The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK

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Influence of teeth resection on the skin temperature and acute phase response in newborn piglets

S Llamas Moya[†‡], LA Boyle*[†], PB Lynch[†] and S Arkins[‡]

[†] Pig Production Department, Teagasc — Moorepark Research Centre, Fermoy, Co Cork, Ireland
[‡] Department of Life Sciences, University of Limerick, Limerick, Ireland
* Correspondence and requests for reprints: Laura.Boyle@teagasc.ie

Abstract

Two experiments were carried out to determine the effect of different teeth resection methods on skin temperature, concentrations of the acute phase proteins C–reactive protein (CRP) and serum amyloid A (SAA), and cortisol in piglets. In Experiment 1, piglets from 60 litters were assigned to three treatments where the 'needle' teeth were clipped (CLIP), ground (GRIND) or left intact (INT) within 12 h of birth; skin temperature was measured immediately afterwards. Fourteen pigs were selected in each treatment for blood sampling at 1 day and 29 days-of-age for the determination of concentrations of CRP, SAA and cortisol. In Experiment 2, a 2 × 2 factorial design was used to determine the effect of teeth clipping and time spent out of the farrowing crate post-clipping on skin temperature. Piglets from 60 litters had their teeth clipped (CLIP) or left intact (INT) and were returned to the farrowing crate immediately or after 1 min. Skin temperature was measured after piglets were returned to the farrowing crate and after 10 min. In Experiment 1, CLIP and GRIND piglets had significantly lower skin temperatures than INT piglets; skin temperature was also significantly reduced in CLIP piglets in Experiment 2. Skin temperature did not differ between time-out groups. Plasma levels of CRP and SAA did not differ between treatments on day 1; however, concentrations of both proteins were significantly higher on day 29. CLIP pigs had significantly higher concentrations of CRP in comparison with GRIND pigs on day 29. Stress caused by teeth resection provoked a transient reduction in skin temperature. Furthermore, both resection methods caused infection and/or inflammation, but to a similar degree as that caused by leaving the teeth intact. These results indicate that the welfare of piglets is better in the short term if their teeth are left intact; however, if teeth resection is necessary grinding can be recommended in preference to clipping.

Keywords: *acute phase proteins, animal welfare, cortisol, pigs, skin temperature, teeth resection*

Introduction

Teeth resection of newborn piglets is widely practised in most commercial pig units. The practice reduces facial injuries to piglets during the establishment of the 'teat order' (Weary & Fraser 1999; Lewis *et al* 2005a) and minimises damage to the sow's udder (Brookes & Lean 1993; Lewis *et al* 2005b). Teeth are generally clipped to the gum line using side-cutting pliers. This can result in mouth lesions attributable to the exposure of the pulp cavity when the stump of the tooth splinters (Hutter *et al* 1993; Lewis *et al* 2005a). The use of rotating electric grinders minimises this problem (Holyoake *et al* 2004; Lewis *et al* 2005a); however, grinding takes longer and therefore involves more handling (Lewis *et al* 2005a). Resection of piglets' teeth is thought to be painful (Scientific Veterinary Committee 1997; Hay *et al* 2004) and changes in the normal behaviour of piglets immediately after teeth resection have been shown (Noonan *et al* 1994; Meunier-Salaün *et al* 2002). However, Noonan *et al* (1994) reported similar behavioural alterations in control piglets, suggesting that the handling and restraint associated with teeth resection is also stressful. Prunier *et al* (2005) reported no variation in cortisol or adrenocorticotropin hormone (ACTH) immediately after teeth resection; however, the influence of teeth resection on measures of immune function or the endocrine response has not been researched extensively.

Studies with rodents have shown that restraint reduces tail skin temperature (Wright & Katovich 1996). The temperature of the skin is under control of the peripheral vascular system, which is sensitive to handling and restraint (Wright & Katovich 1996; Gordon *et al* 2002). In pigs, the reduced skin temperature observed in response to cold stress is associated with increased concentrations of catecholamines (Mayfield *et al* 1989; Lossec *et al* 1998). Therefore, it is possible that the stress inherent with teeth resection (Noonan *et al* 1994; Scientific Veterinary Committee 1997) activates the sympathetic nervous system eliciting a fall in skin temperature (Mayfield *et al* 1989).

