Effects of husbandry and low-dose lipopolysaccharide challenge on the acute phase response of young pigs

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Summary

In recent years, concern has grown for the welfare of domesticated animals in different production systems (Appleby and Hughes, 1997). Poor welfare can result in poor performance and productivity. However, the consumers are also requesting more welfare-friendly systems, as reflected by the importance that 'organic' and 'free-range' products have gained in our markets. Furthermore, there are ethical reasons for safeguarding the welfare of animals in our care. Thus, it is scientists' task to be able to develop methods and techniques that can help to assess the welfare objectively. Traditionally, welfare assessment relied on the study of behaviour and the measurement of endocrine parameters. Acute phase response mediators and products, such as pro-inflammatory cytokines and acute phase proteins, emerged recently as potential indicators of infection and herd health status (Eckersall, 2000; Petersen *et al.*, 2004). Thus, investigating the effects of husbandry and low-dose lipopolysaccharide (LPS) challenge on the acute phase response of young pigs can give valuable information on the use of these immune parameters as health and welfare indicators in pigs.

Pigs reared in intensive production systems are subjected to husbandry conditions, some of which may be detrimental for their welfare from the beginning of their postnatal life. In addition to the relatively immature immune system, neonatal piglets are subjected to invasive managerial practices, such as teeth clipping, ear notching and tail docking, which can cause injuries and trauma (Done *et al.*, 2003; Hay *et al.*, 2004; Lewis *et al.*, 2005a). Increases in the concentrations of plasma tumor necrosis factor-alpha (TNF-α) found in neonatal pigs in these experiments support the hypothesis that immune function starts developing soon after birth (Matteri *et al.*, 1998; Blecha, 2001). However, high cortisol levels in newborn piglets may simulate stress conditions, and therefore lead to the synthesis of serum amyloid A (SAA) and haptoglobin (Hp) by hepatocytes. Thus, it is possible that as suggested by Martín *et al.* (2005), newborn piglets need to overcome a moderate acute phase response after birth.

Several studies have shown that while clipping ameliorates some problems associated with leaving teeth intact, it raises new problems, namely gum and mouth lesions (Holyoake *et al.*, 2004; Lewis *et al.*, 2005a). Grinding appears to combine the best aspects of both procedures, reducing facial injuries as well as gum and mouth lesions compared to leaving teeth intact or clipping respectively (Holyoake *et al.*, 2004; Lewis *et al.*, 2005a). Results from this study show that both grinding and clipping piglets teeth represent a significant, but transient stressor as reflected by the reduction in skin temperature. Furthermore, grinding increased cortisol concentrations one day after teeth resection. Nonetheless, grinding reduced plasma C-reactive protein (CRP) levels at weaning in comparison to clipping, but not to leaving the teeth intact. Thus, leaving teeth intact would seem to pose the least risk to welfare in the long term. However, if resection is necessary, grinding has long term welfare benefits over clipping. The conclusion from these results agrees with the recent work from Hay *et al.* (2004) that investigated the effect of teeth resection alternatives by histological examination.

Surgical castration of male piglets is carried out in many countries to avoid problems with boar taint (EFSA, 2004). In agreement with several publications, this procedure had a significantly effect on the behaviour of pigs, inducing pain-related activities and also altering their normal behaviour (Hay *et al.*, 2003). In general, these behavioural alterations were probably aimed at reducing the stimulation of painful tissues and stopping other animals from inflicting more pain (Mellor *et al.*, 2000; Hay *et al.*, 2003). Results from these experiments also suggest that some behaviours and activities adopted by castrated piglets are aimed at alleviating the pain inflicted by the procedure. Immediate physiological consequences of surgical castration of pigs have been reported by Prunier *et al.* (2005). Hay *et al.* (2003) showed that urinary corticosteroid and

cathecholamines were not useful for evaluating the physiological consequences in the days following castration. The assessment of the acute phase response subsequent to surgical castration did not draw any marked conclusion regarding the welfare of these animals, and suggests that piglets of this age may be sensitive to other husbandry conditions that may be imposed prior to castration.

