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Original article

Differences in skin characteristics in European (Large White) and Caribbean (Creole) growing pigs with reference to thermoregulation*

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Abstract – The effects of breed and season on histological skin characteristics were studied at the experimental facilities of INRA in Guadeloupe (16° Lat. N., 61° Long. W.) in two replicates using a total of 20 Creole (CR) and 20 Large White (LW) pigs. The first replicate was carried out during the warm season (i.e., between February and April) and the second during the hot season (i.e., between August and October). In the warm season, ambient temperature and relative humidity averaged 25.3 °C and 86.0%, respectively. The corresponding values for the hot season were 27.9 °C and 83.6%. At 90 kg BW, all pigs were slaughtered and a 2 to 4 cm² sample of cutaneous tissue from the back region (i.e., at the last dorsal rib level) was taken from each animal before scalding and dehairing. Slices were stained with trichrome blue staining and the thickness of the epidermis and dermis were determined. The density and surface of the sweat glands (SG) were also determined. The mast cell density was measured using slices stained with Giemsa. Epidermis thickness was not affected by breed or season and averaged 55 µm. However, dermis thickness was significantly higher in CR than in LW pigs (3.60 vs. 3.13 mm; $P < 0.01$) but it was not influenced by season (3.36 mm on average; $P = 0.17$). The density of SG was significantly higher in CR than in LW pigs (32.0 vs. 25.4 SG per mm²; $P < 0.01$) but their surface area was lower in CR than in LW pigs (106 vs. $263 \times 10^{-3} \mu\text{m}^2$ per mm²; $P < 0.01$). The SG density tended to be higher in the hot than in the warm season (30.4 vs. 26.9 SG per mm²; $P = 0.0971$). Mast cell density in the dermis was found to be higher in CR than in LW pigs (2.52 vs. 1.38 mast cells per mm²; $P < 0.01$). Irrespective of the breed, mast cell density was higher during the hot than the warm season (2.22 vs. 1.68 mast cells per mm²; $P < 0.01$). In conclusion, our study suggests that the differences in skin histology and/or sweat gland histometry could partly explain the better heat tolerance in CR pigs.

pigs / heat tolerance / breed / skin / histology

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Résumé – Les effets du type génétique et de la saison sur les caractéristiques histologiques de la peau ont été étudiés l'INRA de Guadeloupe (16° Lat. N., 61° Long. W.) sur un total de 20 porcs Créole (CR) et 20 porcs Large White (LW) répartis en deux répétitions. La première répétition s'est déroulée au cours de la saison fraîche (entre février et avril) et la seconde au cours de la saison chaude (entre août et octobre). En saison fraîche, la température et l'humidité relative moyenne étaient respectivement de 25,3 °C et 86,0 %. Les valeurs correspondantes pour la saison chaude étaient de 27,9 °C et 83,6 %. Au poids vif de 90 kg, tous les porcs ont été abattus et un échantillon de peau de 2 à 4 cm² a été prélevé au niveau du dos (i.e., au niveau de la dernière côte dorsale) avant l'échaudage et l'épilation. Les épaisseurs de l'épiderme et du derme ont été déterminées sur des coupes de peau colorées au trichrome bleu. La densité et la surface des glandes sudoripares (SG) ont également été mesurées. La densité des mastocytes a été déterminée sur des coupes colorées au Giemsa. L'épaisseur de l'épiderme n'est pas affectée par le type génétique ou par la saison (55 µm en moyenne). En revanche, l'épaisseur du derme est significativement plus importante chez le CR comparativement au LW (3,60 vs. 3,13 mm; $P < 0,01$) mais n'est pas influencée par la saison (3,36 mm en moyenne; $P = 0,17$). La densité des glandes sudoripares est significativement plus importante chez le porc CR par rapport au LW (32,0 vs. 25,4 SG par mm²; $P < 0,01$) mais leur surface est plus faible chez le porc CR par rapport au LW (106 vs. 263×10^{-3} µm² par mm²; $P < 0,01$). La densité des SG tend à être plus élevée en saison chaude comparativement à la saison fraîche (30,4 vs. 26,9 SG par mm²; $P = 0,097$). Dans le derme, la densité des mastocytes est significativement plus élevée chez le porc CR par rapport au LW (2,52 vs. 1,38 mastocytes par mm²; $P < 0,01$). Quelque soit la race, la densité des mastocytes est plus importante au cours de la saison chaude comparativement à la saison fraîche (2,22 vs. 1,68 mastocytes par mm²; $P < 0,01$). En conclusion, notre étude montre que les différences d'histologie de la peau ou des caractéristiques des glandes sudoripares entre les deux types génétiques pourraient en partie expliquer la meilleure adaptation du porc CR.

