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Meat quality in cold-acclimated chickens

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

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25 **ABSTRACT**

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

31 Ross 308 eggs were incubated under control temperature (**I₀**, 37.6°C) or submitted to 15°C
32 during 30 min on d 18 and 19 of incubation (**I₁**). Chicks from each group were reared in floor
33 pens either at standard rearing temperature (**T₀**), from 32°C at d 0 to 21°C at d 21, or exposed
34 to colder rearing temperature (**T₁**), from 29°C at d 0 to 21°C at d 21 of age. Birds were then
35 all kept at 21°C until slaughter (d 40), when body weights (**BW**), feed conversion ratio
36 (**FCR**), breast muscle yield, meat processing quality and the occurrences of meat defects,
37 hock burns and pododermatitis were recorded. No significant impact of incubation conditions
38 on hatchability was observed. At d 40, BW was greater under T₁ than T₀ condition, with T₀
39 females (but not males) presenting greater BW after I₁ than after I₀ condition. On the whole
40 period, T₁ chickens presented lower FCR than T₀, and greater breast meat yields at d 40. The
41 occurrence of white striping was greater in I₁T₁ males than in all other groups, except for the
42 I₀T₁ males. Hock burns were more frequent in I₁T₁ males than in all females and I₀T₀ males,
43 whereas the occurrence of pododermatitis was lower in T₀ males than in other groups. Despite
44 some positive effects of I₁ incubation on growth after starting under low ambient temperature,
45 this study reveals the limits of such strategy concerning chicken health and welfare,
46 demonstrating that early thermal environment is a major component of the quality and
47 sustainability of chicken meat production.

48

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

INTRODUCTION

50

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

56 It was suggested that the genetic selection of fast-growing broilers on growth rate, feed
57 efficiency and breast meat yield have simultaneously increased the sensitivity of these birds to
58 temperature variations (Piestun et al., 2008; Havenstein et al., 2003a,b; Zuidhof et al., 2014).
59 Because the thermoregulatory system of chicks is immature at hatching (Tzschentke, 2007),
60 they remain very sensitive to postnatal cold temperatures during the first days of breeding
61 (Collin et al., 2003; Mujahid and Furuse, 2009). Environmental conditions during the
62 perinatal period are considered as critical for the chicken later performance and fitness
63 (Guilloteau et al., 2019). Especially, brooding chicks at low temperatures (26.7°C vs. 32.2°C)
64 results in a decreased feed efficiency and increased mortality rate (Renwick and Washburn,
65 1982). Fast-growing broilers exhibit greater ascites prevalence (Druyan et al., 2007) and leg
66 disorders (Yalçın et al., 2007; Zhang et al., 2014; Nyuiadzi et al., 2017) when they are
67 submitted to low rearing temperatures post-hatch.

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

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

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

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

106

107

MATERIALS AND METHODS

108 All experimental procedures were approved by the Ethics Committee for Animal
109 Experimentation Val de Loire (CEEA Val de Loire, Tours, France, N°2014111809444741
110 (APAFIS#70).03).

111 *Incubation Process*

112 A total of 1,200 fertile Ross 308 broilers eggs with 3 d of storage from 36 wk-old breeder
113 flock were provided from a commercial hatchery (Couvoir Perrot, Pommerit-Jaudy, France).
114 Prior to incubation, average egg weight was determined (62.3 ± 0.7 g) and eggs were
115 randomly divided in two treatment groups experiencing control (**I₀** at 37.6°C and 56% RH) or
116 cold incubation (**I₁**) at PEAT INRA Poultry Experimental Facility (2018,
117 <https://doi.org/10.15454/1.5572326250887292E12>). **I₁** consisted in exposing 3 trays of 132
118 eggs to 15°C and 81% RH during 30 min on d 18 and d 19 of incubation by transferring them
119 from the hatcher to a cold room, eggs being incubated at 37.6°C and 56% RH for the
120 remainder of incubation (Figure 1). This treatment resulted in a minimal eggshell temperature
121 (\pm SD) of $26.9^\circ\text{C} \pm 2.1^\circ\text{C}$ and maximal eggshell temperature of $33.1 \pm 0.6^\circ\text{C}$, measured by
122 thermal imaging after 30 minutes of exposure. On d 7 and d 14 of incubation, unfertile and

123 undeveloped eggs were eliminated after candling. At d 18 of incubation, all eggs were moved
124 to a common hatcher set at 37.6°C and 70% RH, with only the 30 min interruption at d 19 for
125 the transfer of group I₁ to the cold room.

