Effect of environmental temperature, floor type and breed on skatole and indole concentrations in fat of females, immuno-castrated and entire males

Antoni Dalmau[a], Tâmara Duarte Borges[b], Eduardo de Mercado[c], Joel González[a], Aranzazu Mateos-San Juan[d], Mariana Huerta-Jiménez[a], Emilio Gómez-Izquierdo[c], Rosil Lizardo[a], Joaquim Pallisera[a], Francesc Borrisser-Pairó[a], Enric Esteve-Garcia[a], Nuria Panella-Riera[a] Ismael Ovejero[d]

[a]IRTA. Finca Camps i Armet, s/n. 17121. Monells. Girona. Spain.

[b]PUCPR. Imaculada Conceição, 1155, Prado Velho. 80215-901. Curitiba – PR. Brazil.

[c]ITACyL. Carretera Burgos, km 119. 47071. Valladolid. Spain.

[d]ETSIAAB. UPM. Avda-Puerta de Hierro, 2-4. 280040. Madrid, Spain

Corresponding author: antoni.dalmau@irta.es; IRTA. Veinat de Sies S/N. 17121. Monells. Spain. Telephone: +34 972 63 00 52

Abstract

The present study was divided in two different trials. The aim of the first trial was to determine if the thresholds of detection of skatole and indole are achieved in females and in males vaccinated against the GnRF housed in two different type of floors and subject to control or high environmental temperatures. The aim of the second trial was to assess the effect of sire (Duroc crossbreed and Pietrain crossbreed) and heat stress on the concentration of skatole and indole in entire males. In the first trial, the animals subjected to heat stress on a concrete floor were found to be dirtier and to present higher skatole and indole concentrations than did animals from the control treatment in 100% slatted floors. In the second trial, although the animals were dirtier when subjected to high temperatures, no effect of the temperature was found in skatole/indole concentrations. The Duroc pigs were dirtier and had higher skatole and indole concentrations than did Pietrain pigs. It is concluded that even females or vaccinated males can reach values of skatole/indole close to the thresholds of sensory detection under conditions of dirtiness and heat stress. However, the relationship between heat, dirtiness and skatole/indole concentrations in fat were not confirmed in trial 2 using entire males.

Key Words

Dirtiness, Duroc, skatole, heat stress, indole, Pietrain

Funding: This work was supported by the Spanish National Institute of Agricultural Research (INIA-RTA2013-00090-CO2) and the CERCA Programme / Generalitat de Catalunya

1. Introduction

Pigs reared for meat production are particularly sensitive to high environmental temperatures mainly due to: 1) the inability of the species to sweat and 2) an increased capacity to produce heat in comparison to their ancestors (wild boar) promoted/enhanced by the genetic selection performed for a great muscular growth (Bellego et al., 2002). Pigs use few strategies to dissipate the heat and most of them are behavioral, such as panting or bathing in fresh (wet) zones to increase evaporative heat loss (Aragogo et al., 1999). However, in intensive farms the dunging area is the only wet place within the pens, so animals get dirty with faeces to reduce body temperature (Olsen et al., 2001).

Skatole and indole are volatile compounds. The only difference between these two components is that indole does not have a methyl group (CH_3). In fact, some authors suggest that indole and skatole concentrations are highly correlated due to the close relationship and similarity between them (Annor-Frempong et al., 1997). They are synthesized in the large intestine by bacterial degradation of tryptophan, exhibiting a faecal-like and naphthalene odour (Vold, 1970). Besides androstenone these two malodorous compounds are believed to contribute to boar taint (Annor-Frempong et al., 1997). In fact, androstenone, which is not present in female and surgically or immuno-castrated males (Dunshea et al., 2001), is hypothesized to inhibit the elimination of skatole (Squires and Lundström, 1997; Babol et al., 1998a, 1998b; 1999), thus enhancing the animal´s sexual odour in entire males.

A part of the skatole/indole is excreted with the faeces, whereas the remaining part is absorbed through the gut-wall. Hansen et al. (1994) suggested that skatole can be lowered by keeping pigs clean and according to Hansen et al. (1991), the effect of dirtiness on the skatole concentration could be also observed in females and castrated males, and not only in entire males. However, the relationship between skatole and

dirtiness is controversial, according to other studies (Aluwé et al., 2011; Bekaert et al., 2012). On the other hand, other factors, such as the stress associated with transportation, have been related to high levels of skatole in entire males (Wesoly et al., 2015). In fact, Claus et al. (1994) considered stress as a modulator for the formation and accumulation of skatole.

Although there is some debate regarding the actual threshold concentrations of skatole and indole that are detectable as taint, the values below 0.05 µg/g fat for both are commonly accepted as out of the range of detection even for trained evaluators (Font i Furnols et al., 2000).

The present study is divided in two different trials. The aim of the first trial is to determine if the thresholds of detection of skatole and indole are achieved in females and males vaccinated against the GnRF housed in two different type of floors (30% vs 100% slatted) and subjected to control or high environmental temperatures. The aim of the second trial is to assess the effect of sire (Duroc and Pietrain) and environmental conditions (control vs. high temperatures) on the concentration of skatole and indole in entire males.

2. Material and Methods

2.1. Trial 1

2.1.1. Animals and experiment design

One hundred and twenty eight pigs in the growing-finishing period (64 females and 64 males) were used. All pigs, a three-breed cross of Large White x Landrace and Pietrain, were reared up to 28 kg ± 0.4 kg (85 days old) in a commercial farm and then transported to IRTA facilities in Monells (Girona, Spain), an experimental farm similar

94 to the commercial one where the trial was performed. Males were vaccinated against the
95 Gonadotropin-releasing factor (GnRF) at Weeks 6 and 9 after arrival at the experimental
96 farm by subcutaneously injecting 2 mL of Improvac® (Zoetis, Spain).