Trauma to the oral mucosa can lead to infectious and inflammatory processes (Hutter *et al* 1993). Therefore, alterations in concentrations of plasma proteins of hepatic origin, known as acute phase proteins may be observed (Gruys *et al* 1994; Eckersall 2000). C–reactive protein (CRP) and serum amyloid A (SAA) are acute phase proteins that are useful indicators of inflammation (Eckersall *et al* 1996) and infection (Heegaard *et al* 1998) in pigs.

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The objective of the first experiment presented in this paper was to determine if two teeth resection procedures influenced skin temperature and activated the acute phase response in pigs. The results indicated an effect on skin temperature of teeth resection but this was confounded by the longer time taken to process and handle piglets that had their teeth resected. In order to elucidate whether the reduction in skin temperature was part of the stress/pain reaction to teeth resection or simply caused by cold stress owing to the prolonged time out of the farrowing crate, a second experiment was devised in which these questions were addressed.

Materials and methods

Experiment 1

Litters of at least 7 piglets from 60 multiparous sows (parity 1–7; Large White × Landrace) in the Moorepark herd were used in this experiment. Litters were housed with their dams in identical farrowing rooms consisting of 10 individual pens (235 × 155 cm, length × width). The floor was fully slatted (Tribar®, Nooyen Roosters BV, Deurne, The Netherlands) with a continuous heat pad (142 × 43 cm, length × width) at both sides of the centrally positioned farrowing crate. The dimensions of the farrowing crate were 235 × 48 cm (length × width). Selected litters over the same week were assigned to the same treatment group. Treatments were applied after the piglets were individually weighed. Although ear notching was required for identification purposes, the tails were not docked to avoid confounding the potential inflammatory and infectious effects of teeth resection. All piglets from the same litter had their teeth clipped (CLIP), ground (GRIND) or left intact (INT). Clipping was performed using clean, sharp side-cutting pliers. Grinding was performed using a high speed diamond coated cylinder enclosed in a fixture that prevented wounds to living tissue, grinding no more than one third of each tooth (Pigmatic 110, SFK Technology A/S, Herlev, Denmark). The 5 mm diameter grindstone operated at 12 500 rpm. The same trained technician performed both resection methods within 12 h of birth. The time taken to select the piglets and impose the management procedures on all animals in each litter was recorded. Skin temperature was measured using an infrared thermometer (Raytek MX4, Milton Keynes, Buckinghamshire, UK). The infrared light was targeted at the rump of the piglets at a 90º angle from a distance of approximately 1 m (Di Costanzo *et al* 1997). Skin temperature readings were obtained in less than 10 s. The use of an infrared thermometer permitted the measurement of skin temperature without restraining the animals. This measurement was recorded immediately after the management procedures were applied once the piglets were back in the farrowing crate.

Twenty-four hours after the managerial procedures were imposed, blood samples (5 ml) from focal piglets (one male and one female) from seven litters per treatment, selected on the basis of being nearest the average litter body weight (BW), were taken in a supine position by anterior vena cava puncture into lithium heparinized syringes (Vacutainer™, Unitech Ltd, Dublin 24, Ireland). Blood samples were collected from the same focal animals 24 h after weaning at 28 days of age using the same blood sampling techniques. Venipuncture required less than 1 min per animal. Immediately after collection, blood samples were centrifuged at 2000 g for 10 min at 5ºC. Plasma samples were stored at –20ºC until analysed. Plasma samples were analysed for concentrations of CRP using a solid phase sandwich immunoassay specific for porcine CRP (Tridelta Development Ltd, Maynooth, Co. Kildare, Ireland). Plasma samples were analysed in duplicate at 1:100, 1:200 and 1:400 dilutions. The sensitivity of the assay was 20 ng ml^{-1}. The assay had an intra- and inter-assay coefficient of variation (CV) of < 11.6% and < 15.1% respectively. SAA concentrations in plasma were determined using a solid phase enzyme-linked immunosorbent assay (ELISA) (Tridelta Development Ltd, Maynooth, Co. Kildare, Ireland). Levels of SAA in plasma were analysed at 1:500, 1:700, 1:1000 and 1:1500 dilutions all in duplicate. The minimum detectable concentration of SAA was 1.9 mg ml^{-1}. The intra- and inter-assay CV were < 7.7% and < 10.8% respectively. Plasma concentrations of cortisol were also determined by an enzyme immunoassay (DRG–Diagnostics, Marburg, Germany). Cortisol concentrations of undiluted plasma samples were analysed in duplicate. The lowest detectable level of cortisol that could be distinguished from the zero standard was 1.14 ng ml^{-1}. The intra- and inter-assay CV were < 4.1% and < 5.5% respectively.

