floor pens either at standard rearing temperature (**T_0**), from 32°C at d 0 to 21°C at d 21, or exposed to colder rearing temperature (**T_1**), from 29°C at d 0 to 21°C at d 21 of age. Birds were then all kept at 21°C until slaughter (d 40), when body weights (**BW**), feed conversion ratio (**FCR**), breast muscle yield, meat processing quality and the occurrences of meat defects, hock burns and pododermatitis were recorded. No significant impact of incubation conditions on hatchability was observed. At d 40, BW was greater under T_1 than T_0 condition, with T_0 females (but not males) presenting greater BW after I_1 than after I_0 condition. On the whole period, T_1 chickens presented lower FCR than T_0, and greater breast meat yields at d 40. The occurrence of white striping was greater in I_1T_1 males than in all other groups, except for the I_0T_1 males. Hock burns were more frequent in I_1T_1 males than in all females and I_0T_0 males, whereas the occurrence of pododermatitis was lower in T_0 males than in other groups. Despite some positive effects of I_1 incubation on growth after starting under low ambient temperature, this study reveals the limits of such strategy concerning chicken health and welfare, demonstrating that early thermal environment is a major component of the quality and sustainability of chicken meat production.

Key words: broiler, incubation, meat quality, white striping, welfare

INTRODUCTION

Chicken production is expected to increase continuously (+1.8%/year) for the next decades (Alexandratos and Bruinsma, 2012), in view of the growing global demand in proteins from animal source and the efficiency of this production (Petracci and Cavani, 2012). However, the environmental, economic and social impacts of the production systems, including meat quality and animal welfare are criteria of concern for consumers and citizens.

It was suggested that the genetic selection of fast-growing broilers on growth rate, feed efficiency and breast meat yield have simultaneously increased the sensitivity of these birds to temperature variations (Piestun et al., 2008; Havenstein et al., 2003a,b; Zuidhof et al., 2014). Because the thermoregulatory system of chicks is immature at hatching (Tzschentke, 2007), they remain very sensitive to postnatal cold temperatures during the first days of breeding (Collin et al., 2003; Mujahid and Furuse, 2009). Environmental conditions during the perinatal period are considered as critical for the chicken later performance and fitness (Guilloteau et al., 2019). Especially, brooding chicks at low temperatures (26.7°C vs. 32.2°C) results in a decreased feed efficiency and increased mortality rate (Renwick and Washburn, 1982). Fast-growing broilers exhibit greater ascites prevalence (Druyan et al., 2007) and leg disorders (Yalçin et al., 2007; Zhang et al., 2014; Nyuiadzi et al., 2017) when they are submitted to low rearing temperatures post-hatch.

Few studies showed that the exposure to cold temperature during embryogenesis reduced the prevalence of ascites and changed thermoregulatory mechanisms in the early life of chicks, helping them to cope with low rearing temperatures post-hatch (Shinder et al., 2011; Akşit et al., 2013). These authors showed that it decreased mortality rate, but it also increased the antioxidant activity of catalase in the liver of chicks at hatching (Loyau et al., 2014). Cold treatment in embryonic life can affect chick behavior (Bertin et al., 2018), but also performance. Van der Pol et al. (2013) showed that cold brooding temperature resulted in

lower BW than normal brooding temperature at 4 d of age. However, long-term positive effects of embryonic and postnatal cold acclimation in broilers were reported on body weight (Shinder et al., 2009) and breast muscle yield (Shinder et al., 2011) when broiler chickens are reared at standard temperature. Their treatment was applied in the last phase of embryogenesis (days 18 and 19 of incubation), just before the blood concentrations of triiodothyronine, a major hormone involved in thermoregulation and hatching process, is reaching a peak (Reyns et al., 2003). The late phase of embryogenesis corresponds to the switch of embryos from the ectothermic phase to the endothermic phase when a greater ability to produce heat is acquired (Minne and Decuypere, 1984; Nichelmann and Tzschentke, 2002; Tzschentke, 2007). Cold stimulations during embryogenesis were shown to induce long lasting effects partially limiting the detrimental effects of postnatal cold temperatures on feed conversion ratio in males reared in cages (Nyuiadzi et al., 2017).

In order to ensure the sustainability of poultry meat production, not only performance but also meat processing quality and animal welfare have to be considered. Metabolic diseases, including ascites, but also muscle myopathies including white striping and wooden breast have recently become a major source of loss for poultry meat production of fast-growing broilers (Julian, 2005; Kuttappan et al., 2016). The rapid growth rate, the increase in breast meat yield and gender are factors affecting the prevalence of white striping (Petracci and Cavani, 2012; Alnahhas et al., 2016). The latter authors have pointed out genetics as the major determinant of this meat defect. To a lesser extent, environmental and management factors, including nutrition (Meloche et al., 2018) also contribute to the variance of the white striping occurrence in breast muscle (Bailey et al., 2015). However, the impacts of rearing temperatures and early life environment on this defect has been poorly investigated, except in the case of heat embryonic exposure (Clark et al., 2017).

To our knowledge no experimentation has been carried out on the effects of cold exposure during embryogenesis on meat processing quality and muscle defects when chickens are later submitted to standard or low environmental temperature. Thus, the objective of the present study was to assess the performance and meat quality at slaughter age in broilers exposed to low or standard temperature incubation in interaction with postnatal low or standard ambient temperature, while recording the impacts of these treatments on animal performance as well a as on some animal welfare criteria.

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°2014111809444741 (APAFIS#70).03).

Incubation Process

A total of 1,200 fertile Ross 308 broilers eggs with 3 d of storage from 36 wk-old breeder flock were provided from a commercial hatchery (Couvoir Perrot, Pommerit-Jaudy, France). Prior to incubation, average egg weight was determined (62.3 ± 0.7 g) and eggs were randomly divided in two treatment groups experiencing control (**I_0** at 37.6°C and 56% RH) or cold incubation (**I_1**) at PEAT INRA Poultry Experimental Facility (2018, https://doi.org/10.15454/1.5572326250887292E12). I_1 consisted in exposing 3 trays of 132 eggs to 15°C and 81% RH during 30 min on d 18 and d 19 of incubation by transferring them from the hatcher to a cold room, eggs being incubated at 37.6°C and 56% RH for the remainder of incubation (Figure 1). This treatment resulted in a minimal eggshell temperature (±SD) of 26.9°C ± 2.1°C and maximal eggshell temperature of 33.1 ± 0.6°C, measured by thermal imaging after 30 minutes of exposure. On d 7 and d 14 of incubation, unfertile and

undeveloped eggs were eliminated after candling. 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_1 to the cold room.