97 The experiment was carried out during summer-autumn 2014 using a three-
98 factorial design : a) two environmental temperatures: control (from 11ºC to 25°C) and
99 heat stress (above 30°C 6 hours per day; from 10:00 h to 16:00 h and from 20ºC to 25ºC
100 the rest of the day, Relative Humidity: 40%-60%), b) two types of floor: totally slatted
101 floor (100% slatted, 18mm slatted and 80mm concrete, each 98mm) and partially slatted
102 concrete floor (30% slatted, 18mm slatted and 80mm concrete, each 98mm) and c) two
103 genders, as 4 males and 4 females were housed in each pen. At halfway through the trial
104 (Week 7) the floor of the pens was cleaned following the usual management practices in
105 the regional commercial farms. Feed and water was provided *ad libitum*. A total of 16
106 pens distributed in four rooms (A, B, C and D) were used, with a total space allowance
107 of 6.75 m^2 per pen (approximately 0.84 m^2 per pig). Rooms A and B had 30% slatted
108 floors and rooms C and D were 100% slatted. In addition, Rooms A and C were subjected
109 to control temperatures and Rooms B and D to heat stress after ten days of adaptation to
110 the facilities. Pens were numbered in all cases from 1 to 4, and each pen contained 8 pigs
111 (individually marked from 1 to 8). In each pen four males (numbered from 1 to 4) and
112 four females (numbered from 5 to 8) were housed.

113 Animals were also distributed in a balanced way according to their initial weight.
114 Air temperature (AT) and relative humidity (RH) were measured at 1.5 m above the floor
115 level in the feeding path, using a thermo hygrometer (HygroLog, Rotronic Hygromer TM
116 C94, sensors Pt100 RTD (1/3 DIN), Switzerland). AT and RH were recorded every five
117 minutes.

118 2.1.2. Performance and stress assessment

At the beginning (previous to the start of the project) and at the end, a fresh blood sample was taken from all animals for a complete hemogram to calculate the neutrophil/lymphocyte ratio as an indicator of stress (Puppe et al., 1997). Animals were weighed at the beginning and at the end of the study.

2.1.3. Dirtiness

The dirtiness of each animal was daily determined, from their arrival until four weeks before slaughter, based on the Welfare Quality Protocol (Welfare Quality®, 2009). This consisted of individually scoring the animals by using a 3-point scale ranging from 0 to 2, scoring '0' when the animal had less than 20% of the body side dirty with manure, '1' if between 20% and 50% of the body side was dirty and '2' when over 50% of the body side had manure.

2.1.4. Slaughter procedures and skatole/indole assessment

Once the animals reached 100 kg ± 10 kg live weight, which occurred at Week 12 after arrival, they were transported a distance of 140 km to a commercial abattoir. They spent around 4-h in the lairage pens, allocated in four groups of 32 animals each. Pigs within the same farm origin room were mixed, but not animals from different rooms. The pigs were stunned by application of CO_2 at high concentrations (90% for 2 minutes and a half) and slaughtered according to common commercial practices.

Subcutaneous fat samples from the dorsal neck region were taken to measure skatole and indole concentration. One-hundred and nineteen samples were finally analysed, this subsample included animals from all of the different treatments and both genders. Skatole and indole concentrations were determined in adipose tissue by HPLC

(García-Regueiro and Rius, 1998). The concentration was expressed as µg of skatole or indole in g of adipose tissue.

2.2. Trial 2

2.2.1. Animals and experiment design

Eighty nine pigs in the growing-finishing period were used. They were reared up to 17 kg ± 0.9 kg (65 days old) in a commercial farm and then transported to the facilities of ITACyL in Hontalbilla (Segovia, Spain) where the trial was performed. The experiment was carried out during summer-autumn 2015 using a factorial experimental model of 2x2, considering: a) two environmental temperatures: control (22ºC-23ºC) and heat stress (6ºC-8º C higher; 28ºC-31ºC) with a RH of 40%-50% and b) two genetic origins: 43 Duroc x (Large White x Landrace) (23 in control and 20 in heat stress) and 46 Pietrain x (Large White x Landrace) (23 in control and 23 in heat stress) entire males. In both cases, piglets came from a pool of five different males and 9 to 10 different females (a maximum of five animals per litter were selected for the trial). Feed and water was provided *ad libitum*. A total of 22 pens distributed in four rooms were used (11 from each genetic origin), with a total space allowance of 1.4 m^2 per pig. Pens were numbered and contained from four to five pigs. The floor was a 100% concrete type with 5 to 10 cm of added straw, cleaned twice per week by taking out the fresh faeces and replacing the straw, and a more thorough cleaning was applied 4 to 5 weeks before the end of the trial, removing any dry faecal residue. Animals were also distributed in a balanced way according to their initial weight. Air temperature (AT) and relative humidity (RH) were measured throughout the experimental period.

2.2.2. Performance and stress assessment

167 At the beginning (previous to the start of the heat treatment) and at the end, two
168 samples of saliva (one in the morning, from 09:00 to 10:00h, and one in the afternoon,
169 from 16:00 to 17.00h, the same day) were taken for cortisol analysis. Animals were
170 weighed at the beginning and at the end of the study.
171
172 2.2.3. Dirtiness
173 The dirtiness of each animal was measured once per week over the last eight
174 weeks before slaughter based on the Welfare Quality Protocol (Welfare Quality®, 2009),
175 with the same scoring system as in Trial 1.
176
177 2.2.4. Slaughter procedures and skatole/indole assessment
178 Once the animals reached 107 kg± 11.2 kg live weight, which occurred at Week
179 16 after arrival, they were transported a distance of 225 km to a commercial abattoir. They
180 spent around 3 h in the lairage pens, allocated in two groups of 43-46 animals each and
181 with a total fasting time of 16 hours. The pigs were stunned by application of CO_2 and
182 slaughtered according to common commercial practices.
183 Subcutaneous fat samples from the rump region (next to the lower back) were
184 taken to measure skatole and indole concentration, and a subsample of fifty-three animals
185 was analysed. This subsample was randomly selected within each treatment (28 Duroc
186 (14 control and 14 heat) and 25 Pietrain (13 control and 12 heat)). Skatole and indole
187 concentration was determined in adipose tissue by HPLC (García-Regueiro and Rius,
188 1998). The concentration was expressed as µg of skatole or indole in g of adipose tissue.
189
190 2.3. Statistical analysis

Statistical analyses were performed by means of the Statistical Analysis System (SAS) (SAS 9.1; software SAS Institute Inc: Cary, NC). Skatole, indole, cortisol in saliva, neutrophil/lymphocytes ratio, body weight and carcass weight were analysed using the PROC MIXED procedure. In Trial 1, the models accounted for the effects of environmental temperature (heat stress vs control), floor type (30% slatted vs 100% slatted), gender (males vs females) and possible interactions. In Trial 2, the models accounted for the effects of environmental temperature (heat stress vs control), genetics (Duroc vs Pietrain) and possible interactions. For salivary cortisol, the moment of the day (morning or afternoon) was also considered. In all cases, the origin pen was included as a random effect in the statistical models. The residual maximum likelihood was used as a method of estimation and the least square means of fixed effects (LSMEANS) was used to carry out multiple comparisons.