126 *Hatching*

127 Into the hatcher, the numbers of hatched chicks in control (I₀) and cold (I₁) incubation were
128 recorded at 480 h of incubation, and then at 504 hours (opening of the hatcher) to calculate
129 hatchability as the ratio of hatched chicks on fertile incubated eggs. Chicks from each
130 treatment were weighed, wing-banded and sexed by cloacal observation. The body
131 temperature (**T_b**) of chicks was measured individually at hatching (on dry chicks after
132 opening the hatcher) by inserting an electronic thermometer (PX-TH519, Tex, Pelimex,
133 Ingwiller, France) in the cloaca. Fifty chicks from both treatment groups were randomly
134 selected for quality measurement according to Tona et al. (2003). Briefly, the score of 8
135 parameters including activity, down and appearance, retracted yolk, eyes, legs, navel area,
136 remaining membrane, and remaining yolk on the navel were measured to determine the chick
137 quality. Each score was summed and scored out of 100, with 100 being the highest score
138 value for the best chick quality.

139 *Rearing Period*

140 Four-hundred chicks from each incubation group were divided randomly into 2 groups and
141 were transferred to 2 identical controlled rooms under standard rearing conditions **T₀**,
142 decreasing from 32°C at d 0 to 21°C at d 21, or under cold rearing conditions **T₁**, consisting in
143 continuously decreasing temperature from 29°C at d 0 to 21°C at d 21, respectively (Figure
144 1). From d 22 to d 40, ambient temperature was maintained at 21°C in both conditions T₀ and
145 T₁. Chicks were reared in groups of same sex per condition with 4 repetitions per incubation
146 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 m²) with wood shaving litter (1.5 kg/m²).
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 (I₀T₀, I₀T₁, I₁T₀, I₁T₁)
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, Barcelona, 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

171 *Pectoralis major* muscles on a scale of 3 classes according to Alnahhas et al. (2016). The
172 intramuscular lipid content was calculated by near-infrared spectroscopy using a Nirflex N-
173 500 spectrometer (Buchi, Rungis, France) from samples of *Pectoralis major* collected at 40 d
174 of age (Alnahhas et al., 2016). The lipid peroxidation value was determined by the
175 quantification of thiobarbituric acid-reactive substance (**TBARS**) using the method developed
176 by Lynch and Frei (1993). Drip loss was determined according to Berri et al. (2007). The
177 cooking loss of the breast muscles was measured as previously described by Baéza et al.
178 (2012) and Alnahhas et al. (2016). The average Warner-Bratzler shear force value (N/cm²) of
179 the meat was determined on 3 samples of cooked breast muscle (1 × 1 × 3 cm) using an
180 Instron universal testing instrument (Instron 5543, Instron S.A., Guyancourt, France) as
181 described by Honikel (1998) and Alnahhas et al. (2016) in order to assess the tenderness of
182 the meat.

183 *Parameters related to chicken welfare*

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

189 *Statistical Analysis*

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

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

202

203

RESULTS

204 *Effects of incubation and postnatal treatment on hatchability, chick quality and* 205 *performance*