Experiment 2

Litters of at least seven piglets from 72 multiparous sows (parity 1–7; Large White × Landrace) farrowing in the same crates described for Experiment 1 were used. Data were collected over a 9-week period. The experiment was designed as a 2 × 2 factorial, completely randomised block design, where the effect of teeth clipping and time spent out of the farrowing crate post-clipping were considered. Therefore, piglets had their teeth left intact (INT) or clipped (CLIP), and were either returned immediately to the farrowing crate (0 min) or held in a box for approximately 1 min from the beginning of the resection treatment, prior to being returned to the crate. This time was selected because it was close to the average time taken to grind piglets' teeth in Experiment 1 (approximately 56 s). Ear notching and teeth resection were imposed after the piglets were weighed within 12 h after birth. All piglets from the same litter were assigned to the same treatment groups. The same trained technician applied all treatments. Clipping was performed as described in Experiment 1. The time taken to select the piglets and impose the management procedures on all piglets of the same litter was recorded. Air temperature was measured in the farrowing room (room T^E) where processing of the piglets took place using an electronic thermocouple thermometer (EIRELEC MT 130C, Sifam Instruments Ltd, Torquay, England). The same device was used to determine air temperature in the farrowing crate (crate T^E) at a distance of 1–2 cm above from the floor on six different locations within each pen (four measurements over each corner of the crate and two measurements over each of the heat pads). On closer inspection of

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these temperatures it was found that the average room T^E on week 8 was significantly lower than the minimum air temperature of 25ºC required by newborn piglets (McGlone & Johnson 2002). This was as a result of an electricity supply cut and the subsequent time lapse until a generator restored power supply. Therefore, litters processed that week were eliminated from the subsequent data analysis.

Skin temperature was measured immediately after piglets were returned to the farrowing crate (skin T^E–0) and 10 min later (skin T^E–10) using the same infrared thermometer and the same methodology as in Experiment 1. The location of each piglet was also recorded at the later time. Litter distribution was subsequently classified as < 45%, 45–65% and > 65% depending on the percentage of piglets in each litter that were located on the heat pads.