A PROC GLIMMIX with binomial distribution was used to analyse the dirtiness in animal´s body for each one of the three categories separately (scores 0, 1 and 2). In this case, the environmental temperature, floor type, gender, their interactions and the week effect were included in the model of Trial 1, and the environmental temperature, genetics, their interactions and the week effect were included in the model of Trial 2. In all cases, the origin pen was included as a random effect in the models. The correlation between skatole and indole was assessed by means of the Proc CORR procedure of SAS. The correlation between Skatole/indole and dirtiness was assessed by means of the Proc CORR SPEARMAN. Significance was fixed at $P < 0.05$ in all cases.

The experiment (Trials 1 and 2) was conducted in compliance with the Spanish guidelines for Use of Animals in Research, and the protocol was approved by the Ethical Animal Committees (IACUC) of IRTA (Barcelona, Spain) and ITACyL (Valladolid, Spain).

3. Results

3.1. Trial 1

3.1.1. Performance and neutrophil/lymphocyte ratio as stress indicator

The neutrophil/lymphocyte ratio was not different between treatments, types of floor or gender at the beginning of the trial, but at the end, an interaction between treatment and type of floor was found ($P = 0.010$). The animals from the stress room with 100% slatted floors had a higher ratio than did the animals from the control room with 100% slatted floors and the animals from the stress room with 30% slatted floors (Figure 1).

An effect of the treatment (control vs heat) was found for body weight (control: 109.0 kg ± 1.10 kg; heat: 100.6 kg ± 1.27 kg; $P < 0.001$) but not for carcass weight (control: 91.0 kg ± 1.16 kg; heat: 88.5 kg ± 1.30 kg; $P > 0.05$). An effect of floor type was found for body weight (30% slatted: 106.4 kg ± 1.21 kg; 100% slatted: 103.0 kg ± 1.15 kg; $P = 0.039$) but not for carcass weight (30% slatted: 89.9 kg ± 1.16 kg; 100% slatted: 89.7 kg ± 1.16 kg; $P > 0.05$). An effect of gender was found for body weight (males: 108.2 kg ± 1.58 kg; females: 102.3 kg ± 1.43 kg; $P < 0.001$) and for carcass weight (males: 93.6 ± 1.22 kg; females: 85.9 ± 1.23 kg; $P < 0.001$).

3.1.2. Dirtiness

For scores 0 (clean animals) and 2 (very dirty animals), there was a treatment effect (control vs heat; $P < 0.001$ in both cases), a type of floor effect (30% vs 100% slatted; $P < 0.001$ in both cases), an interaction between the treatment and the floor type

239 ($P < 0.001$) and a week effect ($P < 0.001$), but no gender effect ($P > 0.05$). Animals were
240 dirtier in the heat stress than in control treatment, and in 30% slatted than in 100% slatted
241 (Figure 2). In general, the animals were dirtier the final weeks (4.1% and 20.3% of pigs
242 with a score of 2 for dirtiness in control or heat stress the last week, respectively) than the
243 first weeks of the trial (4.2% and 8.4% of pigs with a score 2 for dirtiness in control or
244 heat stress treatments the first week, respectively, Figure 3).

245

246 3.1.3. Skatole and indole

247 A treatment effect (control vs heat) was found for skatole ($P= 0.013$), being lower
248 in the control than in the high temperature environment (Table 1). In the case of indole,
249 an effect of treatment ($P = 0.013$), floor type ($P < 0.001$), and an interaction between
250 treatment and floor type was found ($P= 0.001$). The mean concentration of indole in the
251 animals from the heat room was higher than in the control room and higher as well in
252 those of the 30% slatted floor than in 100% slatted(Table 1). The highest value for indole
253 was found in the heat stress room with a 30% slatted floor (0.066 μ/g, Figure 4). The
254 correlation between skatole and indole was $r = 0.67$. No effect of gender was found,
255 females having mean values of 0.04 μ/g and 0.03 μ/g of skatole and indole, respectively,
256 and males having mean values of 0.04 μ/g for both. The correlations between dirtiness
257 and skatole ranged from 0.24 to 0.39 depending of the day inside the same week and the
258 correlations between dirtiness and indole ranged from 0.43 to 0.72 depending of the day
259 inside the same week.

260

261 3.2. Trial 2

262 3.2.1. Performance and cortisol as stress indicator

No treatment effect (control vs heat) or genetic effect was found for saliva cortisol concentrations at the beginning or at the end of the trial. However, differences were found between the basal and the final sampling ($P < 0.001$), being higher at the beginning (7.5 µg ± 0.23 µg cortisol/mg saliva) than at the end of the trial (6.1 µg ± 0.23 µg cortisol/mg saliva).