porcs / tolérance à la chaleur / race / peau / histologie

1. INTRODUCTION

Like other homeothermic animals, the pig maintains a constant body temperature under varying environmental conditions by regulating heat loss and heat production. This ability to regulate body temperature under heat stress is affected by environmental factors (feeding level, space allocation, floor type, relative humidity) and animal related factors (physiological and immune status, body weight, age, genotype) [13]. Breed differences with regard to heat tolerance are reported to exist in halothane positive and negative boars [10] and in high and low producing lines [22]. Recently, the effects of short term and long term exposure to high ambient temperature were found to be attenuated in a local Caribbean (Creole) breed in comparison with a European genotype (Large White) [23]. In these latter studies, mechanisms implicated in better tolerance to heat stress are unknown but can be attributed to a re-

duction in total heat production due to their lower performance and/or increase of heat dissipation capacity (evaporative and sensible heat loss). Because pigs have few sweat glands, a large part of water is lost by evaporation in the respiratory tract [16]. Moreover, the skin plays a key role in thermoregulation in pigs since it controls the rate of sensible heat transfer from the body core to the surface. Within the thermoneutral range of ambient temperature the loss of sensible heat from the body is mainly controlled by adjustment of tissue insulation in particular by change in peripheral blood flow [15]. In accordance with the results obtained in cattle [8], histological changes in the skin structure (number of epithelial strata, dermis thickness...) can also be implicated in body heat transfer.

The objective of this study was to compare skin histology of a Caribbean breed (i.e., Creole) with that of an imported European breed (i.e., Large White) based on

the hypothesis that breed explains differences in heat tolerance level and heat loss capacity at the cutaneous level. The results on the effect of breed and season on growth performance and feeding behaviour have been previously published [24].

2. MATERIALS AND METHODS

A total of 40 castrated pigs (20 Large White and 20 Creole) was used in a trial conducted in two replicates at the experimental facilities of INRA in Guadeloupe (16° Lat. N., 61° Long. W.), an area characterised as a humid tropical climate. One replicate was carried out during the warm season (i.e., between February and April 2003) and the other during the hot season (i.e., between August and October 2003). During the warm season, ambient temperature and relative humidity averaged 25.3 °C and 86.0% respectively. The corresponding values for the hot season were 27.9 °C and 83.6%. The experiment started after a two week adaptation period when the animals were 14-wks old and lasted until the pigs reached 90 kg live BW. For each replicate, the pigs were divided into two groups of 10 animals based on their breed (Creole vs. Large White). The pigs were housed in an open front fattening unit and therefore they were exposed to natural light, temperature and relative humidity levels. The animals had 24-h access to feed (9.0 MJ net energy and 158 g per kg crude protein) and water.

The animals were slaughtered at about 90 kg BW after a 24-h fasting period by manual low voltage electrical stunning. Skin samples of about 2–4 cm² were collected from the last dorsal rib before scalding and dehairing. These samples were fixed in 10% (w/v) buffered formalin fixed and embedded in paraffin. Sections of a thickness of six micrometers were cut both perpendicular and parallel to the surface from a few different areas of each sample

and they were stained with Hematoxylin, Eosin and Safran (HES), Trichrome blue, Fontana, Giemsa, Toluidine blue and Cresyl violet solutions.