Hatching

Into the hatcher, the numbers of hatched chicks in control (I_0) and cold (I_1) incubation were recorded at 480 h of incubation, and then at 504 hours (opening of the hatcher) to calculate hatchability as the ratio of hatched chicks on fertile incubated eggs. Chicks from each treatment were weighed, wing-banded and sexed by cloacal observation. The body temperature (**Tb**) of chicks was measured individually at hatching (on dry chicks after opening the hatcher) by inserting an electronic thermometer (PX-TH519, Tex, Pelimex, Ingwiller, France) in the cloaca. Fifty chicks from both treatment groups were randomly selected for quality measurement according to Tona et al. (2003). Briefly, the score of 8 parameters including activity, down and appearance, retracted yolk, eyes, legs, navel area, remaining membrane, and remaining yolk on the navel were measured to determine the chick quality. Each score was summed and scored out of 100, with 100 being the highest score value for the best chick quality.

Rearing Period

Four-hundred chicks from each incubation group were divided randomly into 2 groups and were transferred to 2 identical controlled rooms under standard rearing conditions **T_0**, decreasing from 32°C at d 0 to 21°C at d 21, or under cold rearing conditions **T_1,** consisting in continuously decreasing temperature from 29°C at d 0 to 21°C at d 21, respectively (Figure 1). From d 22 to d 40, ambient temperature was maintained at 21°C in both conditions T_0 and T_1. Chicks were reared in groups of same sex per condition with 4 repetitions per incubation treatment, sex and treatment group at the rate of 23 males or 27 females from d 0 to d 21,

147 reduced to 19 males or 22 females per group on the basis of the mean BW and standard error
148 of the group from d 22 to d 40 in order to limit the rearing density in pens at slaughter age.
149 Chicks were reared in floor pens (surface = 2.93 m^2) with wood shaving litter (1.5 kg/m^2).
150 Water and pelleted feed were supplied *ad libitum* until slaughter age (d 40).

151 ***Performance***

152 Chickens were weighed at d 0, d 21, and d 40 of age. Feed consumption was recorded on each
153 floor pen from d 0 to d 21 and from d 22 to d 40. Feed conversion ratio (**FCR**) was calculated
154 for both periods as the ratio of feed consumption by body weight (**BW**) gain per pen during
155 both periods and during the entire rearing period.

156 ***Muscle and meat quality parameters***

157 At d 40, 8 males and 8 females per floor pen per treatment group (I_0T_0, I_0T_1, I_1T_0, I_1T_1)
158 representatives of their pen (average weight and SD) were slaughtered after 8 h of feed
159 withdrawal, in the experimental processing plant of INRA (UE PEAT, Nouzilly, France).
160 After evisceration, whole carcasses were stored at 4°C for 18 h until cut and deboned for meat
161 characteristics analyses. Abdominal fat, *Pectoralis major* and *minor* (breast) muscles were
162 weighed to calculate their yields in relation to body weight. The ultimate pH (**pHu**) of the
163 muscle was measured 24 h postmortem using a portable pH meter (model 506; Crison
164 Instruments SA, Alella, Bercelona, Spain) by inserting the electrode in the left *Pectoralis*
165 *major* muscle as described by Berri et al. (2001). The color measurements of the muscle
166 defined by 3 components, lightness (**L***), redness (**a***) and yellowness (**b***) values according
167 to the CIE trichromatic (Girolami et al., 2013), were measured by using a Miniscan
168 Spectrocolorimeter (Hunterlab, Reston, VA) as described by Alnahhas et al. (2014; 2015).
169 The occurrence of meat quality defects (Kuttappan et al., 2012; Sihvo et al., 2014; Mudalal et
170 al., 2015), white striping (**WS**) and wooden breast (**WB**) were evaluated macroscopically in

Pectoralis major muscles on a scale of 3 classes according to Alnahhas et al. (2016). The intramuscular lipid content was calculated by near-infrared spectroscopy using a Nirflex N-500 spectrometer (Buchi, Rungis, France) from samples of *Pectoralis major* collected at 40 d of age (Alnahhas et al., 2016). The lipid peroxidation value was determined by the quantification of thiobarbituric acid-reactive substance (**TBARS**) using the method developed by Lynch and Frei (1993). Drip loss was determined according to Berri et al. (2007). The cooking loss of the breast muscles was measured as previously described by Baéza et al. (2012) and Alnahhas et al. (2016). The average Warner-Bratzler shear force value (N/cm^2) of the meat was determined on 3 samples of cooked breast muscle (1 × 1 × 3 cm) using an Instron universal testing instrument (Instron 5543, Instron S.A., Guyancourt, France) as described by Honikel (1998) and Alnahhas et al. (2016) in order to assess the tenderness of the meat.

Parameters related to chicken welfare

At slaughter (d 40), hock burn and pododermatitis occurrences were recorded on all slaughtered animals. At the end of the experiment, the litter of each floor pen was weighed. After homogenization of the litter amount in the pen at 40 days of age, 1 kg of litter from each floor pen was sampled and analyzed for dry matter (MS) to evaluate the litter moisture depending on the incubation and postnatal treatments.

Statistical Analysis

Data were analyzed using the statistics software Statview (version 5.0; SAS Institute, Cary, NC). The hatchability results were analyzed by using a Chi-square test. The effect of incubation treatment on body weight and body temperature at hatching was determined by one-way ANOVA followed by Student-Newman-Keuls test, while scores of chick quality were analyzed by a non-parametric Kruskal-Wallis test, because of of the heterogeneity of the

variance of the data set. The effects of incubation condition, rearing condition and sex and their interactions on body weight, feed conversion ratio (n = 4 groups), carcass and meat quality (breast meat yield, abdominal fat yield, shear force value, pH_u, L*, a*, b*, cooking loss, drip loss, technological performance) and litter characteristics were performed by ANOVA followed by a Student-Newman-Keuls test. The frequencies of occurrence of pododermatitis, hock burn, wooden breast and white striping were analyzed by Chi-square tests.

