Livestock Science 220 (2019) 46–51

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

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] ESTSIAAB-UPM. Avda-Puerta de Hierro, 2-4. 28040. Madrid. Spain

ARTICLE INFO

Keywords:
Dirtiness
Duroc
Skatole
Heat stress
Indole
Pietrain

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.

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 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 immunocastrated males (Dunshea et al., 2001), is hypothesized to inhibit the elimination of skatole (Squires and Lundström, 1997; Babol et al., 1998a,b, 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

* Corresponding author.
E-mail addresses: antoni.dalmau@irta.es, antoni.dalmau@irta.cat (A. Dalmau).

https://doi.org/10.1016/j.livsci.2018.11.021
Received 24 May 2018; Received in revised form 27 November 2018; Accepted 28 November 2018

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

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

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

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 min 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 2 × 2, considering: a) two environmental temperatures: control (22 °C–23 °C) and heat stress (6 °C-8 °C higher; 28 °C–31 °C) 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 9–10 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 5–10 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 4–5 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

At the beginning (previous to the start of the heat treatment) and at the end, two samples of saliva (one in the morning, from 09:00 to 10:00 h, and one in the afternoon, from 16:00 to 17.00 h, the same day) were taken for cortisol analysis. Animals were weighed at the beginning and at the end of the study.

2.2.3. Dirtiness

The dirtiness of each animal was measured once per week over the last eight weeks before slaughter based on the Welfare Quality Protocol (Welfare Quality®, 2009), with the same scoring system as in Trial 1.

2.2.4. Slaughter procedures and skatole/indole assessment

Once the animals reached 107 kg ± 11.2 kg live weight, which

occurred at Week 16 after arrival, they were transported a distance of 225 km to a commercial abattoir. They spent around 3 h in the lairage pens, allocated in two groups of 43–46 animals each and with a total fasting time of 16 h. The pigs were stunned by application of CO_2 and slaughtered according to common commercial practices.

Subcutaneous fat samples from the rump region (next to the lower back) were taken to measure skatole and indole concentration, and a subsample of fifty-three animals was analysed. This subsample was randomly selected within each treatment (28 Duroc (14 control and 14 heat) and 25 Pietrain (13 control and 12 heat)). Skatole and indole concentration was 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.

2.3. 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 (Fig. 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; $P < 0.001$).

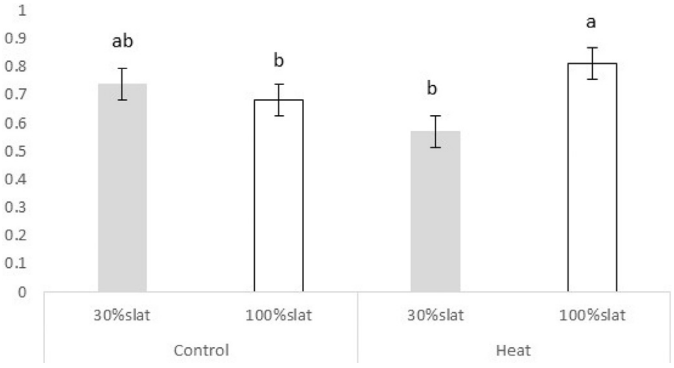

Fig. 1. Neutrophil/lymphocyte ratio (mean ± S.E.) in blood samples of female and vaccinated against the GnRF male pigs subjected to high environmental temperatures (heat) or control in pens with 30%slat or 100%slat.

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

3.1.3. Skatole and indole

A treatment effect (control vs heat) was found for skatole ($P = 0.013$), being lower in the control than in the high temperature environment (Table 1). In the case of indole, an effect of treatment ($P = 0.013$), floor type ($P < 0.001$), and an interaction between treatment and floor type was found ($P = 0.001$). The mean concentration of indole in the animals from the heat room was higher than in the control room and higher as well in those of the 30% slatted floor than in 100% slatted(Table 1). The highest value for indole was found in the heat stress room with a 30% slatted floor (0.066 μ/g, Fig. 4). The correlation between skatole and indole was r = 0.67. No effect of gender was found, females having mean values of 0.04 μ/g and 0.03 μ/g of skatole

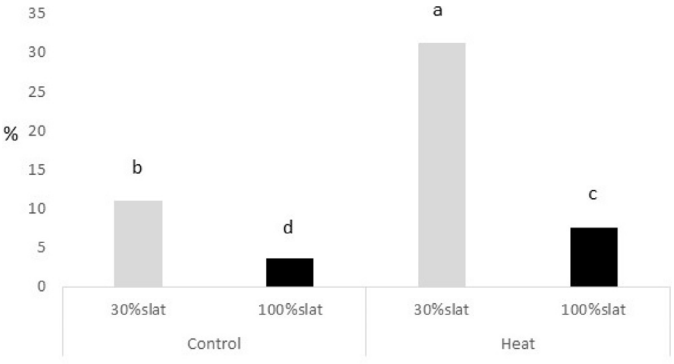

Fig. 2. Percentage of animals with a score of 2 for dirtiness (more than 50% of the body soiled with faeces) at Week 10 of the trial when subjected to high environmental temperatures (heat) or control in pens with 30% slat or 100% slat.