Epidermis and dermis thicknesses were determined from vertical sections of skin (two slices per animal) using the Leica Qwin software (Leica imaging system, Cambridge). Dermis and sweat gland surfaces and hair follicle density were also measured from vertical sections of skin using Image J software (NIH, <http://rsb.info.nih.gov/ij/>). According to Mowafy and Cassens [21] and also based on our results in pigs, sweat glands and hair follicles generally exist in the ratio of one-to-one. In consequence, we assumed that sweat gland density was equal to the number of hair follicles. Mast cells were recognised by their metachromatic granules using slices stained with Giemsa (20% in aqueous solution) and their density was obtained by counting 3 slices per animal.

Epidermis and dermis thicknesses, as well as dermis surface were not evenly distributed and they were submitted to a logarithm transformation. Data were submitted to an analysis of variance including the effect of breed ($n = 2$), season ($n = 2$) and interactions as main factors. The GLM procedure of SAS [28] was used for the statistical analyses and the least squares means procedure (PDIF option) was used to compare means when a significant F-value was obtained. The results from the log-linear transformed variables were presented after back transformation and an approximate standard error in the original scale was calculated using the delta method [19].

3. RESULTS AND DISCUSSION

In the present experiment, CR pigs have slower growth rate (642 vs. 861 g per d) in the 45 to 90 kg BW range and fatter carcasses at slaughter (23.5 vs. 10.4 mm for

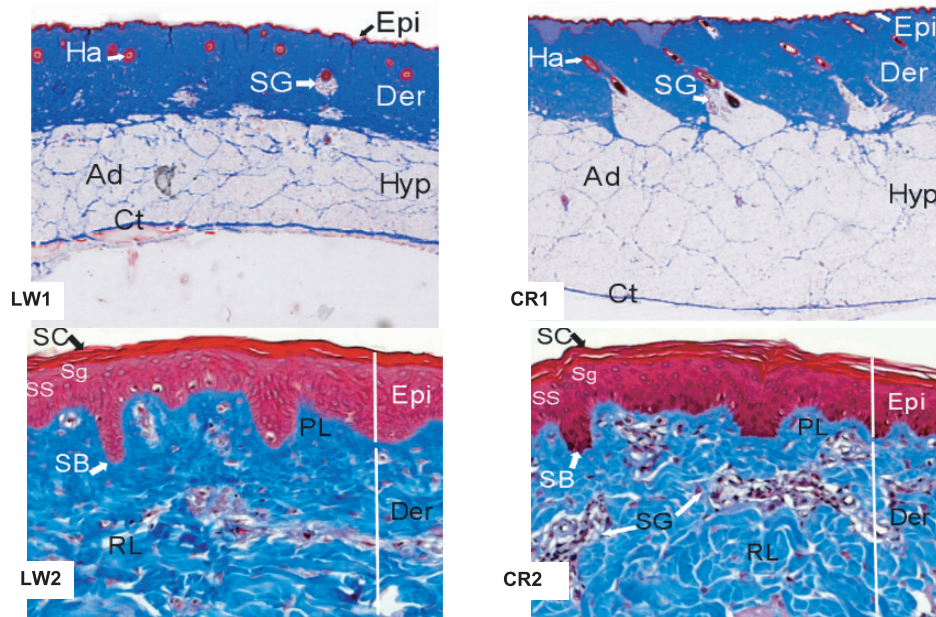


Figure 1. Vertical skin sections from the back of a Large White (LW) and Creole pig (CR). LW1 and CR1 sections show the epidermis (Epi), dermis (Der) with hair (Ha) and sweat gland (SG) structures, and hypodermis (Hyp) with adipocytes (Ad) and connective tissue border (Ct) between the outer and the inner l of subcutaneous adipose tissue (trichrome blue staining, objective: 1×). LW2 and CR2 sections show the three mains layers of strata of the epidermis (Epi) (stratum basal, SB; stratum spinosum, SS; stratum granulosum, Sg; stratum corneum, SC) and the dermis (Der) (papillary layer, PL; reticular layer, RL). (Trichrome blue staining, objective: 10×).

backfat thickness at 150 days) than the LW pigs [25].