RESULTS

Effects of incubation and postnatal treatment on hatchability, chick quality and performance

Hatchability of fertile eggs was not significantly affected by the cold incubation treatment (P = 0.801) with hatchabilities over 95% in both groups in our experimental conditions (Table 1). However, at 480 h of incubation, i.e. 24 h before opening the hatcher, 43% of the I_0 chicks were hatched, but only 36% of the I_1 chicks (P = 0.007). At hatching, neither the male chick percentage nor the quality of chicks from cold-incubated eggs I_1 was different from that of control chicks I_0 (P = 0.765 and 0.415, respectively; Table 1). Body temperature tended to be greater in I_1 than in I_0 chicks at hatching (P = 0.059), but BW (Table 1) was not influenced by the incubation thermal treatment (P = 0.681). There were significant interactions of incubation condition, postnatal rearing temperature and sex on BW at d 21 (P = 0.008) and d 40 of age (P = 0.038; Table 2). At d 21, male BW were not different between I_0 and I_1 at standard temperature T_0, while male BW was greater for I_1 than for I_0 group under cold ambient temperature T_1 (P < 0.05; Table 3). On the contrary, I_1 females exhibited greater BW at d 21 than I_0 ones at T_0 (P < 0.05), whereas there were no difference observed between

incubation conditions under cold ambient temperature T_1. At slaughter age (d 40), body weights of both males and females were greater under cold rearing temperature T_1 than under standard conditions T_0. In females (but not in males) under standard temperature T_0, I_1 group exhibited greater slaughter BW than I_0 ones ($P < 0.05$; Table 3). The postnatal cold temperature and the sex affected the FCR from d 0 to d 21 with greater FCR in females than in males and in chickens reared under lower ambient temperature T_1 than at T_0 ($P < 0.001$ and $P < 0.001$, respectively; Table 2). Between d 22 and d 40 (when ambient temperatures were maintained at 21°C for both groups T_0 and T_1), FCR of chickens incubated in control conditions I_0 was lower in T_1 than in T_0 group, while it did not differ depending on the postnatal temperature in I_1-incubated chickens (Table 3). During this period, females exhibited higher FCR than males ($P < 0.02$). On the whole period from d 0 to d 40 (Table 2), females exhibited greater FCR than males ($P = 0.002$) and T_1 chickens presented lower FCR than T_0 chickens ($P = 0.007$).

Effects of incubation, postnatal treatment and sex on body composition and meat quality parameters

No effect of incubation conditions on breast meat yield and yellowness of the meat were observed ($P = 0.838$ and $P = 0.733$, respectively). The breast meat yields were greater under cold rearing temperature T_1 ($P = 0.033$) than under standard temperature T_0, and females showed greater percentage of fillet than males ($P < 0.001$; Table 2). A greater intensity of yellowness (b*) of the fillet was observed in males compared to females ($P < 0.001$) and in chickens reared under cold temperature T_1 the first 21 days than under T_0 ($P = 0.022$; Table 3). Females exhibited lower thigh yield ($P < 0.001$) and lightness (L*; $P = 0.026$) but greater abdominal fat percentage ($P < 0.001$), pHu ($P < 0.001$) and processing yield ($P < 0.001$) than males (Table 2). The drip loss and the shear force of the cooked breast meat were not influenced by the incubation treatment, rearing conditions or sex ($P > 0.05$, data not shown).

There was a significant interaction of incubation treatment and sex on fillet intramuscular lipids of the breast (P = 0.018). The highest lipid content was observed in I_0 male chickens and the lowest in both females I_0 and I_1 groups (Table 3). A significant interaction of incubation treatment and postnatal condition was measured on the redness intensity (a*; P = 0.032) with a higher intensity of redness measured in I_0T_1 group than in the I_0T_0 and I_1T_0 groups, the I_1T_1 group presenting intermediate value (Table 3).

The observation of breast meat for the detection of meat defects showed no case of wooden breast but a difference in the occurrence of white striping between experimental groups. Indeed, the occurrence of white striping was greater in males from the cold-incubated and exposed I_1T_1 chickens (with 58% occurrence) as compared to all other groups except the I_0T_1 males (Table 3). The lowest occurrences, below 18%, were observed in I_1T_0 males and females and in I_0T_1 females.

Effects of incubation and postnatal treatment on chicken welfare

The occurrence of hock burns and pododermatitis were measured on carcasses at slaughter as parameters related to welfare troubles depending on incubating and rearing conditions and sex. Hock burns were more frequently observed in males experiencing cold both during incubation and post hatching (I_1T_1 with 45% occurrence) as compared to all female groups and males incubated and reared in standard conditions (P < 0.05). The male chickens with only one cold exposure (I_0T_1 and I_1T_0 males) presented intermediate values (Figure 2). The occurrence of pododermatitis at slaughter was lower (below 28%) in males under standard rearing conditions (in both incubation conditions) than in all other groups (Figure 3A), where occurrences were over 64%. Greater occurence of pododermatitis were observed in I_1T_1 males and females than in all other female groups (P < 0.05; Figure 3B).

DISCUSSION

In an attempt for assessing the positive and negative impacts of perinatal thermal environment of chicks, the main objective of this experiment was to study the effects of cold manipulations during incubation in male and female fast-growing chickens that were later exposed to standard or to low postnatal temperature. The parameters considered consequently to these treatments relate to growth performance and feed efficiency, but also meat processing quality and defects, and animal welfare criteria. Previous experiments had permitted to compare the effects of two published methods of cold embryo manipulation (Shinder et al., 2011; Yalçin et al., 2012) combined to continuous or fluctuating 4°C-colder or standard postnatal temperatures (Nyuiadzi et al., 2017) during the first 21 d of age in Ross 308 broilers. These studies conducted in pens for the two first and in cages for the latter allowed determining thermal conditions of incubation and postnatal rearing enhancing cold adaptation of chicks that had little to no impact on performance in the long term. The present experiment, realized in floor pens, confirms the positive effects of these treatments on performance parameters under normal temperature or posthach cold, however in a sex dependent manner. However, the cumulative exposures to cold both during incubation and posthatching had some detrimental effects on chicken welfare and meat quality parameters in the long term, especially in males.