No treatment effect (control vs heat) was found for body weight (control: 106.9 kg ± 1.31 kg; heat: 107.0 kg ± 1.34 kg; $P > 0.05$) and carcass weight (control: 78.8 kg ± 1.00 kg; heat: 78.7 kg ± 0.98 kg; $P > 0.05$). No genetic effect was found for body weight (Duroc x (Large White x Landrace): 107.2 kg ± 1.39 kg; Pietrain x (Large White x Landrace): 106.6 kg ± 1.30 kg; $P > 0.05$) or carcass weight (Duroc x (Large White x Landrace): 78.9 kg ± 1.04 kg; Pietrain x (Large White x Landrace): 78.6 kg ± 0.97 kg; $P > 0.05$)

3.2.2. Dirtiness

For scores 0 (clean animals) and 2 (very dirty animals) there was a treatment effect (control vs heat; $P < 0.001$ in both cases), genetic effect ($P < 0.001$ in both cases), an interaction between the treatment and genetics ($P < 0.001$) and week effect ($P < 0.001$ in both cases). Animals were dirtiest in the heat stress conditions than in control treatment, and Duroc entire males were dirtier than were Pietrain entire males, with Duroc males under heat stress being the dirtiest animals (Figure 5). In relation to the week, animals assessed in the fourth week reached maximum dirtiness, with 82% and 24% of animals having a score of 2 for dirtiness in heat stress and control treatment, respectively, while, for the first and last week, the scores of 2 for dirtiness were 0% and 4.4% in control

286 conditions, respectively, and 53% and 45% in heat stress conditions, respectively (Figure
287 3).

288

289 3.2.3. Skatole and indole

290 Only one effect of genetic origin was found for skatole, (P= 0.037), being higher
291 in Duroc than Pietrain (Table 1). In the case of indole only one effect of genetic origins
292 was also found (P=0.002), being higher in Duroc than in Pietrain (Table 1). The
293 correlation between skatole and indole was r = 0.67.

294

295 **4. Discussion**

296 In both trials of the present study, it was confirmed that high environmental
297 temperature conditions increased dirtiness in intensive housed pigs. In addition, In Trial
298 1, it was confirmed that this effect was higher in older animals and when they were housed
299 on concrete floor. The temperatures applied to the treatment groups were in both trials
300 above the thermoneutral temperature for growing pigs (from 25ºC to 31ºC; Bruce and
301 Clark, 1979). In Trial 1, the neutrophil/lymphocyte ratio was used as the indicator of
302 chronic stress (Puppe et al., 1997) and the results showed that the high environmental
303 temperatures were only stressful for the animals housed in 100% slatted floor. However,
304 in all cases the values of each treatment for neutrophil and lymphocyte were into the
305 ranges described for healthy finishing pigs (Elbers et al., 1992). In fact, in the present
306 study the values for neutrophil ranged from 32% to 38% of the total leucocytes and those
307 for lymphocyte ranged from 52% to 61% of the total leucocytes, Elbers et al., (1992)
308 described a mean of 33.6% (ranging from 18 to 50%) for neutrophil and 62.3% (ranging
309 from 46 to 79%) for lymphocyte. In Trial 2, the salivary cortisol was used as indicator of

310 acute stress (Becker et al., 1985; Breinekova et al., 2007) and did not show a significant
311 temperature effect, at least at the end of the study, when skatole/indole concentrations
312 could be more affected. Therefore, more clearly in Trial 2, and associated with the floor
313 type in Trial 1, the animals were able to cope with the stressor (environmental temperature
314 increase) with just changes in their behaviour before having any physiological effect. In
315 Trial 1, this physiological stress could be observed only in the animals with a 100% slatted
316 floor and high environmental temperatures. Heat stress has an effect on dirtiness because
317 animals tend to dissipate the heat by lying in their faeces and urine to perform a
318 temperature exchange with the environment (Aragogo et al., 1999; Olsen et al., 2001).
319 Pedersen and Ravn (2008), studying four different types of floor, concluded that solid
320 floors had the highest risk of contamination by faeces, requiring much more cleanliness
321 management than for slatted ones. However, one advantage of solid flooring under heat
322 stress conditions is that it provides the animals with a humid surface that helps them to
323 lose temperature and, in consequence, cope better with high environmental temperatures,
324 as happens in the present study in Trial 1 when a 70% concrete floor is compared with a
325 100% slatted floor.

326 The results obtained in the first trial with the skatole/indole concentrations were,
327 perhaps, caused by the dirtiness observed on the animals. However, the relationship
328 between the dirtiness of animals and the concentrations of skatole and indole in fat is very
329 controversial. In fact, it is described that the presence of skatole in the fat tissue it is
330 related to the potential of pigs to metabolise it (Zamaratskaia et al., 2006), being
331 eliminated from the porcine body in the form of several metabolites (Diaz and Squires,
332 2003), and also related to nutritional factors since it is hypothesized that feed ingredients
333 can reduce skatole levels by affecting the rate of skatole production or the absorption of
334 skatole (i.e. supplementing with chicory, inulin or fructooligosaccarides; Aluwé et al.,

2017). Besides,studies with radioactive skatole (Baek et al., 1997) confirmed the absorption of this compound through the skin. Hansen (1998) also reported that skatole can be absorbed through the skin and/or lungs, and Hansen et al. (1994) suggested that skatole can be lowered by keeping pigs clean. However, Aluwé et al. (2011) did not find clear indications towards skatole reduction by improving the cleanliness of pigs and Bekaert et al. (2012), found only a weak correlation between the concentration of skatole in fat with the extent of soiling in 18-week-old male pigs, but not at the other ages. In the present study, the correlation between dirtiness and skatole ranged from 0.24 to 0.39, being higher in the case of indole, ranging from 0.43 to 0.72 depending of the day of the dirtiness assessment. Gibis (1994) described the highest concentrations of skatole in animals slaughtered in the summer rather than in winter. Jensen and Jensen (1998) also described a higher presence of skatole and indole when animals were reared at 38ºC instead of at 15ºC, by far a higher range of temperature than those established in the present study. Nevertheless, other factors, such as the stress associated with transportation, have been related to high levels of skatole in entire males (Wesoly et al., 2015). In fact, Claus et al. (1994) already considered stress as a modulator for the formation and accumulation of skatole. Therefore, it could be concluded that under conditions of heat stress, it is this stress, and not the dirtiness of the animals, which is the factor producing an increase in indoles. Nevertheless, in Trial 1, a higher neutrophil/ lymphocyte ratio was found as indicator of chronic stress in the cleanest rather than in the dirtiest animals, the skatole/indole being higher in the dirtiest ones rather than in the cleanest. Therefore, in this case stress was not associated with the skatole/indole concentrations found.