206 Hatchability of fertile eggs was not significantly affected by the cold incubation treatment (P
207 = 0.801) with hatchabilities over 95% in both groups in our experimental conditions (Table
208 1). However, at 480 h of incubation, i.e. 24 h before opening the hatcher, 43% of the I₀ chicks
209 were hatched, but only 36% of the I₁ chicks (P = 0.007). At hatching, neither the male chick
210 percentage nor the quality of chicks from cold-incubated eggs I₁ was different from that of
211 control chicks I₀ (P = 0.765 and 0.415, respectively; Table 1). Body temperature tended to be
212 greater in I₁ than in I₀ chicks at hatching (P = 0.059), but BW (Table 1) was not influenced by
213 the incubation thermal treatment (P = 0.681). There were significant interactions of
214 incubation condition, postnatal rearing temperature and sex on BW at d 21 (P = 0.008) and d
215 40 of age (P = 0.038; Table 2). At d 21, male BW were not different between I₀ and I₁ at
216 standard temperature T₀, while male BW was greater for I₁ than for I₀ group under cold
217 ambient temperature T₁ (P < 0.05; Table 3). On the contrary, I₁ females exhibited greater BW
218 at d 21 than I₀ ones at T₀ (P < 0.05), whereas there were no difference observed between

219 incubation conditions under cold ambient temperature T_1 . At slaughter age (d 40), body
220 weights of both males and females were greater under cold rearing temperature T_1 than under
221 standard conditions T_0 . In females (but not in males) under standard temperature T_0 , I_1 group
222 exhibited greater slaughter BW than I_0 ones ($P < 0.05$; Table 3). The postnatal cold
223 temperature and the sex affected the FCR from d 0 to d 21 with greater FCR in females than
224 in males and in chickens reared under lower ambient temperature T_1 than at T_0 ($P < 0.001$ and
225 $P < 0.001$, respectively; Table 2). Between d 22 and d 40 (when ambient temperatures were
226 maintained at 21°C for both groups T_0 and T_1), FCR of chickens incubated in control
227 conditions I_0 was lower in T_1 than in T_0 group, while it did not differ depending on the
228 postnatal temperature in I_1 -incubated chickens (Table 3). During this period, females
229 exhibited higher FCR than males ($P < 0.02$). On the whole period from d 0 to d 40 (Table 2),
230 females exhibited greater FCR than males ($P = 0.002$) and T_1 chickens presented lower FCR
231 than T_0 chickens ($P = 0.007$).

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

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

244 There was a significant interaction of incubation treatment and sex on fillet intramuscular
245 lipids of the breast ($P = 0.018$). The highest lipid content was observed in I_0 male chickens
246 and the lowest in both females I_0 and I_1 groups (Table 3). A significant interaction of
247 incubation treatment and postnatal condition was measured on the redness intensity (a^* ; $P =$
248 0.032) with a higher intensity of redness measured in I_0T_1 group than in the I_0T_0 and I_1T_0
249 groups, the I_1T_1 group presenting intermediate value (Table 3).

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

256

257 *Effects of incubation and postnatal treatment on chicken welfare*

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

268

269

DISCUSSION

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

287 Concerning hatching parameters, on the one hand, no negative effect of incubation
288 temperature I_0 or I_1 was observed on hatchability (over 95% in both conditions) or on body
289 temperature neither body weight of hatched-chicks, as previously shown by Shinder et al.
290 (2011) and Nyuiadzi et al. (2017). Nevertheless, cold treatment at the end of incubation
291 slightly slowed down hatching process as demonstrated by the lower percentage of hatched

292 chicks in I₁ compared to I₀ at 480 h of incubation. This result is not surprising since a faster
293 hatching rate was observed following heat-stimulation during embryogenesis (Piestun et al.,
294 2008), with half percentage of hatched chicks at 480 h in heat-treated groups relative to
295 controls, suggesting an impact of thermal treatments during incubation on hatching window.
296 On the other hand, no significant difference between I₀ and I₁ groups was observed on chick
297 quality, contrary to what was recently observed in the study of Nyuiadzi et al. (2017), where
298 the quality score related to the remaining membrane at the navel was lower in I₁ group than
299 the one obtained with control incubation, probably due to a greater chick immaturity at
300 hatching. Sex-ratio was not affected by incubation treatment, whereas the male percentage at
301 hatching was previously reported to be increased by embryonic warm-stimulation during late
302 incubation (Tzschentke and Halle, 2009).