Concerning hatching parameters, on the one hand, no negative effect of incubation temperature I_0 or I_1 was observed on hatchability (over 95% in both conditions) or on body temperature neither body weight of hatched-chicks, as previously shown by Shinder et al. (2011) and Nyuiadzi et al. (2017). Nevertheless, cold treatment at the end of incubation slightly slowed down hatching process as demonstrated by the lower percentage of hatched

chicks in I_1 compared to I_0 at 480 h of incubation. This result is not surprising since a faster hatching rate was observed following heat-stimulation during embryogenesis (Piestun et al., 2008), with half percentage of hatched chicks at 480 h in heat-treated groups relative to controls, suggesting an impact of thermal treatments during incubation on hatching window. On the other hand, no significant difference between I_0 and I_1 groups was observed on chick quality, contrary to what was recently observed in the study of Nyuiadzi et al. (2017), where the quality score related to the remaining membrane at the navel was lower in I_1 group than the one obtained with control incubation, probably due to a greater chick immaturity at hatching. Sex-ratio was not affected by incubation treatment, whereas the male percentage at hatching was previously reported to be increased by embryonic warm-stimulation during late incubation (Tzschentke and Halle, 2009).

As expected, both the sex and the postnatal temperature markedly affected growth and feed efficiency, with, at d 21 and at d 40 of age, a significant interaction between prenatal and postnatal thermal conditions and sex on the body weight of the birds. At d 21, just at the end of the thermal treatments, the double cold exposure of group I_1T_1 was beneficial for growth in males, whereas female growth was stimulated by *in ovo* or post-hatch cold treatments or both. This effect persisted even after the treatment, as shown by the greater body weights at slaughter age exhibited by males reared under cold conditions and all females that have experienced at least one exposition to cold (*in ovo* and/or during rearing). The cold incubation treatment seemed particularly beneficial in terms of growth for males under cold postnatal temperature and in females under normal postnatal temperature. This may be the result of metabolic or endocrine differences between sexes in the early growth phases. In particular, there are sex-specific responses to hormonal factors or inhibitors and nutrients in the embryonic stage (Dainat et al.; 1991; Kocamis et al.; 1998, Bello et al., 2014) that may affect growth in the long term, supporting this hypothesis. Whatever the postnatal temperature

applied, no detrimental effects of incubation condition on growth were observed. This result is consistent with the previous studies reporting positive effects of cold incubation treatments (Shinder et al., 2011) and of postnatal cold-conditioning (Shahir et al., 2012) on BW at slaughter age in males submitted to ascites-inducing conditions. The positive impact of cold on BW was even clearer in the present study compared to the transient effect already observed with the same treatments applied in birds reared in cages until 21 d of age (Nyuiadzi et al., 2017). One hypothesis is that the birds exposed to cold have consumed more food for sustaining lower post-hatching temperatures, chickens regulating their energy intake depending on ambient temperature post-hatching. However, this is expected to lower feed efficiency while increasing heat production. This was actually the case between d 0 and d 21 during the postnatal cold treatment, with greater feed conversion ratios observed in T_1 groups, consistent with our previous results (Nyuiadzi et al. 2017) and those of Aksit et al. (2013) recorded from d 0 to d 42 in chickens issued from old flocks and postnatally exposed to cold temperatures. However, because FCR became lower from d 22 onward, especially in the I_0 groups submitted to T_1, broilers submitted to cold challenge during rearing were more efficient on the overall period than those reared in standard conditions, whatever the incubation treatment considered. This result supports the hypothesis that chickens submitted to slightly colder conditions during the first 3 weeks of age can gain efficiency by retaining more energy and tissue when placed in standard thermal conditions afterwards. This suggests that metabolic adaptation might have occurred following the cold treatments at young age, in the line of changes of the expression of metabolic enzymes previously reported by Loyau et al. (2014) in case of embryonic exposure to 1°C lower incubation temperatures.

One other remarkable outcome of the present study was the 4% higher body weight observed in I_1 compared to I_0 females reared under standard postnatal conditions, whereas BW of males remains unaffected to cold treatment during incubation. This suggests a sex-specific response

of females to cold incubation temperature that would enhance their growth during the postnatal period. The underlying mechanisms explaining this sex-difference remain to be elucidated.

Consistently with the higher body weight measured at slaughter age, our results showed + 0.4 percentage points in breast meat yield in the groups having experienced postnatal cold conditions. However, the increase in breast meat yields previously reported by Shinder et al. (2011) following the same cold incubation treatment was not observed in the present experiment. This discrepancy could be due to different environmental rearing conditions of T_0 broilers in both experiments. The highest value of breast meat yield was obtained in female groups, consistent with previous results (Collin et al., 2007). Breast meat of female also exhibited higher pHu, probably resulting of a lower muscle energy (glycogen) store (Le Bihan-Duval et al., 2008). This result is in accordance with the higher L* values observed in males than in females, this parameter having been reported to negatively correlate with pHu. Unexpected lower values of the fillet redness a* were observed in I_0T_1 group compared to other groups reared in standard postnatal conditions. The redness of the breast meat was previously associated to chicken behavior before slaughter and early rate of post-mortem glycolysis in muscle, the most active chickens producing the most red meat (Berri et al., 2005; Chabault et al., 2012). Our study suggested a possible impact of postnatal cold on meat redness, probably in relation with a modification of the chicken metabolic rate (Collin et al., 2003) or of muscle fiber metabolic type switch towards more oxidative one as already suggested by Ueda et al. (2004).

White striping is described as white striations parallel to muscle fibers that occur in both pectoral and thigh muscles (Kuttappan et al 2013b) and correspond to inclusion of lipids between fibers, affecting many criteria including color, tenderness, cooked processing and nutritional value of meat (Kuttappan et al 2013a). In the recent study of Clark et al. (2017),

broilers experiencing cyclical heat exposures during incubation were less prone to exhibit moderate to severe myopathic defects compared to controls. In this case, the lower rate of myopathy was associated to lower breast meat yields. In the present study, cold stimulations during incubation (I_1) did not affect the occurrence of white striping under control postnatal temperature. In contrast, combining cold stimulation during incubation with cold post-natal experience (I_1T_1) resulted in greater scores of white striping in males that were also characterized by the greatest body weights at d 40 compared to all T_0 males and all female groups. This result points out the importance of the perinatal thermal environment on the later development of muscle, at least in males. According to Kuttappan et al. (2012), a high growth rate of chickens over a short period leading to heavier broilers would trigger the appearance of non-infectious quality defects in broilers such as white striping. This could explain the higher occurrence of WS of I_1T_1 males, but not the lower occurrence observed in other I_1 birds. *In ovo* temperature alteration and rearing temperature of chicks may influence the embryonic muscle development with consequences on both metabolism and structure. Several authors have shown that embryonic heat-stimulation during incubation modified adipogenic and growth factor expression, myogenesis and cells proliferation (Halevy et al., 2006; Piestun et al., 2009; 2011; Al-Musawi et al., 2012). We can hypothesize that the combined exposure to acute cold at the end of incubation (d 18 and d 19), resulting in a drop of eggshell temperature by 5 to 11°C (data not shown) and to postnatal cold rearing temperature, may have enhanced the proliferation of adipogenic precursors and further lipid deposition in the breast muscle.