LPS challenges are used as models to study the pro-inflammatory cytokine and acute phase protein response in pigs and other species. Administration of a low dose of LPS can be used to simulate sub-acute inflammation/infection. As reported by other authors, TNF-α synthesis increased significantly subsequent to the low-dose LPS challenge (Warren *et al.*, 1997; Webel *et al.*, 1997). In addition, CRP and SAA levels also increased after LPS administration. Thus, these acute phase proteins can be used as markers of sub acute inflammation/infection. Pro-inflammatory cytokines were detectable in saliva of pigs, which agrees with studies in humans (Dugué *et al.*, 1996). Finally, gender differences were observed. Females presented a more responsive hypothalamus-pituitary-adrenal (HPA) axis, associated with decreased concentrations of pro-inflammatory cytokines. In this regard, high concentrations of male gonadal steroids have been described in the early postnatal life of male pigs (Schwarzenberger *et al.*, 1993). Results from these experiments also showed higher testosterone levels in the saliva of pigs during this period, and the removal of the testes inhibited this increase. The interaction between the HPA axis, the hypothalamus-pituitary-gonadal (HPG) axis and the immune system may account for these gender differences (Da Silva, 1999). Hence, gender differences need to be considered in the early postnatal period.

Administration of LPS has also been used to model sickness behaviour in pigs (Johnson and von Borell, 1994; Warren *et al.*, 1997). Sickness behaviour, the set of non-specific behavioural changes that develop in animals during the course of infection, is regulated by pro-inflammatory cytokines (Dantzer, 2001). It is probable that the synthesis of pro-inflammatory cytokines may have caused the synchronicity in the occurrence of behaviours characteristics of sickness subsequent to low-dose LPS administration. Depressed general activity, anorexia and loss of interest in the surrounding areas were the most noticeable symptoms elicited by LPS.

Surgical castration of male pigs can also affect the health and welfare of these animals in the long-term (Lessard *et al.*, 2002; Frank *et al.*, 2005). Exposure to acute stressors, in particular at young age, may have an effect on the subsequent response to other stressors (Kanitz *et al.*, 2004). This could be of special importance at weaning, which is a time associated with poor performance due to the severe nutritional, physical and psychological challenges. The absence of male gonadal steroids due to castration reduces aggression among growing and fattening pigs (EFSA, 2004). This study showed that the number of agonistic interactions is also reduced in groups of castrated pigs after weaning. In addition, surgical castration altered the exhibition of sickness behaviours induced by low-dose LPS. Castrated pigs did not display general behavioural symptoms of sickness, which indicates that this surgical procedure may affect the coping mechanisms of these animals upon encountering subsequent stressors. Taking into consideration the beneficial effects of sickness behaviour on recovery from infection (Hart *et al.*, 1988), it is likely that entire males were more efficient in overcoming the challenge.

This project evaluated the effect of husbandry and low-dose LPS challenge on the acute phase response of young pigs, assessing the use of pro-inflammatory cytokines and acute phase proteins as health and welfare indicators. Results indicate that pro-inflammatory cytokines and acute phase proteins can be useful for evaluating the effect of husbandry and sub acute inflammation/infection. Nonetheless, the results also highlight the relevance of other indicators in the rather difficult task of assessing the welfare of animals.

Introduction

In recent years, interest in the welfare of domestic animals has escalated rapidly. This is largely due to the demand for more welfare-friendly production systems by consumer groups (Appleby and Hughes, 1997). In addition, poor animal welfare practices can increase morbidity and mortality rates and can also affect reproduction of animals. Such practices can result in great economic losses (Lay *et al.*, 2002). Thus, it is essential to be able to objectively assess welfare (SVC, 1997). In this regard, scientists agree that animal welfare can only be properly assessed if several indicators are measured (Moberg, 2000). This takes into account that poor welfare can result in behavioural and physiological alterations, which can affect the endocrine, autonomic and immune responses of animals (Moberg, 2000).