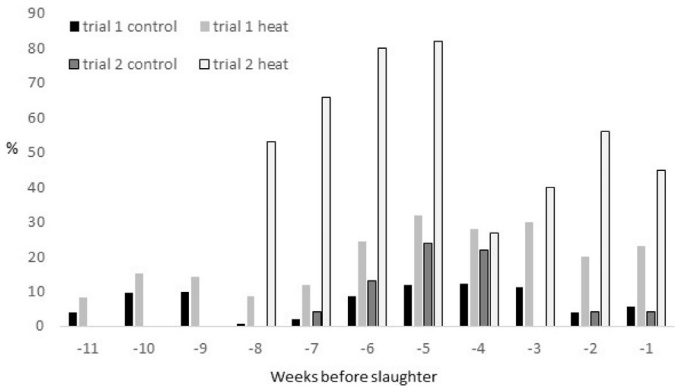

Fig. 3. Percentage of animals with a score of 2 for dirtiness (more than 50% of the body soiled with faeces) in control and heat stress pens in Trials 1 and 2 by week. In Trial 1, the assessments were carried out from Week 1 to Week 10, and in Trial 2 the assessments were carried out from Week 8 to Week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

Table 1
Skatole and indole concentrations (mean ± SE) in μg/g of fat obtained in 119 and 53 pigs in Trial 1 and 2, respectively.

			Skatole (μg/g)	Indole (μg/g)
Trial 1				
	Stress	Control	0.035[b] ± 0.0024	0.029[b] ± 0.0035
		Heat	0.044[a] ± 0.0024	0.046[a] ± 0.0034
	Floor type	30% slatted	0.047 ± 0.0024	0.051[a] ± 0.0034
		100% slatted	0.041 ± 0.0023	0.025[b] ± 0.0034
Trial 2				
	Stress	Control	0.133 ± 0.0141	0.070 ± 0.0077
		Heat	0.158 ± 0.0138	0.071 ± 0.0075
	Genetics	Duroc	0.166[a] ± 0.0137	0.088 ± 0.0076
		Pietrain	0.125[b] ± 0.0135	0.054 ± 0.0075

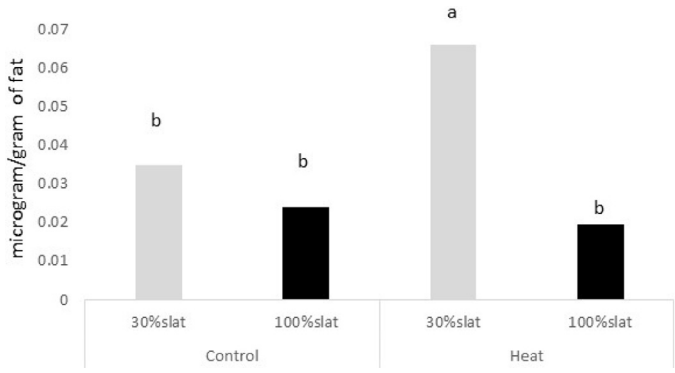

Fig. 4. Concentration (micrograms/gram of fat) of indole in females and vaccinated against the GnRF males subjected to high environmental temperatures (heat) or control temperatures in pens with 30% slat or 100% slat.

and indole, respectively, and males having mean values of 0.04 μ/g for both. The correlations between dirtiness and skatole ranged from 0.24 to 0.39 depending of the day inside the same week and the correlations between dirtiness and indole ranged from 0.43 to 0.72 depending of the day inside the same week.

3.2. Trial 2

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).

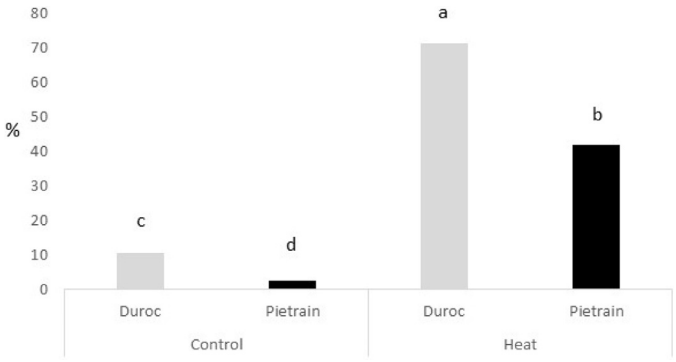

Fig. 5. Percentage of animals with a score of 2 for dirtiness (more than 50% of the body soiled with faeces) in the last week of assessment when subjected to high environmental temperatures (heat) or control temperatures in Duroc and Pietrain pigs.