Unexpectedly, the higher white striping scores observed in the I_1T_1 male group was not associated with higher values of intramuscular fat content (IMF). Indeed, it had been previously reported that chickens with moderate to severe white striping scores highlighted higher IMF percentage than non-affected chickens (Alnahhas et al., 2016). However, we showed low IMF percentage in breast muscles of females compared to males, in accordance

with previous results of Zerehdaran et al. (2004). The authors related this difference to the lower body weight or muscle fatty acid metabolism of females. Consistently with variations of IMF, in which the liposoluble pigments accumulate, meat of males appeared more yellow than that of females. The meat of broilers that experienced cold postnatal conditions T_1 also appeared more yellow compared to control T_0. Altogether, these results highlighted the role of early life thermal experience in determining breast muscle development and meat quality, especially in male broilers exhibiting a high growth rate.

Beyond the observed effects on the quality of the meat, our study indicates that cold challenge *in ovo* and/or after hatch negatively affects animal welfare evaluated through the occurrence of hock burns and pododermatitis at slaughter. The combined cold treatments I_1T_1 increased the occurrence of hock burns in males and of pododermatitis in both sexes, whereas under standard postnatal temperature, these welfare parameters were not affected by incubation temperature. It is likely that hock burns increase was in part due to the increase in body weights observed in I_1T_1 males at 42 d. Indeed, Baéza et al. (2012) demonstrated a negative impact of increased age and body weight on these types of injuries in relation with the lower walking ability measured in broilers. However, the greater body weight cannot explain by itself the higher occurrence of pododermatitis observed in both I_1T_1 males and females compared to other groups, except I_0T_1. Variations in the occurrence of pododermatitis between groups may also be influenced by environmental rearing conditions such as litter moisture, which was significantly greater in T_1 than in T_0 male pens, and intermediate in all female pens (data not shown). Indeed, low litter moisture percentages, for example in case of low crude protein content of the diet (Li et al., 2018), resulted in lower rate of footpad dermatitis in broilers or in turkeys (Mayne et al., 2007; Tullo et al., 2017). It is therefore possible that the lower post hatching temperature and the greater animal density of female pens (22 vs. 19 for males to account for the body weight differences between sexes from d 22

to 40 of age) have increased litter moisture and hence pododermatitis occurrence in T_1 males and all female groups. This might be explained by a higher water consumption in less efficient birds in this group, which remains to be explored, or to the lower drying of the litter due to the lower ambient temperature. These observations are also consistent with our previous study in which a greater occurrence of leg problems was reported in birds reared in cages at low post hatching temperature (28°C instead of 33°C at d 0, Nyuiadzi et al., 2017). It is also in accordance with the recent work of Steenfeldt et al. (2019) showing that cold temperature (15°C) during the second half of the growth period resulted in a colder and wetter litter, resulting in more footpad pododermatitis than standard temperature (21°C) in fast-growing chickens. Nevertheless, the present study pointed to potential limitations of short and acute cold exposures at the end of incubation when chickens were later reared under postnatal cold temperatures. These limitations could be relative to the timing, intensity, homogeneity and duration of the treatment, possibly favoring the chicken muscle growth at the detriment of other health-related biological functions.

Based on these results, it appears that short cold stimulations at the end of incubation has positive effects on growth in females, and no deleterious effects on feed efficiency, muscle white striping and pododermatitis under normal postnatal temperatures. Despite the positive effects of cold stimulations during embryonic incubation combined to low temperature at start on performance and feed efficiency in broiler chickens, the present study also revealed the limits of such strategy to improve bird adaptive capacities to cold environment. Indeed, thermal manipulation during embryogenesis and rearing period have altered some aspects of welfare and meat quality, with a recrudescence of hock burns, pododermatitis and white striping defects in males exposed to cold both before and after hatching. Our results also confirmed that early management affects in the long term both animal welfare and product

quality, two important components of the image and competitiveness of the poultry production systems. It is now necessary to understand the cellular, molecular and pathological disturbances involved in the observed changes to propose early management strategies allowing efficient and sustainable production systems, limiting current meat defects in broilers and improving welfare.

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Table 1. Hatchability of fertile eggs, sex-ratio, quality score of chicks and body temperature at hatch depending on incubation temperature.

	Incubation temperature[1]		
Traits[2]	I_0	I_1	*P*-value
Rate of hatched chicks at 480h of incubation, % of fertile eggs	43	36	**0.007**
Hatchability, % of fertile eggs	95.4	95.7	0.801
Male chick percentage, %	52.8	51.9	0.765
Total chick quality score[3] (/100)	93.3 ± 1.3	94.3 ± 0.5	0.415
Body weight at hatch, g	42.7 ± 0.1	42.6 ± 0.1	0.681
Body temperature at hatch, °C	38.8 ± 0.1	39.0 ± 0.1	0.059

[1]Control incubation I_0: eggs were incubated until hatch at 37.6°C and 56% RH; I_1: eggs were incubated in the same conditions but exposed for 30 min to 15°C at d 18 and d 19 of incubation.

[2]For each parameter, values are presented as mean ± standard error. Hatchability of fertile eggs was calculated for n = 581 I_0 and 576 I_1 fertile eggs. Body weight and body temperature were measured on n=548 and n = 50 per incubation treatment, respectively.

[3]Chick quality was calculated according to Tona et al. (2003).

Table 2. Chicken performance and meat quality parameters and yields depending on incubation and postnatal rearing temperatures and sex at slaughter age, and measured on meat cut 24h postmortem[1].