In pig production, the preweaning period is a critical time in which extra effort is required in order to guarantee the survival of piglets. After birth, piglets are very susceptible to hypothermia. The provision of thermoneutral conditions is an essential requirement (Lay *et al.*, 2002; McGlone and Johnson, 2002). Furthermore, piglets are born with a relatively immature immune system, and colostrum ingestion is necessary for the absorption of immunoglobulins within 24 h of birth (Gaskins and Kelley, 1995; Blecha, 2001). Piglets are also subjected to invasive husbandry practices that are known to cause short-term pain and discomfort (McGlone *et al.*, 1993; Noonan *et al.*, 1994; Lewis *et al.*, 2005a). These practices may affect the performance and coping responses of animals to later unavoidable stresses. Weaning, which is characterised by nutritional, environmental and social changes (Pluske and Williams, 1996), is associated with an increase in disease susceptibility (Kanitz *et al.*, 2002; Carstensen *et al.*, 2005).

In addition to the more traditional behavioural, health and endocrine parameters, the use of immune-related indicators is being successfully developed for the assessment of animal welfare. The acute phase response is a prominent systemic reaction of the organism to local or systemic disturbances in its homeostasis caused by infection, tissue injury, trauma or immunological disorders. This response is induced by cytokines, which are signalling molecules within and between the immune system (Baumann and Gauldie, 1994). The pro-inflammatory cytokines - tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) regulate not only local inflammatory processes, but also induce a variety of responses centrally (Rothwell and Hopkins, 1995). These include the hypothalamus-pituitary-adrenal (HPA) axis and hepatic acute phase responses. The latter refer to the production of specific plasma proteins by the liver, known as acute phase proteins (Baumann and Gauldie, 1994). Acute phase proteins, such as C-reactive protein (CRP), serum amyloid A (SAA) and haptoglobin (Hp), are recognised as promising tools to assess welfare, health and performance in animal production (Gruys *et al.*, 1994; Eckersall, 2000; Petersen *et al.*, 2004). Hence, this project aimed at evaluating the effect of husbandry and low-dose lipopolysaccharide (LPS) challenges on the acute phase response of young pigs.

Pro-inflammatory cytokines and acute phase proteins have been utilised successfully as markers of infection and herd disease levels in pigs (Balaji *et al.*, 2002; Hultén *et al.*, 2003; Petersen *et al.*, 2002; Lauritzen *et al.*, 2003). However, there is little information on their suitability as indicators of the impact of husbandry practices and sub acute inflammation/infection conditions that may also compromise the health and welfare of pigs.

The objectives of this project were to investigate:

- the levels of pro-inflammatory cytokines and major porcine acute phase proteins during the first week of life of pigs; and the effect of the mentioned husbandry practices on possible age-related changes in these immunomodulators.

- the effect of two methods of teeth resection and leaving the teeth intact of the skin temperature of piglets; and the impact of these teeth resection alternatives on the acute phase protein response after resection and at weaning.

- the behavioural and physiological reactions of 5-day-old piglets after surgical castration.

- the acute phase response elicited by low-dose LPS challenge, establishing the time-response of cytokines and acute phase proteins in both the plasma and saliva of pigs; and possible gender differences in the acute phase response in the early postweaning period.

- the effect of surgical castration of 5-day-old pigs on subsequent behavioural, endocrine and immune responses elicited by low-dose LPS challenge in pigs after weaning.

Experiment 1. Age-related changes in pro-inflammatory cytokines, acute phase proteins and cortisol concentrations in neonatal piglets