The skin of the pig is composed of three major layers with different structures and/or functions: the epidermis, the dermis and the hypodermis (Fig. 1). The hypodermis is composed of two layers of adipose tissue containing lobules of adipocytes; the two adipose layers are separated by a thin collagenous stratum; only the outer layer is represented in Figure 1.

3.1. Epidermis

In pigs, the epidermis consists of four major layers (Fig. 1): a thin residual cornified layer (stratum corneum), a discrete granulosum layer (stratum granulosum), a

thick spinous layer (stratum spinosum) and a basal monolayer (stratum basal) which sits on the dermis [11]. The main function of the epidermis is to protect against harmful agents from the external environment (germs, ultraviolet light, chemicals) and to preserve a balanced internal environment. Moreover, the epidermis helps to control body temperature. In fact, according to the results obtained by Carvalho et al. [8], the number of cellular layers of the epidermis is negatively correlated with body temperature in cows. In other words, these results suggest that heat transfer by simple physical diffusion of excess heat from the body tissues is superior in animals with a thinner epidermis [26]. In our study, the thickness of the epidermis was affected neither

Table I. Effect of breed and season on skin characteristics of castrated growing pigs (adjusted means).

| | Breed | | Season | | | Statistical analysis ² | | |
|---|--------|-------------|--------|------|------------------|-----------------------------------|--------|----------------|
| | Creole | Large White | Warm | Hot | RSD ¹ | Breed | Season | Breed × Season |
| Nb. of pigs | 20 | 20 | 20 | 20 | | | | |
| Age at slaughter (days) | 190 | 153 | 171 | 173 | 11 | 0.0001 | 0.6097 | 0.8162 |
| Skin thickness | | | | | | | | |
| Epidermis (μm) | 54 | 56 | 55 | 55 | 20 | 0.3804 | 0.8941 | 0.3517 |
| Dermis (mm) | 3.60 | 3.13 | 3.46 | 3.26 | 1.21 | 0.0019 | 0.1693 | 0.6293 |
| Sweat gland characteristics | | | | | | | | |
| Density (nb. per cm^2) | 32.0 | 25.4 | 26.9 | 30.4 | 6.5 | 0.0028 | 0.0971 | 0.2504 |
| Surface ($10^{-3} \times \mu\text{m}^2$ per mm^2) | 106 | 263 | 134 | 207 | 159 | 0.0033 | 0.1401 | 0.2090 |
| Mast cell | | | | | | | | |
| Density (nb. per mm^2) | 2.52 | 1.38 | 1.68 | 2.22 | 0.51 | 0.0001 | 0.0026 | 0.8857 |

¹ Residual standard deviation.² Analysis of variance including the effects of breed (Creole vs. Large White), season (Warm vs. Hot season) and interaction.

by breed nor by season and it averaged $55 \mu\text{m}$ (Tab. I). As a result, the superiority of the CR breed with respect to heat tolerance is not related to a variation in epidermis thickness. Owing to their black skin, CR pigs have a large amount of melanin pigment in the basal layer of the epidermis (Fig. 2). In contrast, no melanin pigments are found in the skin of the LW pigs. Many studies have demonstrated that melanin provides protection against UV light (see review of [29]). According to Johansson Moller et al. [17], the dominant white coat in pigs is related to a mutation in the KIT receptor which arises with domestication.

3.2. Dermis

In pigs, the dermis is divided into two layers that blend together with no clear demarcation: the papillary (superficial) layer and the reticular (deep) layer (Fig. 1). According to Mowafy and Cassens [21], the

dermis consists largely of dense irregular connective tissue with collagenous fibres. On the back, the pig's dermis thickness averaged 3.82 mm . However, it was influenced by genotype (Tab. I): dermis thickness was significantly higher in CR than in LW pigs (3.60 vs. 3.13 mm ; $P < 0.001$). In contrast, Daghash et al. [9] found that buffaloes had a significantly thicker dermis than heat tolerant Jersey cows (4.57 vs. 1.43 mm). They hypothesised that heat tolerance is improved with the thinning of the dermis. In addition, dermis thickness was found to be similar in Indian zebu cattle and European cattle [6]. Moreover, Dowling [12] showed that skin thickness was not essential for adaptability to a hot environment in cattle and that the functional capacity of sweat glands and type of hair appeared to be more important than the actual skin thickness in hot climates.