Factors[1]	Incubation temperature[2] (I)			Postnatal temperature[3] (P)			Sex (S)			*P*-values of interactions			
	I_0	I_1	*P*-value	T_0	T_1	*P*-value	Male	Female	*P*-value	I×P	I×S	P×S	I×P×S
Performance and litter parameters[4]													
d 21 BW (g)	851±4	863±4	**0.026**	847±4	868±4	**<0.001**	901±4	822±4	**<0.001**	0.708	0.856	0.513	**0.008**
d 40 BW (g)	2500±16	2537±16	**0.034**	2462±16	2574±16	**<0.001**	2740±14	2337±11	**<0.001**	0.547	0.542	0.896	**0.038**
d 0 - d 21 FCR (g/g)	1.38±0.01	1.38±0.01	0.504	1.36±0.00β	1.39±0.00α	**<0.001**	1.37±0.00b	1.39±0.01a	**<0.001**	0.940	0.076	0.050	0.490
d 22 - d 40 FCR (g/g)	1.82±0.02	1.82±0.01	0.864	1.85±0.01	1.79±0.01	**<0.001**	1.80±0.01b	1.84±0.01a	**0.001**	**0.042**	0.173	0.653	0.231
d 0 - d 40 FCR (g/g)	1.65±0.01	1.66±0.01	0.678	1.66±0.01α	1.64±0.01β	**0.008**	1.64±0.01b	1.67±0.01a	**0.002**	0.065	0.075	0.979	0.412
Litter moisture (%)	39.0±0.7	38.9±0.8	0.926	37.9±0.7	40.0±0.8	**0.047**	38.9±0.8	39.0±0.8	0.897	0.403	0.429	**0.037**	0.570
Meat yields and parameters of carcass quality[5]													
Breast meat yield (%)	20.7±0.1	20.7±0.1	0.838	20.5±0.1β	20.9±0.1α	**0.033**	20.2±0.1b	21.1±0.1a	**<0.001**	0.978	0.960	0.547	0.609
Thigh Yield (%)	22.1±0.1	22.1±0.1	0.911	22.0±0.0	22.2±0.0	0.213	22.5±0.0b	21.8±0.0a	**<0.001**	0.240	0.413	0.945	0.902
Abdominal Fat (%)	2.2±0.0	2.2±0.0	0.236	2.2±0.0	2.2±0.0	0.155	2.1±0.0b	2.3±0.0a	**<0.001**	0.968	0.861	0.618	0.461
Processing yield (%)	82.3±0.5	82.5±0.5	0.855	82.1±0.6	82.8±0.5	0.281	80.6±0.5b	83.9±0.5a	**<0.001**	0.989	0..311	0.978	0.765
Ultimate pH	5.84±0.01	5.83±0.01	0.882	5.84±0.01	5.83±0.01	0.609	5.80±0.01b	5.87±0.01a	**<0.001**	0.147	0.696	0.289	0.192
Yellowness, b*	10.2±0.1	10.2±0.1	0.733	10.0±0.1β	10.4±0.1α	**0.014**	10.70±0.11a	9.77±0.11b	**<0.001**	0.159	0.277	0.664	0.302
Redness a*	-0.38±0.05	-0.39±0.05	0.808	-0.50±0.05	-0.27±0.05	**0.003**	-0.32±0.06	-0.45±0.05	0.092	**0.038**	0.525	0.629	0.165
Lightness L*	50.9±0.2	50.8±0.3	0.825	50.8±0.3	50.9±0.2	0.944	51.2±0.3a	50.5±0.2b	**0.026**	0.313	0756	0.674	0.319
Fillet intramuscular lipids (%)	1.17±0.09	1.12±0.08	0.298	1.11±0.07	1.18±0.10	0.576	1.42±0.10	0.92±0.04	**<0.001**	0.853	**0.018**	0.548	0.167

[1] Statistical effects of Sex (S), Postnatal temperature (P) and Incubation temperature (I) and of their interactions were analyzed by ANOVA. a, b: different letters correspond to significant differences ($P < 0.05$) between sexes and α, β: different letters correspond to significant differences ($P < 0.05$) between postnatal temperatures.

[2] Control incubation I_0: eggs were incubated until hatch at 37.6°C and 56% RH; I_1: eggs were incubated in the same conditions but exposed for 30 min at 15°C at d 18 and d 19 of incubation.

[3] Control temperature T_0 from 32°C at d 0 to 21°C at d 21 or Cold temperature T_1 from 29°C at d 0 to 21°C at d 21, then T_0 and T_1 were maintained at 21°C from d 22 to d 40.

[4] Body weights (BW) were measured on n = 92 to 108 chickens per group at d 21 and 76 to 88 at d 40, and for Feed conversion ratio (FCR) and litter moisture percentage, values are presented as mean ± standard error (n = 4 per group).

[5] Parameters were measured on n = 29 to 35, except for processing yield measured on n = 11 to 13, and for Fillet intramuscular lipid % measured on n = 6 to 9.

Table 3. Performance of chickens and meat quality parameters depending on incubation and postnatal rearing temperatures and sex when at least one interaction is significant between these factors. Body weights were measured at d 21 and d 40 of age. Feed conversion ratio (FCR) was calculated between d 22 and d 40.

Sex (S)	Males				Females										
Postnatal temperature[1] (P)	T_0		T_1		T_0		T_1		Statistical effects[3]						
Incubation temperature[2] (I)	I_0	I_1	I_0	I_1	I_0	I_1	I_0	I_1	I	P	S	I×P	I×S	P×S	I×P×S
d 21 BW (g)	892 ± 8[b]	893 ± 9[b]	896 ± 7[b]	920 ± 7[a]	797 ± 7[d]	823 ± 7[c]	835 ± 6[c]	830 ± 6[c]	**0.026**	**<0.001**	**<0.001**	0.708	0.856	0.513	**0.008**
d 40 BW (g)	2685 ± 31[b]	2686 ± 27[b]	2769 ± 24[a]	2818 ± 27[a]	2240 ± 18[e]	2330 ± 23[d]	2388 ± 19[c]	2390 ± 21[c]	**0.034**	**<0.001**	**<0.001**	0.547	0.542	0.896	**0.038**
d 22 - d 40 FCR (g/g)	1.85 ± 0.03	1.80 ± 0.01	1.76 ± 0.02	1.78 ± 0.01	1.87 ± 0.01	1.88 ±0.01	1.79 ± 0.02	1.82 ± 0.02	0.864	**<0.001**	**0.001**	**0.042**	0.173	0.653	0.231
Litter moisture (%)	37.0 ± 1.1	36.4 ± 1.0	41.6 ± 1.3	40.5 ± 1.2	38.0 ± 1.1	40.1 ± 1.8	39.3 ± 1.6	38.6 ± 2.2	0.926	**0.047**	0.897	0.403	0.429	**0.037**	0.570
Redness a*	-0.45 ± 0.12	-0.37 ± 0.12	-0.22 ± 0.09	-0.24 ± 0.13	-0.66 ± 0.10	-0.47 ± 0.10	-0.16 ± 0.08	-0.47 ± 0.08	0.808	**0.003**	0.092	**0.038**	0.525	0.629	0.165
Fillet intramuscular lipids (%)	1.63 ± 012	1.13 ± 0.09	1.59 ± 0.23	1.40 ± 0.27	0.80 ± 0.05	1.06 ± 0.10	0.92 ± 0.06	0.93 ± 0.12	0.298	0.576	**<0.001**	0.853	**0.018**	0.548	0.167

[1] Control temperature T_0 from 32°C at d 0 to 21°C at d 21 or Cold temperature T_1 from 29°C at d 0 to 21°C at d 21, then T_0 and T_1 were maintained at 21°C from d 22 to d 40.