Introduction

In piglets, the neonatal period is a critical time during which they must adapt to the extra-uterine environment. These adaptations include qualitative and quantitative changes in the immune and endocrine systems (Kanitz *et al.*, 1999). In general, neonates have a depressed immune system compared to that of the adult (Blecha, 2001). This is in part due to the immaturity of the immune system, since immunity of newborn piglets is highly dependent on immunoglobulin acquisition through colostrum intake during the first 24 hours after birth (Wilson, 1974; Blecha, 2001). Due to this hormone's role in initiating parturition (First and Bosc, 1979; Whittemore, 1993), high concentrations of plasma cortisol are found in newborn piglets, which start to decrease almost immediately after birth (Kattesh *et al.*, 1990; Kanitz *et al.*, 1999). It is possible that this hormone is also responsible in part for depressing the immune function of neonates. A recent study showed that levels of the acute phase proteins haptoglobin (Hp) and major acute phase protein (pig-MAP) also vary during the postnatal life of the pig (Martín *et al.*, 2005). Thus, it is possible that these pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β), and other major acute phase proteins in pigs, such as C-reactive protein (CRP) and serum amyloid A (SAA) may be subjected to age-related changes. In addition, acute phase proteins are important in the recovery from and limitation of infection or tissue injury and trauma caused by inflammation (Eckersall, 2000). In most commercial pig units, newborn piglets are routinely subjected to managerial practices such as teeth clipping, ear notching and tail docking, which can cause injuries (Done *et al.*, 2003; Lewis *et al.*, 2005a) that could lead to an acute phase response. Hence, the objective of this study was to determine if age-related changes occur in the production of TNF-α, IL-1β, CRP, SAA and Hp in the neonatal pig; and verifying whether the invasive managerial practices to which newborn piglets are subjected, elicit an acute phase response.

Materials and Methods

Litters of not less than 7 piglets from 24 multiparous sows in the Moorepark minimal disease herd were used in this experiment. Litters were housed with their dams in identical farrowing rooms. On the day of birth, 2 male and 2 female piglets were selected in each litter on the basis of being nearest to the average body weight (BW) of the litter. Experimental piglets were randomly assigned to one of two treatment groups (1 male and 1 female per treatment and litter). Thus, piglets were ear notched, teeth clipped and tail docked (NCD) or left untreated (CON). Litters were randomly assigned to one of four sampling times at 1, 3, 5 or 7 days of age.. Blood samples from experimental piglets were taken by anterior vena cava puncture into lithium heparinised syringes [Vacutainer™, Unitech Ltd., Dublin 24, Ireland]. All samples were taken between 0845 and 0915 h. Plasma TNF-α and IL-1β were measured using commercially available solid phase ELISA tests specific for these porcine pro-inflammatory cytokines [Biosource International, Camarillo, CA, USA]. Concentrations of CRP, SAA and Hp were determined using a solid phase sandwich immunoassay [Tridelta Development Ltd., Maynooth, Co. Kildare, Ireland]. Plasma cortisol was determined by an enzyme immunoassay [DRG-Diagnostics, Marburg, Germany]. Concentrations of TNF-α, IL-1β, CRP, SAA, Hp and cortisol were analysed after log transformation using the GLM procedure [SAS®, 1999]. Data were

subjected to ANOVA to test for the main effects of age, treatment and gender, and for any interactions between these factors. The effect of gender was not significant ($P > 0.10$).

Results

Plasma levels of TNF-α were significantly affected by age ($P < 0.001$; Figure 1A). The lowest concentrations of TNF-α were found on day 1. Peak TNF-α concentrations were found on day 5, and remained elevated on day 7. Plasma TNF-α concentrations did not differ between the two treatment groups ($P > 0.10$). Plasma concentrations of IL-1β and CRP did not vary with age ($P > 0.10$) and did not differ between treatment groups ($P > 0.10$). There was a significant effect of age in plasma SAA level ($P < 0.001$; Figure 1B). Plasma SAA level was elevated on days 1, 3 and 5. Subsequently, there was a significant reduction on day 7. Concentration of plasma SAA was not affected by treatment ($P > 0.10$). Concentration of Hp varied significantly with age ($P < 0.001$; Figure 1C). Plasma Hp level was lowest on day 1 and increased with age. Plasma concentration of Hp tended to differ between treatment groups ($P = 0.066$). Levels of Hp in plasma of NCD pigs were higher than in CON animals. Plasma cortisol varied with #age of the animals ($P < 0.001$; Figure 1D). Peak plasma cortisol concentration was found on day 1 and progressively decreased with age until day 7. No significant effect of treatment was found ($P > 0.10$).