303 As expected, both the sex and the postnatal temperature markedly affected growth and feed
304 efficiency, with, at d 21 and at d 40 of age, a significant interaction between prenatal and
305 postnatal thermal conditions and sex on the body weight of the birds. At d 21, just at the end
306 of the thermal treatments, the double cold exposure of group I₁T₁ was beneficial for growth in
307 males, whereas female growth was stimulated by *in ovo* or post-hatch cold treatments or both.
308 This effect persisted even after the treatment, as shown by the greater body weights at
309 slaughter age exhibited by males reared under cold conditions and all females that have
310 experienced at least one exposition to cold (*in ovo* and/or during rearing). The cold incubation
311 treatment seemed particularly beneficial in terms of growth for males under cold postnatal
312 temperature and in females under normal postnatal temperature. This may be the result of
313 metabolic or endocrine differences between sexes in the early growth phases. In particular,
314 there are sex-specific responses to hormonal factors or inhibitors and nutrients in the
315 embryonic stage (Dainat et al.; 1991; Kocamis et al.; 1998, Bello et al., 2014) that may affect
316 growth in the long term, supporting this hypothesis. Whatever the postnatal temperature

317 applied, no detrimental effects of incubation condition on growth were observed. This result
318 is consistent with the previous studies reporting positive effects of cold incubation treatments
319 (Shinder et al., 2011) and of postnatal cold-conditioning (Shahir et al., 2012) on BW at
320 slaughter age in males submitted to ascites-inducing conditions. The positive impact of cold
321 on BW was even clearer in the present study compared to the transient effect already observed
322 with the same treatments applied in birds reared in cages until 21 d of age (Nyuiadzi et al.,
323 2017). One hypothesis is that the birds exposed to cold have consumed more food for
324 sustaining lower post-hatching temperatures, chickens regulating their energy intake
325 depending on ambient temperature post-hatching. However, this is expected to lower feed
326 efficiency while increasing heat production. This was actually the case between d 0 and d 21
327 during the postnatal cold treatment, with greater feed conversion ratios observed in T₁ groups,
328 consistent with our previous results (Nyuiadzi et al. 2017) and those of Aksit et al. (2013)
329 recorded from d 0 to d 42 in chickens issued from old flocks and postnatally exposed to cold
330 temperatures. However, because FCR became lower from d 22 onward, especially in the I₀
331 groups submitted to T₁, broilers submitted to cold challenge during rearing were more
332 efficient on the overall period than those reared in standard conditions, whatever the
333 incubation treatment considered. This result supports the hypothesis that chickens submitted
334 to slightly colder conditions during the first 3 weeks of age can gain efficiency by retaining
335 more energy and tissue when placed in standard thermal conditions afterwards. This suggests
336 that metabolic adaptation might have occurred following the cold treatments at young age, in
337 the line of changes of the expression of metabolic enzymes previously reported by Loyau et
338 al. (2014) in case of embryonic exposure to 1°C lower incubation temperatures.

339 One other remarkable outcome of the present study was the 4% higher body weight observed
340 in I₁ compared to I₀ females reared under standard postnatal conditions, whereas BW of males
341 remains unaffected to cold treatment during incubation. This suggests a sex-specific response

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

345 Consistently with the higher body weight measured at slaughter age, our results showed + 0.4
346 percentage points in breast meat yield in the groups having experienced postnatal cold
347 conditions. However, the increase in breast meat yields previously reported by Shinder et al.
348 (2011) following the same cold incubation treatment was not observed in the present
349 experiment. This discrepancy could be due to different environmental rearing conditions of T₀
350 broilers in both experiments. The highest value of breast meat yield was obtained in female
351 groups, consistent with previous results (Collin et al., 2007). Breast meat of female also
352 exhibited higher pHu, probably resulting of a lower muscle energy (glycogen) store (Le
353 Bihan-Duval et al., 2008). This result is in accordance with the higher L* values observed in
354 males than in females, this parameter having been reported to negatively correlate with pHu.
355 Unexpected lower values of the fillet redness a* were observed in I₀T₁ group compared to
356 other groups reared in standard postnatal conditions. The redness of the breast meat was
357 previously associated to chicken behavior before slaughter and early rate of post-mortem
358 glycolysis in muscle, the most active chickens producing the most red meat (Berri et al., 2005;
359 Chabault et al., 2012). Our study suggested a possible impact of postnatal cold on meat
360 redness, probably in relation with a modification of the chicken metabolic rate (Collin et al.,
361 2003) or of muscle fiber metabolic type switch towards more oxidative one as already
362 suggested by Ueda et al. (2004).