[2] Control incubation I_0: eggs were incubated until hatch at 37.6°C and 56% RH; I_1: eggs were incubated in the same conditions but exposed for 30 min at 15°C at d 18 and d 19 of incubation.

[3] Statistical effects of Sex (S), Postnatal temperature (P) and Incubation temperature (I) and of their interactions were analyzed by ANOVA.

For FCR and litter moisture, values are presented as mean ± standard error (n = 4 per group).

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

FIGURE LEGENDS

Figure 1. Experimental design. Number of birds per treatment are reported in the Materials and Methods section.

Figure 2. Effects of sex, incubation condition and postnatal rearing condition on the occurrence of white striping on the breast meat observed 24 h after slaughter at d 40. Control incubation I_0 corresponds to egg incubation at 37.6°C, 56% RH. Cold incubation I_1 corresponds to egg exposure for 30 min at 15°C at d 18 and 19 of incubation, eggs being incubated in the same conditions than for I_0 the remaining time. Chickens were reared in floor pens either under control temperature T_0 from 32°C at d 0 to 21°C at d 21 or under cold temperature T_1 from 29°C at d 0 to 21°C at d 21. From d 22 to d 40, the ambient temperature in T_0 and T_1 was maintained at 21°C. Different letters (a, b, c) correspond to significant differences ($P < 0.05$) between groups.

Figure 3. Effects of sex, incubation condition and postnatal rearing condition on parameters related to welfare troubles observed on carcasses after slaughter at d 40. A- Occurrence of hock burns. B- Occurrence of pododermatitis. Control incubation I_0 corresponds to egg incubation at 37.6°C, 56% RH. Cold incubation I_1 corresponds to egg exposure for 30 min at 15°C at d 18 and 19 of incubation, eggs being incubated in the same conditions than for I_0 the remaining time. Chickens were reared in floor pens either under control temperature T_0 from 32°C at d 0 to 21°C at d 21 or under cold temperature T_1 from 29°C at d 0 to 21°C at d 21. From d 22 to d 40, the ambient temperature in T_0 and T_1 was maintained at 21°C. Different letters (a, b, c) correspond to significant differences ($P < 0.05$) between groups.

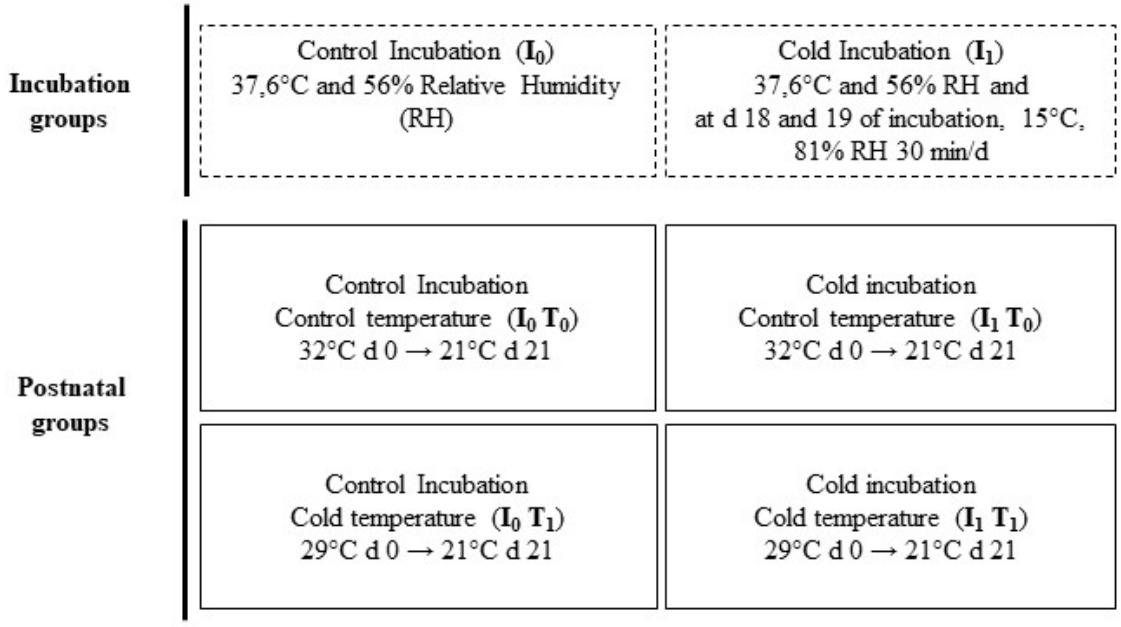
Incubation groups
Control Incubation (I_0)
37,6°C and 56% Relative Humidity (RH)
Cold Incubation (I_1)
37,6°C and 56% RH and at d 18 and 19 of incubation, 15°C, 81% RH 30 min/d
Postnatal groups
Control Incubation
Control temperature (I_0 T_0)
32°C d 0 → 21°C d 21
Cold incubation
Control temperature (I_1 T_0)
32°C d 0 → 21°C d 21
Control Incubation
Cold temperature (I_0 T_1)
29°C d 0 → 21°C d 21
Cold incubation
Cold temperature (I_1 T_1)
29°C d 0 → 21°C d 21