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 (Fig. 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 conditions, respectively, and 53% and 45% in heat stress conditions, respectively (Fig. 3).

3.2.3. Skatole and indole

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

4. Discussion

In both trials of the present study, it was confirmed that high environmental temperature conditions increased dirtiness in intensive housed pigs. In addition, In Trial 1, it was confirmed that this effect was higher in older animals and when they were housed on concrete floor. The temperatures applied to the treatment groups were in both trials above the thermoneutral temperature for growing pigs (from 25 °C to 31 °C; Bruce and Clark, 1979). In Trial 1, the neutrophil/lymphocyte ratio was used as the indicator of chronic stress (Puppe et al., 1997) and the results showed that the high environmental temperatures were only stressful for the animals housed in 100% slatted floor. However, in all cases the values of each treatment for neutrophil and lymphocyte were into the ranges described for healthy finishing pigs (Elbers et al., 1992). In fact, in the present study the values for neutrophil ranged from 32%

to 38% of the total leucocytes and those for lymphocyte ranged from 52% to 61% of the total leucocytes. Elbers et al. (1992) described a mean of 33.6% (ranging from 18 to 50%) for neutrophil and 62.3% (ranging from 46 to 79%) for lymphocyte. In Trial 2, the salivary cortisol was used as indicator of acute stress (Becker et al., 1985; Breinekova et al., 2007) and did not show a significant temperature effect, at least at the end of the study, when skatole/indole concentrations could be more affected. Therefore, more clearly in Trial 2, and associated with the floor type in Trial 1, the animals were able to cope with the stressor (environmental temperature increase) with just changes in their behaviour before having any physiological effect. In Trial 1, this physiological stress could be observed only in the animals with a 100% slatted floor and high environmental temperatures. Heat stress has an effect on dirtiness because animals tend to dissipate the heat by lying in their faeces and urine to perform a temperature exchange with the environment (Aragogo et al., 1999; Olsen et al., 2001). Pedersen and Ravn (2008), studying four different types of floor, concluded that solid floors had the highest risk of contamination by faeces, requiring much more cleanliness management than for slatted ones. However, one advantage of solid flooring under heat stress conditions is that it provides the animals with a humid surface that helps them to lose temperature and, in consequence, cope better with high environmental temperatures, as happens in the present study in Trial 1 when a 70% concrete floor is compared with a 100% slatted floor.

The results obtained in the first trial with the skatole/indole concentrations were, perhaps, caused by the dirtiness observed on the animals. However, the relationship between the dirtiness of animals and the concentrations of skatole and indole in fat is very controversial. In fact, it is described that the presence of skatole in the fat tissue it is related to the potential of pigs to metabolise it (Zamaratskaia et al., 2006), being eliminated from the porcine body in the form of several metabolites (Diaz and Squires, 2003), and also related to nutritional factors since it is hypothesized that feed ingredients can reduce skatole levels by affecting the rate of skatole production or the absorption of 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 38 °C instead of at 15 °C, 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, 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, no differences were found between females and vaccinated males in skatole/indole concentrations.

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

5. Conclusions

In Trial 1, the applied heat affected the performance and the physiological state of the animals. In Trial 2, neither performance nor physiological state were affected by the applied heat treatment.

However, in both cases, the animals were dirtier when subjected to high environmental conditions than to control conditions. This would confirm that changes in behaviour (i.e. lying on the faeces) and their consequences (dirtiness, studied in the present work), may be first before any other change in physiological parameters or performance. Although a possible relationship between dirtiness and skatole/indole concentrations could be inferred from some of the results obtained in the present work, the lack of differences between heat treatments in Trial 2 does not allow for this hypothesis to be entirely confirmed.

Funding

This work was supported by the Spanish National Institute of Agricultural Research (INIA-RTA 2013-00090-C02-01 and RTA2013-00090-C02-02) and the CERCA Programme / Generalitat de Catalunya.

Conflict of interest statement

No one of the authors have any conflict of interest with the content of the paper.

Acknowledgements

Thanks are due to Joaquim Soler, Albert Fontquerna and Carlos Millán for their help in taking care of the pigs. This project was funded by the Spanish Research Program, INIA (RTA 2013-00090-C02-01 and RTA2013-00090-C02-02).