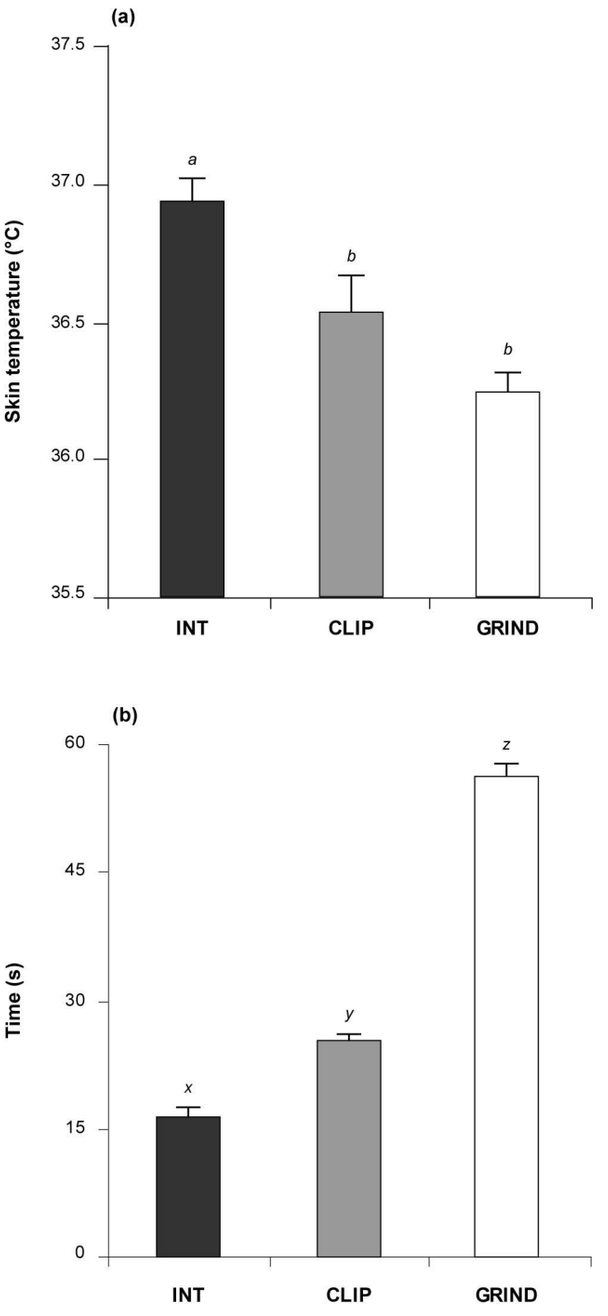
All procedures were carried out under licence, issued by the Department of Health and Children, in accordance with the European Communities (Amendment of Cruelty to Animals Act 1876) Regulations 2002. This study was considered ethically justifiable by the authors because the piglets, being part of the commercial herd at Moorepark, would have had their teeth resected anyway as it is routine management practice on the unit.

Statistical analysis

Data collected in Experiment 1 were analysed as a complete randomised design using the general linear models (GLM) procedures of SAS® (Statistical Analysis System [SAS] Institute Inc, Cary, NC, USA [1999]). Skin T^E data were subjected to analysis of variance (ANOVA) for the main effect of treatment (CLIP, GRIND or INT) using the time taken to impose the procedure and week as covariates; the litter was the experimental unit. Concentrations of CRP, SAA and cortisol on day 1 were analysed by ANOVA to test for the main effects of treatment (CLIP, GRIND or INT) and gender (male or female), and their interaction. Data from samples collected on day 29 were analysed similarly using data from day 1 as a covariate. The effect of gender was not significant ($P > 0.1$); therefore, gender was not included in subsequent analyses. Single degree-of-freedom orthogonal contrast analysis was used to study the statistical differences between the treatment groups.

In Experiment 2, the general linear model (GLM) procedure of the statistical package SAS® was also used. Skin T^E–0 data were subjected to ANOVA for the main effects of treatment (CLIP or INT) and time-out (0 min or 1 min), using room T^E, time taken to apply the procedures, BW and crate T^E as covariates. Skin T^E–10 was also subjected to ANOVA for the main effects of treatment, time and litter distribution (percentage of piglets on the heat pad < 45%, 45–65% and > 65%) and all possible interactions. Skin T^E–0 was used as a covariate in the analysis of skin T^E–10 data. Data were normally distributed ($P > 0.1$) and had equal variance ($P > 0.1$); all data are presented as mean ± standard error of the mean (SEM).

Figure 1



(a) Skin temperature **(b)** and time taken to apply the procedures in pigs with intact (INT), clipped (CLIP) or ground (GRIND) teeth (*a*,*b*,*c*: $P < 0.05$; *x*,*y*,*z*: $P < 0.001$).