Figure 1

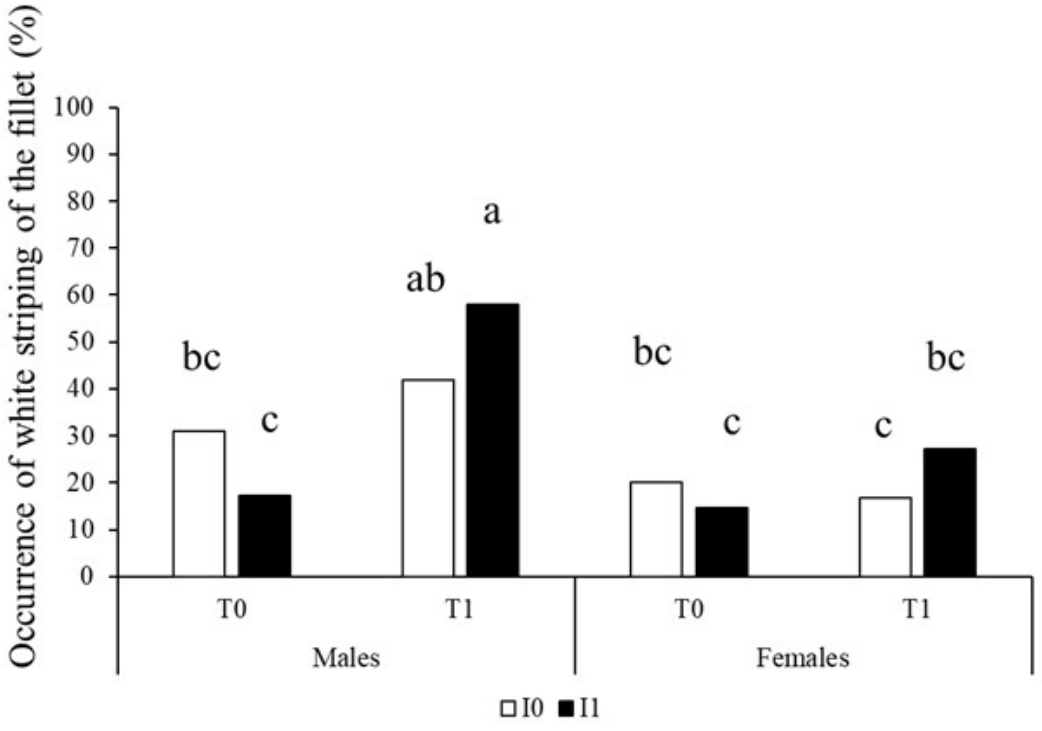
Occurrence of white striping of the fillet (%)
100
90
80
70
60
50
40
30
20
10
0
bc
c
ab
a
bc
c
c
bc
T0
T1
T0
T1
Males
Females
I0
I1

Figure 2

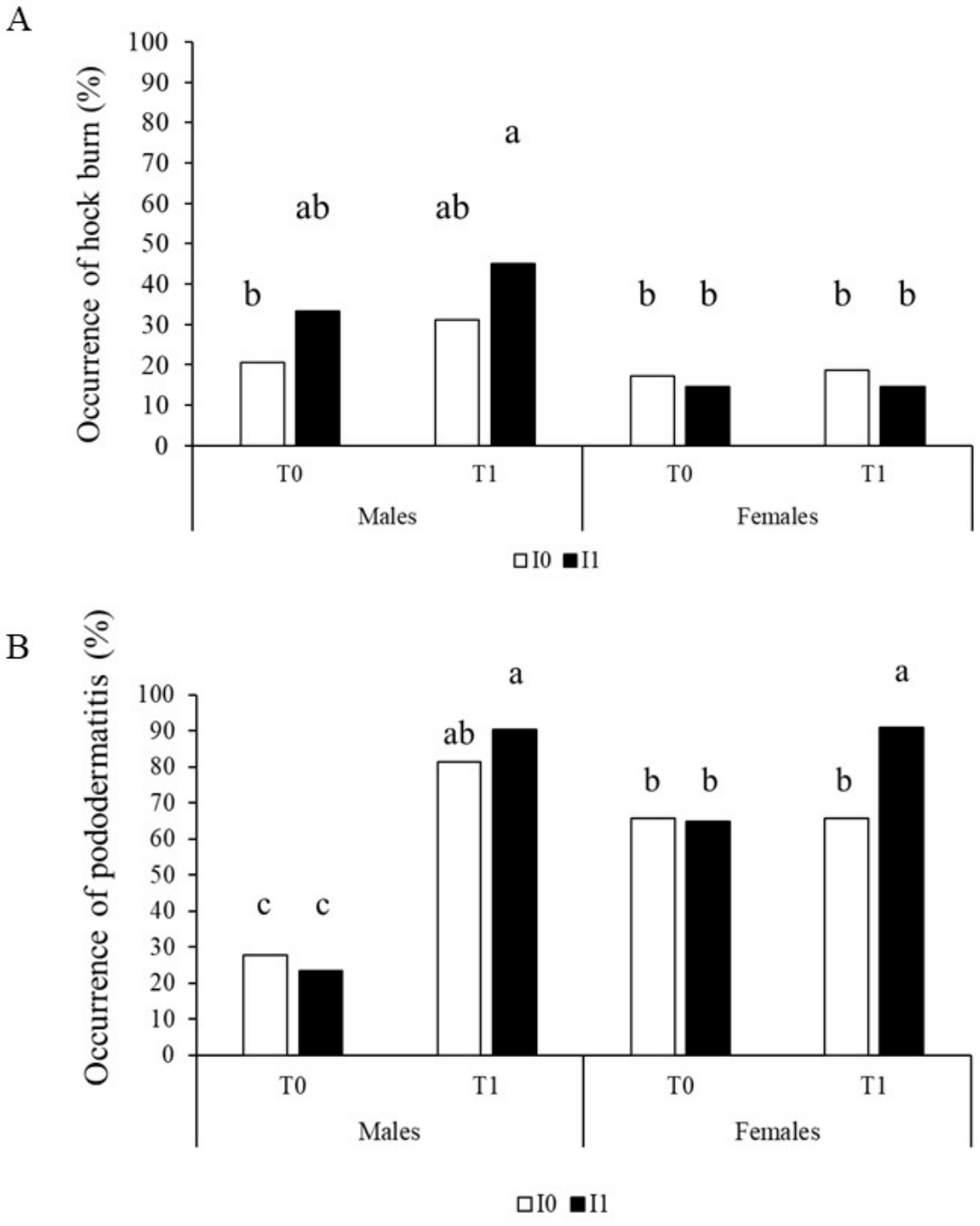
A
Occurrence of hock burn (%)
100
90
80
70
60
50
40
30
20
10
0
b
ab
ab
a
b
b
b
b
T0
T1
T0
T1
Males
Females
I0
I1
B
Occurrence of pododermatitis (%)
100
90
80
70
60
50
40
30
20
10
0
c
c
ab
a
b
b
b
a
T0
T1
T0
T1
Males
Females
I0
I1

Figure 3