According to Figures 1 and 3, the pigs' sweat glands (SG) were generally distributed throughout the dermis usually in a one-to-one ratio with hair follicles in

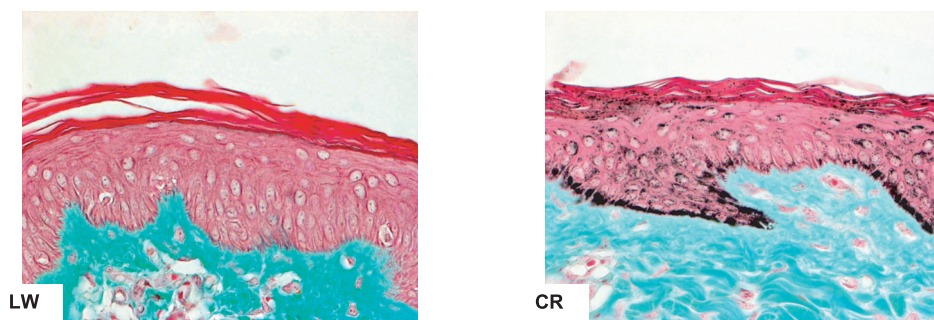


Figure 2. Vertical skin sections from the back of a Large White (LW) and Creole pig (CR). The figure shows a large amount of melanin pigments in the basal layer of the epidermis in CR and no melanin pigments in LW pigs (Fontana staining, objective: 20×).

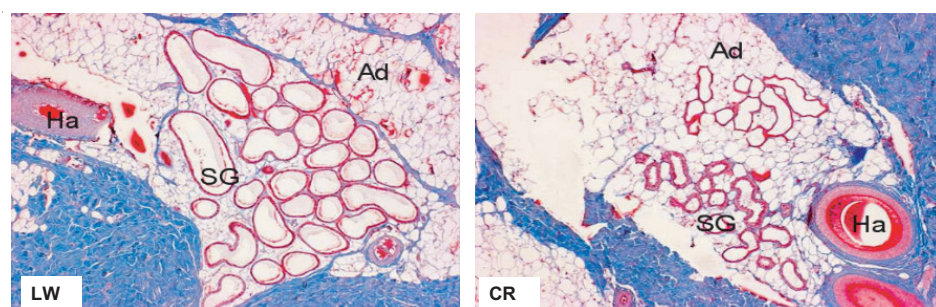


Figure 3. Vertical skin sections from the back of a Large White (LW) and Creole pig (CR); Ad, Adipocytes, SG, sweat gland and Ha, Hair structure. (Trichrome blue staining, objective: 4×).

accordance with the observations of De Melo et al. [11]. These authors also reported that pigs' SG are of a simple, coiled, tubular and apocrine type with a secretory tubular portion located in the lower portion of the dermis and an excretory duct parallel to the hair follicles. Even though the secretory activity of SG in pigs has been demonstrated by Ingram [14], their ability to evaporate cutaneous moisture is very small in comparison with other animals such as cows [16]. In the present study, SG density for the dorsal area averaged 30 SG per cm^2 , which was 10 to 100-fold lower than the values reviewed by Berbigier [5] in cattle. According to these authors, this large variability can be explained by many factors including the body localisation of

the skin sample [1] and animal related factors such as age and body weight ranges [7] or breed type [3]. Sweat gland density was significantly higher in CR than in LW (i.e., 32.0 vs. 25.5 SG per cm^2 in CR and in LW pigs, respectively; $P = 0.003$). Moreover, SG surface was significantly lower in CR than in LW pigs (106 vs. $263 \times 10^{-3} \mu\text{m}^2$ per mm^2 ; $P = 0.0033$). According to Amakiri and Mordi [2], the rate of cutaneous evaporative loss appears to be related to a high SG density rather than SG volume. This result suggests an increase in heat loss efficiency from SG in CR pigs.