Some authors suggest that indole can be predicted from skatole due to the close relationship and similarity between them (Annor-Frempong et al. 1997). However, the

correlation between them was only moderately high (0.67), being exactly the same in both trials. Although there is some debate regarding the actual threshold concentrations of skatole and indole that are detectable as taint, the values below 0.02 µg/g of fat for both are commonly accepted as out of the range of detection even for experts, and values higher than 0.05 µg/g are considered as detectable by untrained consumers (Desmoulin et al., 1982; Bonneau et al., 1992; Bonneau, 1998, Font i Furnols et al., 2000; Font i Furnols et al., 2003). Therefore, the levels of skatole and indole found in Trial 1 are close to the threshold considered as detectable (In fact 36 animals had values above 0.05 µg/g and three even above 0.10 µg/g). In consequence, although this should be confirmed by means of a consumer panel, it is suspected that the dirtiest animals of the present work, even being females or vaccinated against the GnRF males, had the risk to produce rejection in the consumers due to boar taint. This is especially important in the case of animals housed on a 30% slatted floor for the indole component, where mean values of 0.6 µg per fat gram were reached. The results found are in accordance with those reported by Hansen et al. (1991), where the effect of dirtiness on the skatole concentration could be observed in females and castrated males and not only in entire males. However, it is also true that the levels of skatole/indole in Trial 2, where entire males were reared, are by far higher than the levels found in the animals of Trial 1 (maximum mean level found in Trial 1 being 0.044 and minimum mean level found in Trial 2 being 0,12 µg of skatole/g of fat). This is not surprising, as androstenone, which is not present in females and surgically or immune-castrated males (Dunshea et al., 2001), may inhibit the elimination of skatole (Babol et al., 1999), enhancing the animal´s sexual odor in entire males. In fact, Font i Furnols et al. (2012) define the concentration reduction of skatole as one of the effects of the vaccination against GnRF in male pigs. Accordingly, in the present study,

384 no differences were found between females and vaccinated males in skatole/indole
385 concentrations.

386 On the other hand, in Trial 2 a breed effect in skatole/indole concentration was
387 found, with the Duroc males having higher levels than Pietrain males. In fact, Duroc is a
388 genetic line already described with a tendency for high levels of androstenone compared
389 as with other genetic lines (Squires and Lou, 1995; Xue et al., 1996; Hortóset al. 2000).
390 Therefore, an effect on skatole levels was expected due to the inhibition of its elimination
391 in the liver (Squires and Lundström, 1997; Babol et al., 1998a, 1998b; Babol et al., 2004).
392 In addition, the Duroc males were also dirtier than were Pietrain males, and it is not
393 possible to ascertain which of the two factors (genetics or dirtiness) had a greater effect
394 on skatole/indole concentrations. As both types of animals were housed in the same
395 conditions (concrete floor with 5 to 10 cm of straw) and the dirtiest animals were the
396 Duroc males in conditions of high environmental temperature, this suggests that these
397 animals had more difficulties in coping with the environmental temperatures than did
398 Pietrain males. However, here, again, it seems that animals could have adapted to the
399 situation by behavioural changes before any effect was found at a physiological level, at
400 least in terms of cortisol levels in saliva or even in performance. Finally, in Trial 2,
401 although a clear effect of dirtiness was found in relation to the heat treatment, the same
402 treatment effect was not found for skatole/indole concentrations. The reason could be the
403 high percentage of very dirty animals in the control room in Trial 2 four to five weeks
404 before slaughtering the animals (See Figure 3), as the percentages (22% and 24%) were
405 similar to the mean values found in the heat stress room of Trial 1 (19.3%) four to five
406 weeks before slaughtering them. In general, the dirtiness of pigs in Trial 2 was higher
407 than it was in Trial 1, but several confounding factors (i.e. straw bedding, entire males vs

408 females or vaccinated against GnRF males, different breeds) impede us to make a
409 conclusion about this difference.

410 **5. Conclusions**

411 In Trial 1, the applied heat affected the performance and the physiological state of
412 the animals. In Trial 2, neither performance nor physiological state were affected by the
413 applied heat treatment. However, in both cases, the animals were dirtier when subjected
414 to high environmental conditions than to control conditions. This would confirm that
415 changes in behaviour (i.e. lying on the faeces) and their consequences (dirtiness, studied
416 in the present work), may be first before any other change in physiological parameters or
417 performance. Although a possible relationship between dirtiness and skatole/indole
418 concentrations could be inferred from some of the results obtained in the present work,
419 the lack of differences between heat treatments in Trial 2 does not allow for this
420 hypothesis to be entirely confirmed.

421

422 **6. Acknowledgements**

423 Thanks are due to Joaquim Soler, Albert Fontquerna and Carlos Millán for their
424 help in taking care of the pigs. This project was funded by the Spanish Research
425 Program, INIA (RTA2013-00090-C02).

426

427 **7. References**

428 Aluwé, M., Bekaert, K.M., Tuyttens, F.A.M., Vanhaecke, L., De Smet, S., De Brabander,
429 H.F., De Brabander, D.L., Millet, S., 2011. Influence of soiling on boar taint in boars.
430 Meat science. 87, 175-179.

Aluwé, M., Heyrman, E., Theis, S., Sieland, C., Thurman, K., Millet, S., 2017. Chicory fructans in pig diet reduce skatole in back fat of entire male pigs. Research in Veterinary Science. 115, 340-344.

Annor-Frempong, I.E., Nute, G.R., Whittington, F.W., Wood, J.D., 1997. The problem of taint in pork – III. Odour profile of pork fat and the interrelationship between androstenone, skatole and indole concentrations. Meat Science. 47, 63-76.

Aragogo, J., Zhang, R.H., Riskowski, G.L., Christianson, L.L., Day D.L., 1999. Mass transfer coefficient of ammonia in liquid swine manure and aqueous solutions. Journal of Agricultural Engineering Research. 73, 77-86.

Babol, J., Squires, E.J., Lundström, K., 1998a. Relationship between oxidation and conjugation metabolism of skatole in pig liver and levels of skatole in fat. Journal of Animal Science. 76, 829-838.

Babol, J., Squires, E.J., Lundström, K., 1998b. Hepatic metabolism of skatole in pigs by cytochrome P450IIE1. Journal of Animal Science. 76, 822-828.