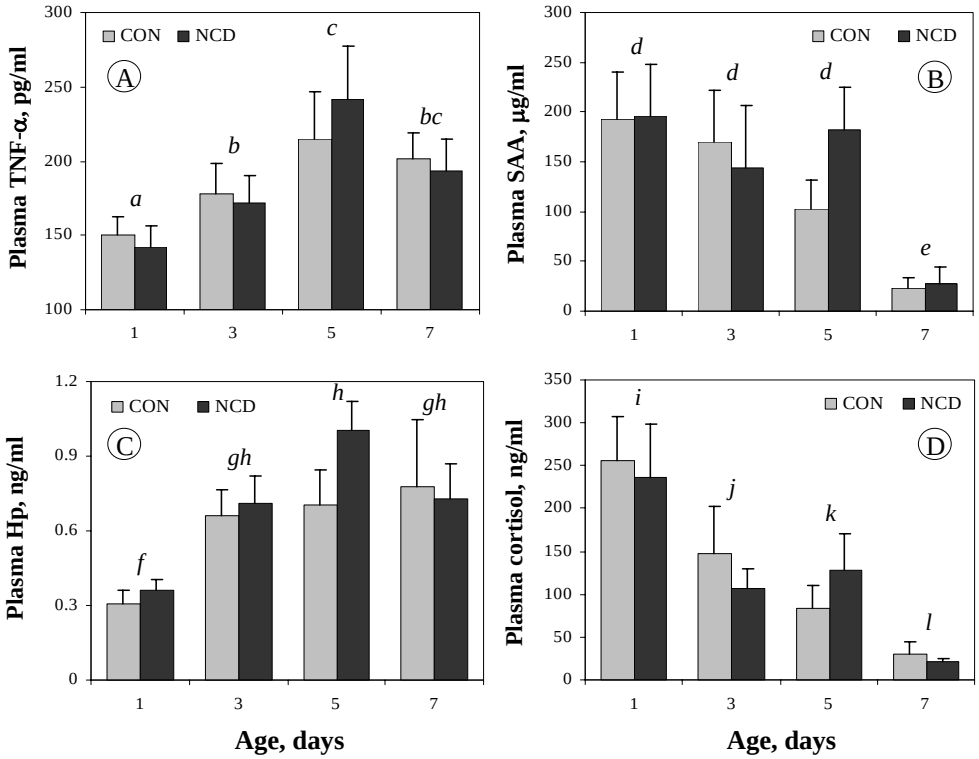

Figure 1. Plasma concentrations of [A] tumor necrosis factor-alpha (TNF-α), [B] serum amyloid A (SAA), [C] haptoglobin (Hp), and [D] cortisol in 1, 3, 5 or 7 day-old piglets subjected to ear notching, teeth clipping and tail docking (NCD) or left untreated (CON).