By using light microscopy, mast cells in the dermis were characterised by their metachromatic granules with a Giemsa staining substance and were found in the

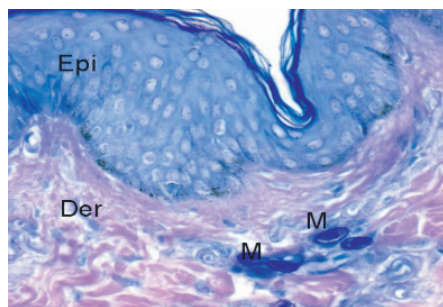


Figure 4. Vertical skin sections of a CR pig showing mast cells (M) found in the dermis (Der) (Giemsa staining, objective: 20 \times).

dermis (Fig. 4). The effects of breed and season on mast cell density are presented in Table I. On average, mast cell density was 2 cells per mm² which was comparable to the values reported by Monteiro-Riviere and Stromberg [20] in pigs at 14 weeks of age (i.e., 2.9 mast cells per mm²) but much lower than values reported in dogs and humans (i.e., 30 to 50 mast cells per mm²) [4]. Irrespective of the breed, mast cell density was higher during the hot season (2.22 vs. 1.68 mast cells per mm² in the warm season; $P < 0.001$). In rodents, Schmelz and Petersen [27] suggested that mast cells could be implicated in vasodilatation in skin vessels during an experimental thermal stimulation. In other words, the high density of mast cells during the hot season would imply an increase of heat dissipation. Moreover, a higher density of mast cells was found in the dermis of CR pigs (2.52 vs. 1.38 mast cells per mm²; $P < 0.001$). Similarly, Komatsu et al. [18] reported a higher mast cell density in the dermis of the Meishan pig when it was compared with Duroc, LW or Landrace pigs. In addition, the Meishan breed was also characterised by a severe eosinophil infiltration in the dermis and high anaphylactic peptide levels in the serum. According to these histopathological findings, Komatsu et al. [18] suggested a genetic variation

in skin immune responsiveness between Meishan and conventional breeds. Our results support this hypothesis but the mechanisms underlying this breed difference in skin sensitivity to disease remains to be investigated. Moreover, it can be hypothesised that unconventional breeds such as the Meishan or Creole breed could be used for comparative studies designed to assess mast cell function. According to the possible implication of mast cells in cutaneous vasodilatation, our results would suggest a higher capacity of the CR pigs to redirect blood from the internal organs to the skin and thereby increase heat loss.

3.3. Hypodermis

The hypodermis consisted largely of adipose tissue and loose connective tissue (Fig. 1). The adipose tissue sometimes formed a dome-like structure around the roots of the hair follicle particularly in CR pigs (Fig. 3). Moreover, the outer layer of subcutaneous adipose tissue appeared to be thicker in CR than in LW pigs. This effect was connected to their higher backfat thickness [12]. In the present work, the feed offered to CR pigs was formulated according to the recommendations reported for LW pigs. Since the nutritional requirements for growth are critically lower in CR than in LW pigs (Renaudeau et al., unpublished results), the higher backfat thickness in CR pigs is mainly related to an inadequate nutrient supply relative to their growth potential.

4. CONCLUSION

In conclusion, it is doubtful that differences in skin histology and/or sweat gland histometry alone explain those observed in heat tolerance between breeds in previous studies ([4] Renaudeau et al., unpublished results) but would support a better heat tolerance in CR than in LW pigs. In addition,

the LW pigs used in this trial have been living for several generations in tropical humid conditions. From this, it can also be hypothesised that the lack of a marked effect of breed in skin characteristics could be related to the acclimatisation of LW pigs to local climatic conditions.

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