Babol, J., Squires, E.J., Lundstrom, K., 1999. Relationship between metabolism of androstenone and skatole in intact male pigs. Journal of Animal Science. 77, 84-92.

Babol, J, Zamaratskaia, G, Juneja, R.K, Lundstrom, K., 2004. The effect of age on distribution of skatole and indole levels in entire male pigs in four breeds: Yorkshire, Landrace, Hampshire and Duroc. Meat Science. 67, 351-358.

Baek, C., Hansen-Moller. J., Friss. C., Cornett. C., Hansen, S.H., 1997. Identification of selected metabolities of skatole in plasma ans urine from pigs. Journal of Agricultural Food Chemistry. 45, 2332-2340.

Becker, B.A., Ford, J.J., Christenson, R.K., Manak, R.C., Hahn, G.L., Deshazer, J.A., 1985. Cortisol response of gilts in tether stalls. Journal of Animal Science. 60, 264-270.

455 Bekaert, K.M, Aluwé, M., Millet, S., Goethals, K., Nijs, G., Isebaert, S., De Brabander,
456 D.L., Verheyden, K., De Brabander, H.F., Vanhaecke, L., Tuyttens F.A.M., 2012.
457 Predicting the likelihood of developing boar taint: Early physical indicators in entire
458 male pigs. Meat Science. 92, 382-385.

459 Bellego, L., Van milgen, J., Noblet J., 2002. Effect of high temperature and low-protein
460 diets on the performance of growing-finishing pigs. Journal of Animal Science. 80, 691-
461 701.Bonneau, M., Le Denmat, M., Vaudelet, J.C., Veloso Nunes, J.R., Mortensen, A.B.,
462 Mortensen, H.P., 1992. Contributions of fat androstenone and skatole to boar taint: I.
463 Sensory attributes of fat and pork meat. Livestock Production Science. 32, 63-80.

464 Bonneau, M., 1998. Use of entire males for pig meat in the European Union. In:
465 *Proceedings of the 44th ICOMST*, p.192-205, Barcelona, Spain, 1998.

466 Breinekova, K., Svoboda, M., Smutna, M., Vorlova L., 2007. Markers of acute stress in
467 pigs. Physiological Research. 56, 323–329.

468 Bruce, J.M, Clark, J.J., 1979. Models of heat production and critical temperatures for
469 growing pigs. Journal of Animal Science. 28, 353-369.

470 Claus, R., Weiler, U., Herzog A., 1994. Physiological aspects of androstenone and
471 skatole formation in the boar—A review with experimental data. Meat Science. 38,
472 289–305.

473 Desmoulin, B., Bonneau, M., Frouin, A., Bidard J.P., 1982. Consumer testing of pork and
474 processed meat from boars: the influence of fat androstenone level. Livestock Production
475 Science. 9, 707-715.

476 Diaz, G. J., Squires, E. J., 2003. Phase II in vitro metabolism of 3methylindole
477 metabolites in porcine liver. Xenobiotica. 33, 485–498.

478 Dunshea, F.R., Colantoni, C., Howard, K. M.C., Cauley, I., Jackson, P., Long, K.A.,
479 Lopaticki, S., Nugent, E.A, Simons, J.A., Walker, J., Hennessy, D.P., 2001. Vaccination
480 of boars with a GnRH vaccine (Improvac) eliminates boar taint and increases growth
481 performance. Journal of Animal Science. 79, 2524-2535.

482 Elbers, A.R.W., Counotte, G.H.M., Tielen, M.J.M., 1992. Haemtological and
483 clinicochemical blood profiles in slaughter pigs. Veterinary Quarterly, 14: 57-62.

484 Font-i-Furnols, M., Gispert, M., Diestre, A., Oliver, M.A., 2000. Sensory characterization
485 of boar taint in entire male pigs. Journal of Sensory studies. 15, 393-409.

486 Font i Furnols, M., Gispert, M., Diestre, A., Oliver, M.A., 2003. Acceptability of boar
487 meat by consumers depending on their age, gender, culinary habits, and sensitivity and
488 appreciation of androstenone odour. Meat science. 64, 433-440.

489 Font-i-Furnols, M., Gispert, M., Soler, J., Diaz, M., Garcia-Regueiro, J.A., Diaz, I.,
490 Pearce, M.C., 2012. Effect of vaccination against gonadotrophin-releasing factor on
491 growth performance, carcass, meat and fat quality of male Duroc pigs for dry-cured
492 ham production. Meat Science. 81, 148-154.

493 García-Regueiro, J.A., Rius, M.A., 1998 Rapid determination of skatole and indole in pig
494 back fat by normal-phase liquid chromatography. Journal Chromatography. 809, 246-
495 251.

496 Gibis, M., 1994. *Einfluss der Substanzen Indol und Skatol auf die Schweinefleischqualität*
497 *(Influence of substance indole and skatole on meat quality)*. Doctoral thesis, Allgemeine
498 und Angewandte Naturwissenschaften der Universität Hohenheim, Germany

499 Hansen, L.L., Barton-Gade, P., Vorup, P., 1991. Effect of mixing "peaceful" or
500 aggressive" pigs at abattoirs on their behaviour and meat quality. Proceedings of the
501 international congress of the Society for Veterinary Ethology, twenty-fifth anniversary

1966-1991, on applied animal behaviour. Ed. Appleby, M.C., Horrel, R.I., Petherick, J.C., Rutter, S.M., p.88. Universities Federation for Animal Welfare.

Hansen, L.L., Larsen, A.E., Jensen, B.B., Hansen-Moller, J., Barton-Gade, P., 1994. Influence of stocking rate and faeces deposition in the pen at different temperatures on skatole concentration (boar taint) in subcutaneous fat. Animal Production. 59, 99−110.

Hansen, L. L., 1998. Influence of environmental factors and antibiotics on skatole in pigs. In W. K. Jensen (Ed.), Skatole and boar taint (pp. 137−150). Roskilde, Denmark: Danish Meat Research Institute.

Hortós, M., Rius, M. A., Devries, A., Lacoste, A., Gispert, M., Diestre, A., 2000. Variation on boar taint compounds in back-fat from divergent genetic lines. Proceedings of the 46th International Congress of Meat Science and Technology, vol. 1. (pp. 98–99) Buenos Aires, Argentina.