Results

Experiment 1

Treatment had a significant effect on skin temperature ($P < 0.05$; Figure 1) with the time taken to apply the procedure having a significant influence ($P < 0.01$). The skin temperature of piglets from the CLIP and GRIND

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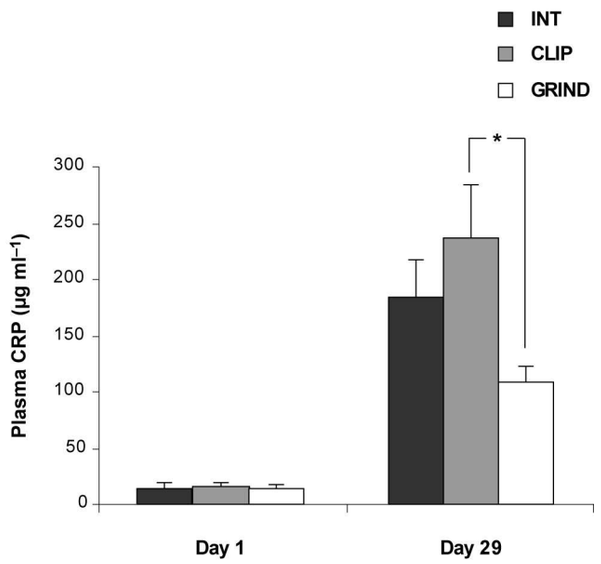
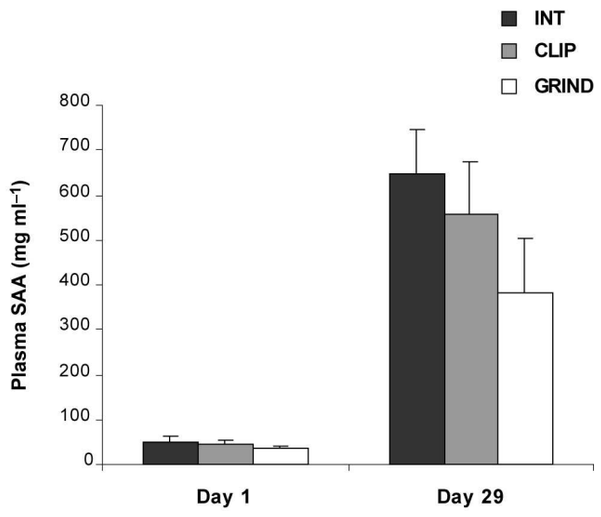
Figure 2



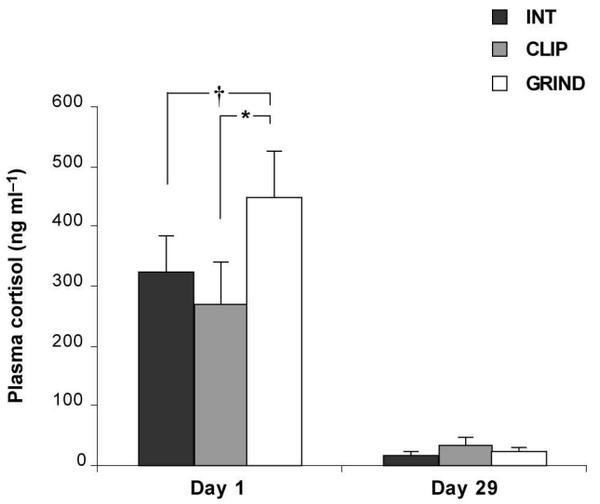
Plasma concentrations of CRP of pigs with intact (INT), clipped (CLIP) or ground (GRIND) teeth on day 1 and day 29 of age (* $P < 0.05$).

Figure 3



Plasma concentrations of SAA of pigs with intact (INT), clipped (CLIP) or ground (GRIND) teeth on day 1 and day 29 of age.

Figure 4



Plasma concentrations of cortisol of pigs with intact (INT), clipped (CLIP) or ground (GRIND) teeth on day 1 and day 29 of age († $P < 0.1$; * $P < 0.05$).

treatment groups was significantly lower compared with piglets from the INT group ($P < 0.05$). However, no significant differences were found in skin temperature between GRIND and CLIP piglets ($P > 0.1$).

The time taken to impose the procedures was significantly different between all three treatments ($P < 0.001$; Figure 1). The average time required to select a piglet, notch its ears and return it to the farrowing crate (INT group) was 16 s. This was significantly lower than the 25 s or 56 s required to additionally clip ($P < 0.001$) or grind ($P < 0.001$) the teeth, respectively. In addition, it took significantly longer to grind piglets' teeth than to clip them ($P < 0.001$).