Conclusions

Piglets from the present study showed elevated concentrations of cortisol after birth on day 1 (Kattesh *et al.*, 1990). Subsequently, a 90% decrease in plasma cortisol was observed between day 1 and day 7. Furthermore, cortisol concentration on day 7 was similar to the level previously reported in piglets of this age (Kattesh *et al.*, 1990; Otten *et al.*, 2001). High cortisol levels are related to the initiation of parturition (First and Bosc, 1979). This study indicates that these elevated cortisol levels are still detectable in 1 day-old piglets. It is possible that parturition itself may have caused a rise in adrenal output, as is observed in human infants (Mears *et al.*, 2004). Plasma cortisol levels determined on day 1 to day 5 were higher than the normal concentrations of this hormone in pigs in resting conditions (Hessing *et al.*, 1994; Prunier *et al.*, 2005), and may arise from stress. During stress, cortisol provokes proteolysis in the muscle (Klassing, 1988). The amino acids released due to protein degradation during the acute phase response are subsequently utilised for acute phase protein synthesis by the liver (Klassing, 1988; Johnson, 1997). Thus, it is possible that the elevated levels of SAA and Hp found in the present study result from the high cortisol concentrations exhibited. In accordance with Martín *et al.* (2005), Hp level increased with age after birth. Although plasma SAA concentration also varied with age, the response of this acute phase protein was different to that for Hp. These age-related variations in SAA levels are similar to that described during the neonatal period in pigs for α_1-acid glycoprotein (α_1-AGP) (Martín *et al.*, 2005). Both SAA and α_1-AGP are considered to be type 1 acute phase proteins, which are characterised by a rapid response to stimuli (Petersen *et al.*, 2004). In contrast, type 2 acute phase proteins, such as Hp, present a later response with concentrations remaining elevated for weeks subsequent to the stimulus (Petersen *et al.*, 2004). Results from this study showed that levels of TNF-α in plasma increased after birth and peaked on day 5. The survival of newborn piglets is strongly dependent on the ingestion of colostrum, which provides them with passive immunity via intestinal uptake of immunoglobulins (Blecha, 2001; Martín *et al.*, 2005). In addition to immunoglobulins, porcine mammary secretions also contain cytokines, which may contribute to the protection of the neonate (Blecha, 2001). Nonetheless, these age-related increases in TNF-α levels found in pigs from the present study reflect maturation of immune cells and immune function (Matteri *et al.*, 1998.

In the present study, husbandry practices like teeth clipping, ear notching and tail docking did not cause changes in the parameters measured, with one exception. Hp levels in pigs subjected to these practices tended to be higher that those of pigs left untreated. Teeth clipping, ear notching and tail docking are known to cause injuries, including mouth and gum lesions, trauma and necrosis of the tail (Done *et al.*, 2003; Lewis *et al.*, 2005a), which probably elicited Hp synthesis and release by hepatocytes. In contrast, CRP and SAA concentrations were not affected by these procedures. Thus, Hp may be a more sensitive indicator of the trauma and tissue injury caused.

In conclusion, results from this study describe age-related changes in the concentration of pro-inflammatory cytokines and acute phase proteins in the neonate. These may reflect the development of the relatively immature immune system in the newborn piglet, but also suggests that the high cortisol levels exhibited after birth may influence the synthesis of acute phase reactants during this period.

Experiment 2. Influence of teeth resection on the skin temperature and acute phase response in newborn piglets

Introduction

Teeth resection of newborn piglets is widely practised. It reduces facial injuries to piglets during establishment of the 'teat order' (Lewis *et al.*, 2005a) and minimises damage to the sow's udder (Lewis *et al.*, 2005b). Teeth are generally clipped to the gum line using side-cutting pliers. This can result in mouth lesions due to the exposure of the pulp cavity when the stump of the tooth splinters (Lewis *et al.*, 2005a). The use of rotating electric grinders minimises this problem (Lewis *et al.*, 2005a). However, grinding takes longer and hence involves more handling (Lewis *et al.*, 2005a). Resection of piglets' teeth is thought to be painful (SVC, 1997; Hay *et al.*, 2004) and changes in the normal behaviour of piglets immediately after teeth resection have been shown (Noonan *et al.*, 1994). However, the influence of teeth resection on measures of immune function or the endocrine response has not been researched.

Studies with rodents showed that restraint reduces tail-skin temperature (Wright and Katovich, 1996). The temperature of the skin is under control of the peripheral vascular system, which is sensitive to handling and restraint (Wright and Katovich, 1996). Hence, it is possible that the stress inherent in teeth resection (Noonan *et al.*, 1994; SVC, 1997) activates the sympathetic nervous system eliciting a fall in skin temperature. In addition, trauma to the oral mucosa can lead to infectious and inflammatory processes. Hence, alterations in concentrations of 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 (Heegard *et al.*, 1998) in pigs.