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

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

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

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

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

417 to 40 of age) have increased litter moisture and hence pododermatitis occurrence in T₁ males
418 and all female groups. This might be explained by a higher water consumption in less
419 efficient birds in this group, which remains to be explored, or to the lower drying of the litter
420 due to the lower ambient temperature. These observations are also consistent with our
421 previous study in which a greater occurrence of leg problems was reported in birds reared in
422 cages at low post hatching temperature (28°C instead of 33°C at d 0, Nyuiadzi et al., 2017). It
423 is also in accordance with the recent work of Steinfeldt et al. (2019) showing that cold
424 temperature (15°C) during the second half of the growth period resulted in a colder and wetter
425 litter, resulting in more footpad pododermatitis than standard temperature (21°C) in fast-
426 growing chickens. Nevertheless, the present study pointed to potential limitations of short and
427 acute cold exposures at the end of incubation when chickens were later reared under postnatal
428 cold temperatures. These limitations could be relative to the timing, intensity, homogeneity
429 and duration of the treatment, possibly favoring the chicken muscle growth at the detriment of
430 other health-related biological functions.

431

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

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

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448

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

Traits ²	Incubation temperature ¹		P-value
	I ₀	I ₁	
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

660 ¹Control incubation I₀: eggs were incubated until hatch at 37.6°C and 56% RH; I₁: eggs were incubated in the same conditions but exposed for 30 min to 15°C at d 18 and d
 661 19 of incubation.

662 ²For each parameter, values are presented as mean ± standard error. Hatchability of fertile eggs was calculated for n = 581 I₀ and 576 I₁ fertile eggs. Body weight and body
 663 temperature were measured on n=548 and n = 50 per incubation treatment, respectively.

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

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670 **Table 2.** Chicken performance and meat quality parameters and yields depending on incubation and postnatal rearing temperatures and sex at
 671 slaughter age, and measured on meat cut 24h postmortem¹.

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
<i>Performance and litter parameters⁴</i>													
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±0.00 β	1.39±0.00 α	<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±0.01 α	1.64±0.01 β	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
<i>Meat yields and parameters of carcass quality⁵</i>													
Breast meat yield (%)	20.7±0.1	20.7±0.1	0.838	20.5±0.1 β	20.9±0.1 α	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±0.1 β	10.4±0.1 α	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	0.756	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

672 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
 673 to significant differences (P < 0.05) between sexes and α , β : different letters correspond to significant differences (P < 0.05) between postnatal temperatures.

674 ² Control incubation I₀: eggs were incubated until hatch at 37.6°C and 56% RH; I₁: eggs were incubated in the same conditions but exposed for 30 min at 15°C at d 18 and d
 675 19 of incubation.

676 ³ Control temperature T₀ from 32°C at d 0 to 21°C at d 21 or Cold temperature T₁ from 29°C at d 0 to 21°C at d 21, then T₀ and T₁ were maintained at 21°C from d 22 to d 40.

677 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,
 678 values are presented as mean ± standard error (n = 4 per group).

679 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.

680

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

Sex (S)	Males				Females				Statistical effects ³						
	T ₀		T ₁		T ₀		T ₁								
Postnatal temperature ¹ (P)	T ₀		T ₁		T ₀		T ₁		I	P	S	I×P	I×S	P×S	I×P×S
Incubation temperature ² (I)	I ₀	I ₁													
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 ^e	2390 ± 21 ^e	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 ± 0.12	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

684 ¹ Control temperature T₀ from 32°C at d 0 to 21°C at d 21 or Cold temperature T₁ from 29°C at d 0 to 21°C at d 21, then T₀ and T₁ were maintained at 21°C from d 22 to d 40.