Plasma CRP concentrations on day 1 did not differ between treatments ($P > 0.1$; Figure 2). Plasma CRP levels determined on 29 day-old pigs were significantly higher in comparison with the concentrations measured on day 1 ($P < 0.001$). Furthermore, a significant effect of treatment was found on day 29 ($P < 0.05$). Pigs in the CLIP treatment group had higher plasma concentrations of CRP than pigs in the GRIND treatment group ($P < 0.05$; Figure 2).

No effect of treatment was found in SAA plasma concentrations of 1-day-old and 29-day-old pigs ($P > 0.1$; Figure 3); however, plasma SAA levels on day 29 were significantly elevated in comparison to the concentrations determined on day 1 ($P < 0.001$).

A significant treatment effect was found in plasma cortisol concentrations on day 1 ($P < 0.05$). GRIND piglets had significantly higher levels of plasma cortisol in comparison to CLIP piglets ($P < 0.05$; Figure 4) and were also significantly different to the INT group, albeit at the 10% level ($P = 0.0783$; Figure 4). On day 29, plasma concentrations of cortisol were significantly reduced in comparison with cortisol levels determined on day 1 ($P < 0.001$). There was no significant effect of treatment ($P > 0.1$; Figure 4)

Experiment 2

Clipping piglets teeth caused a reduction in skin T^E–0 ($P = 0.051$; Figure 5). No statistical differences were found between the two time-out groups ($P > 0.1$; Figure 5). The time taken to apply the procedures tended to have a significant effect on skin T^E–0 ($P = 0.059$). The average time taken to process the piglets also differed significantly between the

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two treatments ($P < 0.001$). An average of 27 s was required to process one piglet in the CLIP treatment, in comparison with 13 s to process a piglet in the INT group. Crate T^E ($P < 0.001$) and room T^E ($P < 0.05$) also significantly influenced skin T^E–0 measurements. There was a positive correlation between the average room T^E (24.85 ± 0.09°C) and the average crate T^E (25.20 ± 0.11°C; $r = 0.802$, $P < 0.001$). In spite of using a randomisation table to assign litters to treatment groups, significant differences were found in the average BW of the litters ($P < 0.05$). Piglets from the INT group were significantly heavier than CLIP piglets (mean ± SEM, INT versus CLIP: 1.63 ± 0.04 kg versus 1.50 ± 0.04 kg); however, BW did not influence skin T^E–0 measurements ($P > 0.1$).

There were no treatment or time-out effects regarding skin T^E–10 data ($P > 0.1$). Skin T^E–10 was influenced significantly by skin T^E–0 ($P < 0.001$). Litter distribution significantly affected skin T^E–10 ($P < 0.001$; Figure 6) irrespective of the treatment ($P > 0.1$). Skin T^E–10 was significantly reduced when less than 45% of the piglets within a litter were located on the heat pads in comparison with 45–65% ($P < 0.05$) and > 65% of the piglets on the heat pads ($P < 0.001$). In 39 litters (65%) used in this experiment, more than 65% of the piglets in the litter were located on the heat pad. Five (8.3%) and 16 (26.7%) litters were classified respectively as having 45–65% and < 45% of piglets located on the heat pad.