References

Aluwé, M., Bekaert, K.M., Tuyttens, F.A.M., Vanhaecke, L., De Smet, S., De Brabander, H.F., De Brabander, D.L., Millet, S., 2011. Influence of soiling on boar taint in boars. Meat Sci. 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. Res. Vet. Sci. 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 Sci. 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. J. Agricult. Eng. Res. 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. J. Anim. Sci. 76, 829–838.

Babol, J., Squires, E.J., Lundström, K., 1998b. Hepatic metabolism of skatole in pigs by cytochrome P450IIE1. J. Anim. Sci. 76, 822–828.

Babol, J., Squires, E.J., Lundstrom, K., 1999. Relationship between metabolism of androstenone and skatole in intact male pigs. J. Anim. Sci. 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 Sci. 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. J. Agricult. Food Chem. 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. J. Anim. Sci. 60, 264–270.

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

Bellego, L., Van milgen, J., Noblet, J., 2002. Effect of high temperature and low-protein diets on the performance of growing-finishing pigs. J. Anim. Sci. 80, 691–701.

Bonneau, M., Le Denmat, M., Vaudelet, J.C., Veloso Nunes, J.R., Mortensen, A.B., Mortensen, H.P., 1992. Contributions of fat androstenone and skatole to boar taint: I. Sensory attributes of fat and pork meat. Livestock Prod. Sci. 32, 63–80.

Bonneau, M., 1998. Use of entire males for pig meat in the European Union. In In: Proceedings of the 44th ICOMST. Spain. Barcelona, pp. 192–205 1998.

Breinekova, K., Svoboda, M., Smutna, M., Vorlova, L., 2007. Markers of acute stress in pigs. Physiol. Res. 56, 323–329.

Bruce, J.M, Clark, J.J., 1979. Models of heat production and critical temperatures for growing pigs. J. Anim. Sci. 28, 353–369.

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

Desmoulin, B., Bonneau, M., Frouin, A., Bidard, J.P., 1982. Consumer testing of pork and processed meat from boars: the influence of fat androstenone level. Livestock Prod. Sci. 9, 707–715.

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

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

Elbers, A.R.W., Counotte, G.H.M., Tielen, M.J.M., 1992. Haemtological and clinicochemical blood profiles in slaughter pigs. Vet. Q. 14, 57–62.

Font-i-Furnols, M., Gispert, M., Diestre, A., Oliver, M.A., 2000. Sensory characterization of boar taint in entire male pigs. J. Sens. Stud. 15, 393–409.

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

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

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

Gibis, M., 1994. Doctoral thesis. Allgemeine und Angewandte Naturwissenschaften der Universität Hohenheim, Germany.

on applied animal behaviourEdHansen, L.L., Barton-Gade, P., Vorup, P., 1991. Effect of mixing “peaceful” or aggressive” pigs at abattoirs on their behaviour and meat quality. In: Appleby, M.C., Horrel, R.I., Petherick, J.C., Rutter, S.M. (Eds.), Proceedings of the international congress of the Society for Veterinary Ethology, twenty-fifth anniversary 1966-1991. Universities Federation for Animal Welfare, pp. 88 on applied animal behaviourEd.

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. Anim. Prod. 59, 99–110.

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

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. In: Proceedings of the 46th International Congress of Meat Science and Technology. Buenos Aires, Argentina. 1. pp. 98–99.

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

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 Prod. Sci. 69, 265–278.

Pedersen, B., Ravn, P., 2008. Characteristics of floors for pig pens: friction, shock absorption, ammonia emission and heat conduction. Agricult. Eng. Int 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, neutrophil/lymphocyte ratio, and plasma glucose levels in pigs. Livestock Prod. Sci. 48, 157–164.

Squires, E.J., Lundström, K., 1997. Relationship between cytochrome P450IIE1 in liver and levels of skatole and its metabolites in entire male pigs. J. Anim. Sci. 75, 2506–2511.

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

Vold, E., 1970. Fleischproduktionseigenschaften bei ebern und kastraten IV: organoleptische und gaschromatographische untersuchungen wasserdampfflüchtiger stoffe des rücken-speckes von ebern. Meldinger fra Norges Landbrugshogskole 49, 1–15.

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

Xue, J., Dial, G.D., Holton, E.E., Vickers, Z., Squires, E.J., Lou, Y, Godbout, D., Morel, N., 1996. Breed differences in boar taint: relationship between tissue levels of boar taint compounds and sensory analysis of taint. J. Anim. Sci. 74, 2170–2177.

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