685 ² Control incubation I₀: eggs were incubated until hatch at 37.6°C and 56% RH; I₁: eggs were incubated in the same conditions but exposed for 30 min at 15°C at d 18 and d
 686 19 of incubation.

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

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

689 ^{a,b,c,d} Different letters correspond to significant differences (*P* < 0.05) between treatments groups.

690

691

692

693

FIGURE LEGENDS

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

696

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

706

707 Figure 3. Effects of sex, incubation condition and postnatal rearing condition on parameters
708 related to welfare troubles observed on carcasses after slaughter at d 40. A- Occurrence of
709 hock burns. B- Occurrence of pododermatitis. Control incubation I_0 corresponds to egg
710 incubation at 37.6°C, 56% RH. Cold incubation I_1 corresponds to egg exposure for 30 min at
711 15°C at d 18 and 19 of incubation, eggs being incubated in the same conditions than for I_0 the
712 remaining time. Chickens were reared in floor pens either under control temperature T_0 from
713 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.
714 From d 22 to d 40, the ambient temperature in T_0 and T_1 was maintained at 21°C. Different
715 letters (a, b, c) correspond to significant differences ($P < 0.05$) between groups.

Incubation groups	Control Incubation (I₀) 37,6°C and 56% Relative Humidity (RH)	Cold Incubation (I₁) 37,6°C and 56% RH and at d 18 and 19 of incubation, 15°C, 81% RH 30 min/d
	Control Incubation Control temperature (I₀ T₀) 32°C d 0 → 21°C d 21	Cold incubation Control temperature (I₁ T₀) 32°C d 0 → 21°C d 21
Postnatal groups	Control Incubation Cold temperature (I₀ T₁) 29°C d 0 → 21°C d 21	Cold incubation Cold temperature (I₁ T₁) 29°C d 0 → 21°C d 21