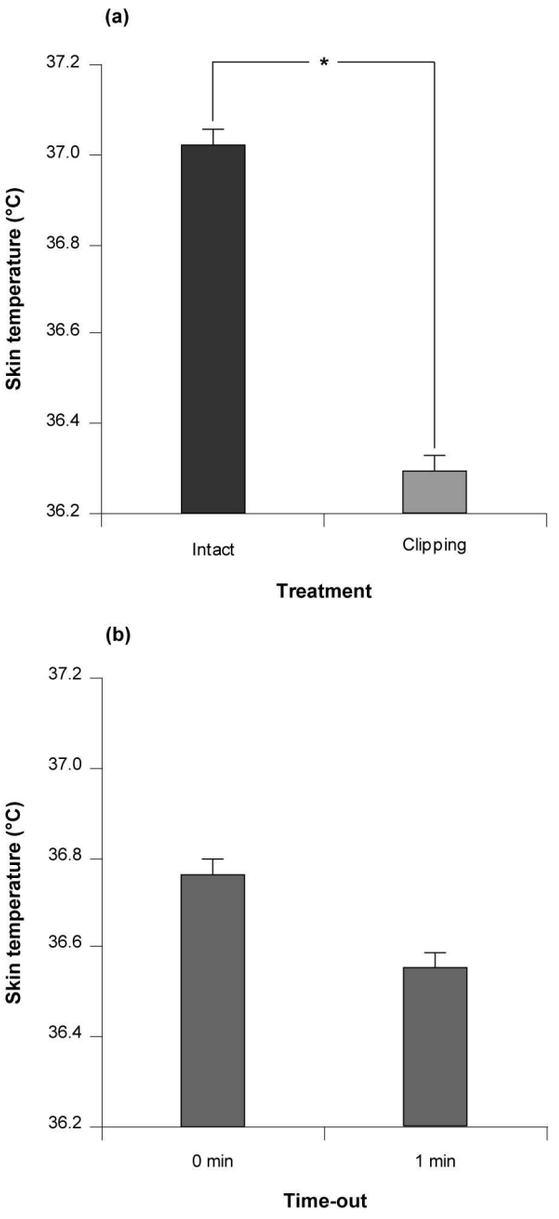
Discussion

The results from both of the experiments described in this paper indicate that teeth resection caused a reduction in skin temperature that is not simply a product of cold stress. This is due to the perception by the piglets of teeth resection as a stressor (Noonan *et al* 1994; Scientific Veterinary Committee 1997) leading to activation of the sympathetic nervous system. This has been previously shown to increase plasma norepinephrine concentrations (Mayfield *et al* 1989; Lossec *et al* 1998). Norepinephrine acts as a vasoconstrictor (Charkoudian 2003), which causes a reduction in blood flow to the skin. Teeth resection constitutes a stressor not only because of the pain derived from the resection of the teeth themselves (Hay *et al* 2004) but also because of the handling and restraint that is involved (Noonan *et al* 1994). In this regard, the time taken to impose the teeth resection methods also influenced skin temperature. However, the longer handling times inherent to grinding did not result in a more pronounced fall in skin temperature relative to clipping. Therefore, it is likely that the pain associated with teeth resection was a more important factor in the reduction of skin temperature than handling and restraint in the current studies.

In Experiment 2, BW at birth differed between pigs from the intact and clipped groups. Low-birth weight animals are extremely prone to hypothermia (Lay *et al* 2002; McGlone & Johnson 2002); therefore, it could be argued that the lower skin temperature in piglets with clipped teeth resulted from the higher sensitivity of these piglets to cold environments. However, BW did not influence measurements of skin temperature. Furthermore, in spite of the treatment differences in BW, piglets used in this experiment could not be considered low-birth weight animals (BW < 900 g) (Campbell & Dunkin 1982).

The reduction in skin temperature was short-lived and was no longer evident 10 min after teeth resection; therefore, teeth resection is a transient stressor. These findings also show that providing extra sources of heat within the farrowing crate could alleviate the stressful effects of teeth resection. Air temperature in the farrowing crate was over

Figure 5



Effect of (a) treatment (intact or clipping) and (b) time-out (0 min or 1 min) on skin temperature of piglets immediately after returning to the farrowing crate (skin T^E–0) (* $P < 0.05$).