Jensen, B. B., Jensen, M.T., 1998. Microbial production of skatole in the digestive tract of entire male pigs. In: Jensen, W. K. (ed.), Skatole and Boar Taint, pp. 41–75. Danish Meat Research Institute, Roskilde.

Olsen, A.W., Dybkjaer, L., Simonsen, H.B., 2001. Behaviour of growing pigs kept in pens with outdoor runs. II Temperature regulatory behavior, comfort behabiour and dunging preferences. Livestock Production Science. 69, 265-278.

Pedersen, B., Ravn. P., 2008. Characteristics of floors for pig pens: friction, shock absorption, ammonia emission and heat conduction. Agricultural Engineering International: CIGR E journal X, Manuscript BC 08 005.

Puppe, B., Tuchscherer, M., Tuchscherer, A., 1997. The effect of housing conditions and social environment immediately after weaning on the agonistic behaviour,

525 netutrophil/lymphocyte ratio, and plasma glucose levels in pigs. Livestock Production
526 Science. 48, 157-164.

527 Squires, E.J., Lundström, K., 1997. Relationship between cytochrome P450IIE1 in liver
528 and levels of skatole and its metabolites in entire male pigs. Journal of Animal Science.
529 75, 2506-2511.

530 Squires, E.J., Lou, Y., 1995. Levels of boar taint in purebred entire male pigs in Canada.
531 *Proceedings of the EAAP Working Group on the Production and Utilization of Meat from*
532 *Entire Male Pigs*, Milton Keynes, U.K., Sept 27-29.

533 Vold, E., 1970. Fleischproduktionseigenschaften bei Ebern und Kastraten IV:
534 Organoleptische und gaschromatographische Untersuchungen wasserdampfflüchtiger
535 Stoffe des Rücken-speckes von Ebern. *Meldinger fra Norges Landbrugshogskole*, 49, 1-
536 15.

537 Wesoly, R., Jungbluth, I., Stefanski, V., Weiler, U., 2015. Pre-slaughter conditions
538 influence skatole and androstenone in adipose tissue of boars. Meat Science. 99, 60-67.

539 Xue, J., Dial, G.D., Holton, E.E., Vickers, Z., Squires, E.J., Lou, Y, Godbout, D., Morel,
540 N., 1996. Breed differences in boar taint: Relationship between tissue levels of boar taint
541 compounds and sensory analysis of taint. Journal of. Animal Science. 74, 2170-2177.

542 Zamaratskaia, G., Chen, G., Lundström, K., 2006. Effects of sex, weight, diet and hCG
543 administration on levels of skatole and indole in the liver and hepatic activities of
544 cytochromes P4502E1 and P4502A6 in pigs. Meat Science. 72, 331-338.

545

546

547

548

Figure 1. Neutrophil/lymphocyte ratio (mean ± S.E.) in blood samples of female and vaccinated against the GnRF male pigs subjected to high environmental temperatures (heat) or control in pens with 30%slat or 100%slat.

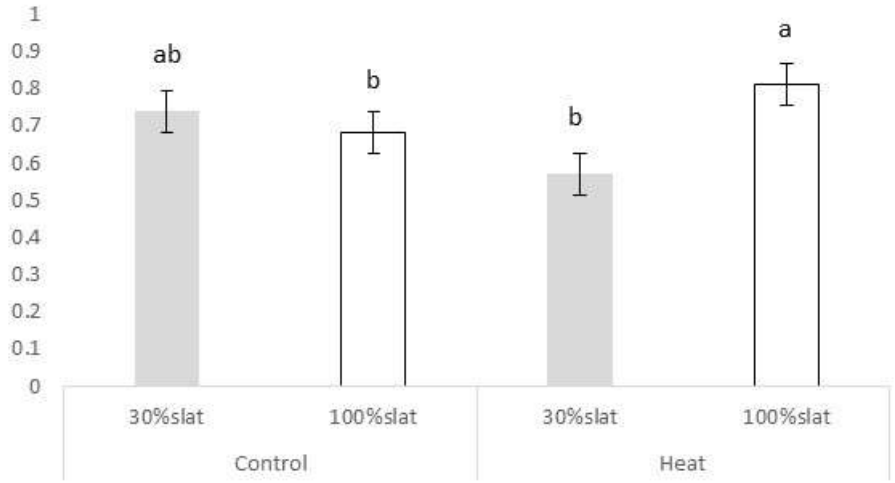
Figure 2. Percentage of animals with a score of 2 for dirtiness (more than 50% of the body soiled with faeces) at Week 10 of the trial when subjected to high environmental temperatures (heat) or control in pens with 30% slat or 100% slat.

Figure 3. Percentage of animals with a score of 2 for dirtiness (more than 50% of the body soiled with faeces) in control and heat stress pens in Trials 1 and 2 by week. In Trial 1, the assessments were carried out from Week 1 to Week 10, and in Trial 2 the assessments were carried out from Week 8 to Week 15.

Figure 4. Concentration (micrograms/gram of fat) of indole in females and vaccinated against the GnRF males subjected to high environmental temperatures (heat) or control temperatures in pens with 30% slat or 100% slat.

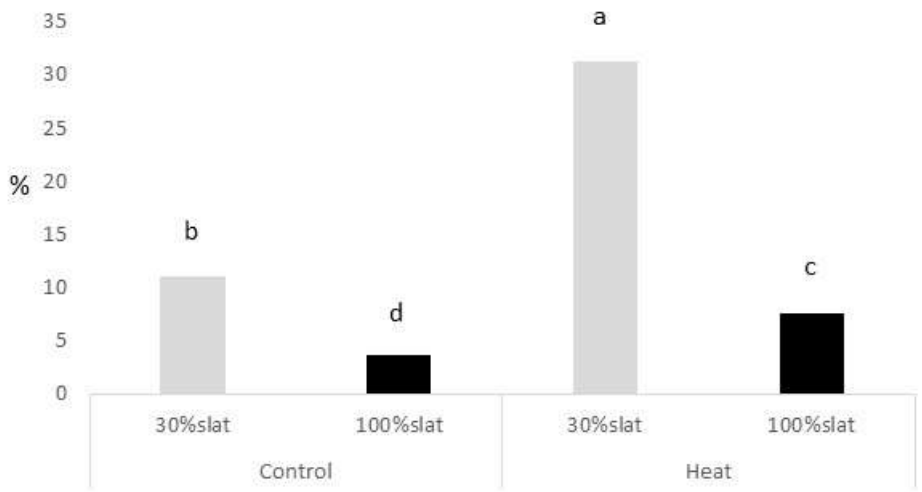
Figure 5. Percentage of animals with a score of 2 for dirtiness (more than 50% of the body soiled with faeces) in the last week of assessment when subjected to high environmental temperatures (heat) or control temperatures in Duroc and Pietrain pigs.