Figure 1

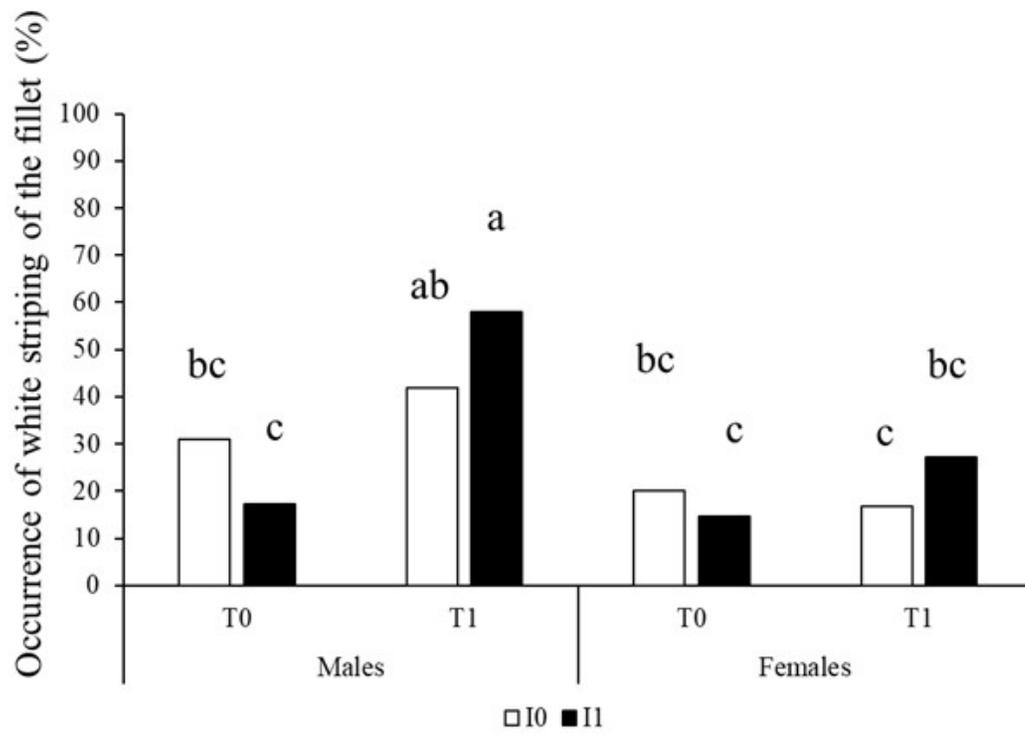


Figure 2

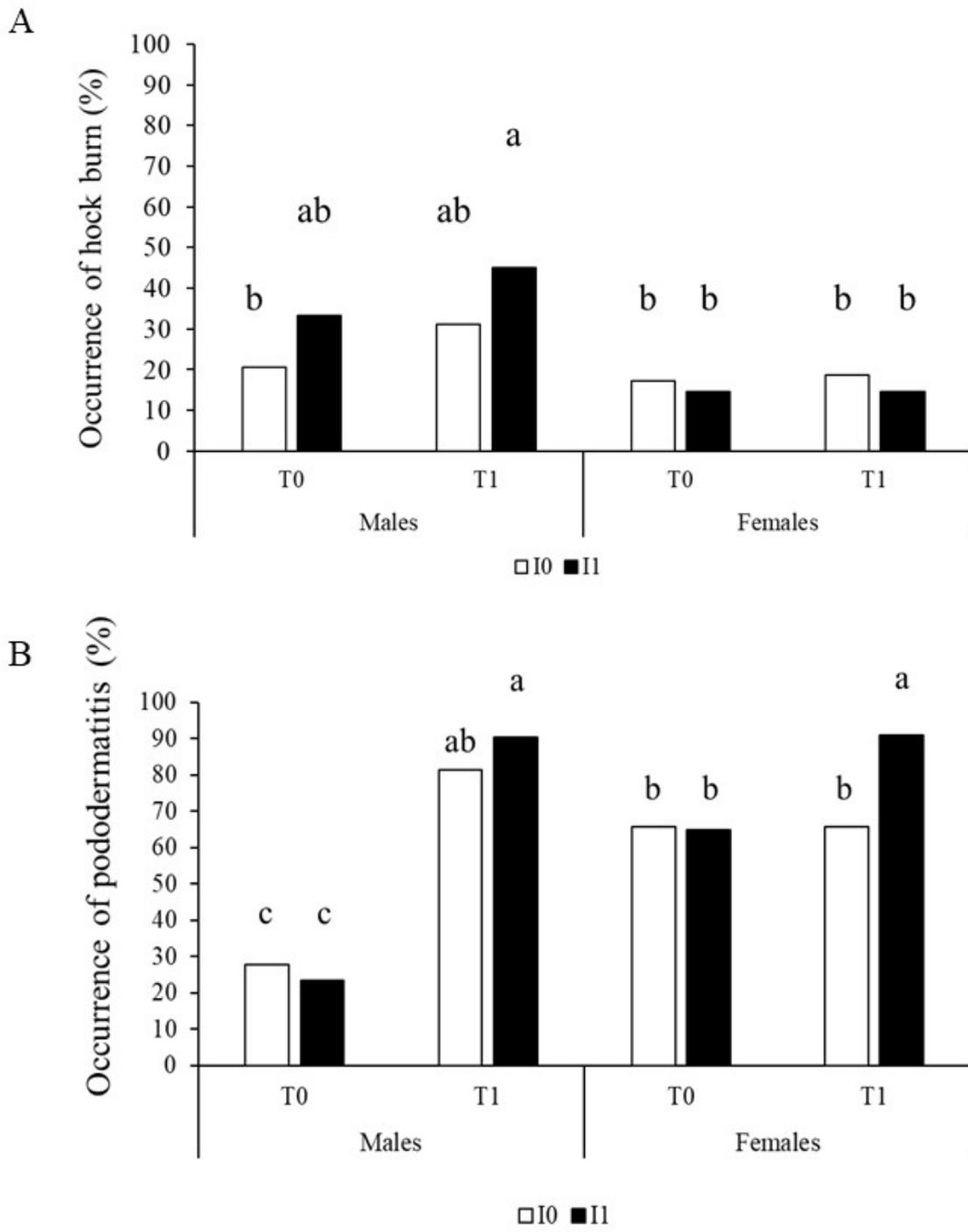


Figure 3