The objective of the first experiment presented here was to determine if two teeth resection procedures influence skin temperature and activate the acute phase response. The results indicated an effect of teeth resection on skin temperature but this was confounded by the longer time taken to process/handle piglets. In order to elucidate whether the reduction in temperature was part of the stress/pain reaction to 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

In experiment 1, litters of not less than 7 piglets from 60 multiparous sows in the Moorepark herd were used. Litters were housed with their dams in identical farrowing rooms. Treatments were applied after the piglets were weighed individually. 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 either 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 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, allowing the measurement of skin

temperature without restraint. This measurement was recorded immediately after the management procedures were applied once the piglet was back in the farrowing crate. Twenty-four hours after the managerial procedures, blood samples (5 ml) from focal piglets (1 male and 1 female) from 7 litters per treatment selected on the basis of being nearest the average litter body weight (BW) were taken by anterior vena cava puncture into lithium heparinised syringes (Vacutainer^{TM}, Unitech Ltd., Dublin 24, Ireland). The same focal animals were blood sampled 24 h after weaning at 28 days of age using the same technique. Plasma samples were analysed for concentrations of CRP and SAA using solid phase sandwich immunoassays (Tridelta Development Ltd., Maynooth, Co. Kildare, Ireland). Plasma cortisol was also determined by an enzyme immunoassay [DRG-Diagnostics, Marburg, Germany].

In experiment 2, litters of not less than 7 piglets from 72 multiparous sows were used. Data were collected over a 9-week period. The experiment was designed as a 2 x 2 factorial, completely randomised block design, where the effect of teeth clipping and time spent out of the farrowing crate were considered. Thus, piglets had their teeth left intact (INT) or clipped (CLIP), and were either returned immediately to the farrowing crate (0-minute) or held in a box for approximately 1-minute 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 teeth in experiment 1 (approximately 56 seconds). 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. 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). Skin temperature was measured immediately after piglets were returned to the farrowing crate (SkinT^E-0) and 10 minutes later (SkinT^E-10) using the same infrared thermometer. 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.

Data collected in experiment 1 were analysed as a complete randomised design using the GLM procedures of SAS® [1999]. Skin temperature data were subjected to ANOVA for the main effect of treatment using the time taken to impose the procedure and week as covariates. Concentrations of CRP, SAA and cortisol on day 1 were analysed by ANOVA to test for main effects of treatment and gender, 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 ($P > 0.10$). Single degree-of-freedom orthogonal contrast analysis was used to study the statistical differences between the treatment groups. In experiment 2, the GLM procedure was also used. SkinT^E-0 data were subjected to ANOVA for the main effects of treatment and time-out, using room T^E, time taken to apply the procedures, BW and crate T^E as covariates. SkinT^E-10 was also subjected to ANOVA for the main effects of treatment, time and litter distribution and all possible interactions. SkinT^E-0 was used as a covariate in the analysis of SkinT^E-10 data.

Results

Results from experiment 1 showed that treatment had a significant effect on skin temperature ($P < 0.05$; Figure 2) 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 treatment groups was significantly lower in comparison to piglets from the INT group ($P < 0.05$). However, no significant differences were found in skin temperature between GRIND and CLIP piglets ($P > 0.10$). The time taken to impose the procedures was different between all three treatments ($P < 0.001$; Figure 2).

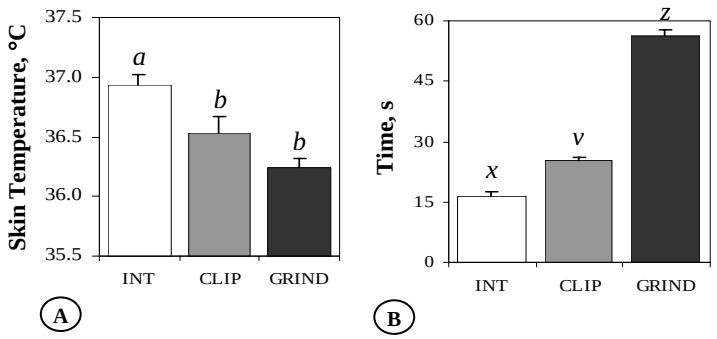

Figure 2. Skin temperature [A] and time taken to apply the procedures [B] in pigs with intact (INT), clipped (CLIP) or ground (GRIND) teeth.