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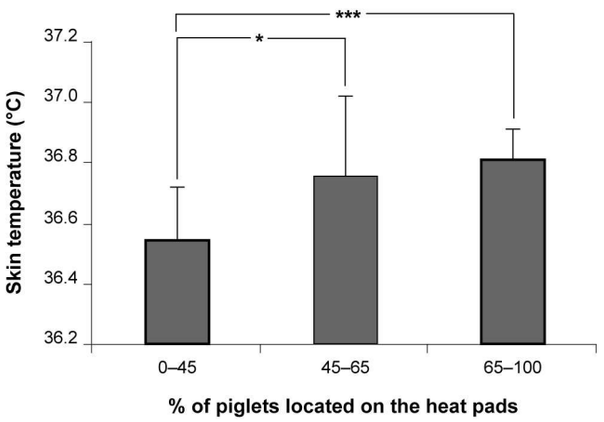
Figure 6



Effect of litter distribution (< 45%, 45–65% or > 65% of piglets located on the heat pads) on skin temperature of piglets 10 min after returning to the farrowing crate (skin T^{ε}–10) (* $P < 0.05$; *** $P < 0.001$).

2°C higher over the heat pads. In litters where at least 45% of the piglets were located on the heat pads higher skin temperatures were recorded 10 min after imposing the teeth resection treatments.

If teeth resection did elicit an acute phase response, it was not observable 24 h after treatments were imposed. Levels of CRP and SAA determined in 1 day-old piglets were lower in comparison with the nearly 10-fold increase in the concentrations of these acute phase proteins on day 29. The stress of weaning could have been responsible for this increase (Spurlock *et al* 1996; Piñeiro *et al* 2003). Nevertheless, a study of the facial skin and mouth lesions of the same piglets used in this experiment found that a high proportion of piglets in litters with intact teeth were affected by facial skin lesions at weaning (Lewis *et al* 2005a). These were more severe than the facial lesions recorded in pigs with clipped or ground teeth. Furthermore, piglets with resected teeth had more mouth lesions immediately prior to weaning (Lewis *et al* 2005a). Teeth resection can also cause teeth abnormalities, such as pulp cavity exposure, haemorrhage, infiltration, abscesses and necrosis (Hay *et al* 2004). Therefore, the inflammatory and infectious processes were likely to have been activated by all three treatments. Nonetheless, plasma concentrations of CRP in pigs with ground teeth were lower in comparison with pigs that had their teeth clipped. Lewis *et al* (2005a) reported that the pigs with ground teeth had fewer gum lesions prior to weaning than the pigs that had their teeth clipped. In addition, although pigs with clipped teeth present teeth fractures at 27 days-of-age, no teeth fractures exist at the same age in pigs with intact or ground teeth (Hay *et al* 2004). Finally, Holyoake *et al* (2004) reported a lower occurrence of facial scars in pigs with ground teeth 1-week post-weaning compared to intact and clipped piglets. Therefore, it appears that the acute phase response at 29 days-of-age may have been activated to a lesser extent by grinding in comparison to clipping. However, there was no difference between pigs with intact teeth and pigs with clipped or ground teeth, which indicates that both facial and mouth injuries may activate the immune/inflammatory response to a similar degree.

One-day-old piglets with ground teeth had higher levels of cortisol than piglets from both the intact and clipped groups. The longer handling times inherent to grinding probably resulted in an enhanced response of the hypothalamic-pituitary-adrenal (HPA) axis when these piglets were subjected to the subsequent stress of blood sampling (Kanitz *et al* 2004). Plasma cortisol levels of pigs vary with age: newborn piglets have higher concentrations of cortisol than weaned pigs (Kattesh *et al* 1990; Otten *et al* 2001); however, the concentrations of cortisol in 1-day-old piglets determined in this study were higher than the values reported in other studies (Kattesh *et al* 1990). It is possible that the HPA axis is highly responsive to the handling inherent to blood sampling (Baldi *et al* 1989), in part because of the novelty and lack of predictability that blood sampling represents to 1-day-old piglets (Griffin 1989).

Animal welfare implications

Measurements of skin temperature and plasma acute phase proteins concentrations were useful in elucidating the impact of methods of teeth resection or leaving the teeth intact on piglet welfare. These findings confirm that teeth resection constitutes an acute, though transient stressor and that the welfare of newborn piglets is better in the short term if their teeth are left intact. Nevertheless, in situations where teeth resection is deemed necessary grinding the teeth is preferable to clipping because the latter method may have more negative welfare implications.

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