Figure 1



Different letter means differences at $p < 0.05$.

Figure 2



Different letter means differences at $p < 0.05$.

Figure 3

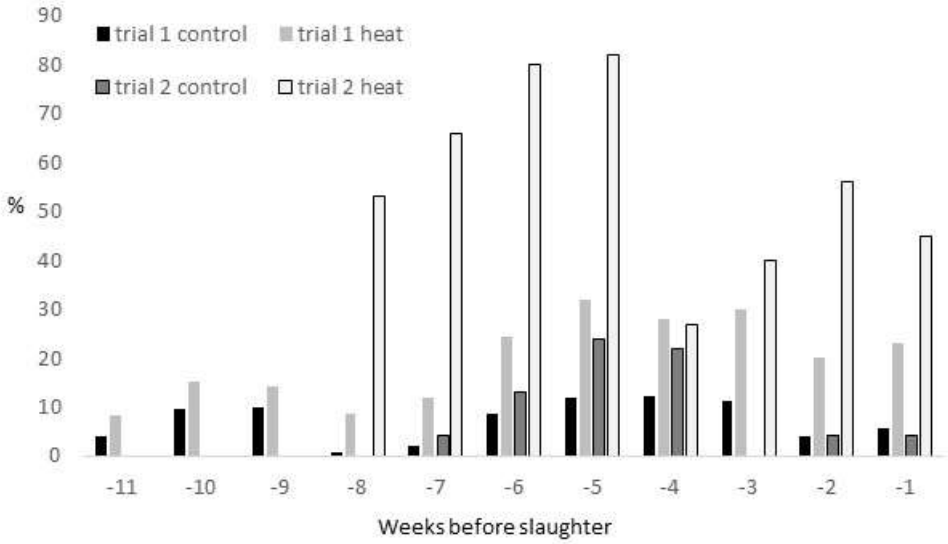
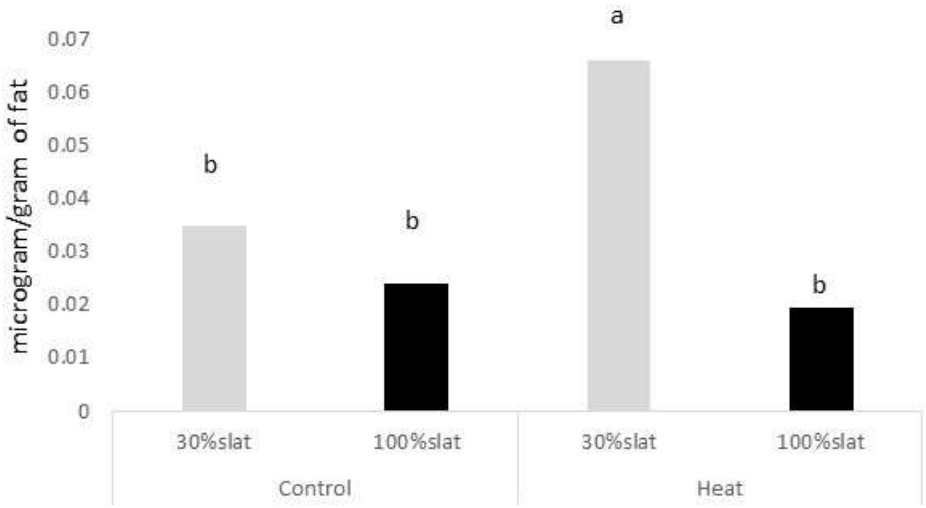
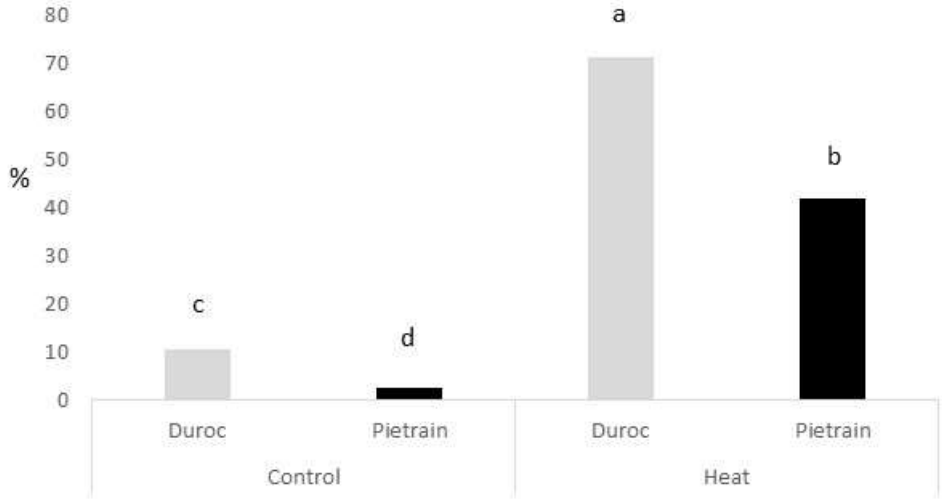

90
80
70
60
%
50
40
30
20
10
0
trial 1 control
trial 1 heat
trial 2 control
trial 2 heat
-11
-10
-9
-8
-7
-6
-5
-4
-3
-2
-1
Weeks before slaughter

Figure 4



Different letter means differences at $p < 0.05$.

628 **Figure 5**



629

630 Different letter means differences at $p < 0.05$.