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 on the CLIP treatment had higher plasma concentrations of CRP than pigs on the GRIND treatment ($P < 0.05$; Figure 3). No effect of treatment was found in SAA plasma concentrations of 1-day-old and 29-day-old pigs ($P > 0.10$). 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 ($P < 0.05$; Figure 3). GRIND piglets had significantly higher levels of plasma cortisol in comparison to CLIP piglets ($P < 0.05$) and were also significantly different to the INT group, albeit at the 10% level ($P = 0.078$). On day 29, plasma concentrations of cortisol were significantly reduced in comparison with cortisol levels determined on day 1 ($P < 0.001$).

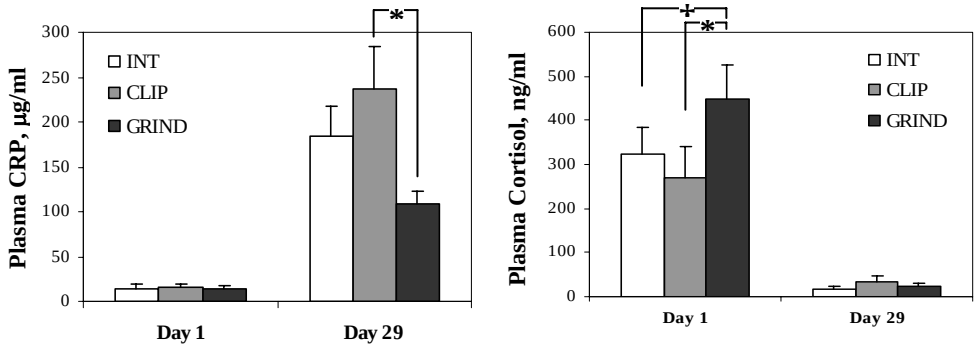

Figure 3. Plasma concentrations of C-reactive protein (CRP) and cortisol of pigs with intact (INT), clipped (CLIP) or ground (GRIND) teeth on days 1 and 29 of age.

Results from experiment 2 showed that clipping caused a reduction in skinT^{E}-0 ($P = 0.051$; Figure 4). No significant differences were found between the two time-out groups ($P > 0.1$;

Figure 4). The time taken to apply the procedures tended to have a significant effect on skinT^E-0 (P = 0.059). The average time taken to process the piglets also differed significantly between the two treatments (P < 0.001). Crate T^E (P < 0.001) and room T^E (P < 0.05) significantly influenced skinT^E-0 measurements.

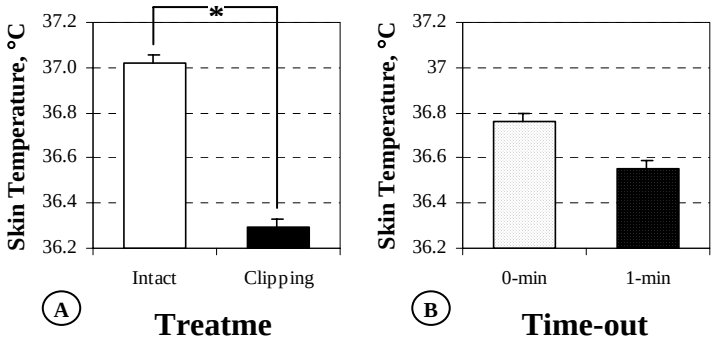

Figure 4. Effect of [A] treatment (intact or clipping) and [B] time-out (0 or 1 minute) on skin temperature of piglets immediately after returning to the farrowing crate [skinT^E-0].

There were no treatment or time-out effects regarding skinT^E-10 data (P > 0.10). SkinT^E-10 was influenced significantly by skinT^E-0 (P < 0.001). Litter distribution significantly affected skinT^E-10 (P < 0.001; Figure 5.6) irrespective of the treatment (P > 0.10). SkinT^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).

Conclusion

The results from both experiments indicate that teeth resection causes 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; SVC, 1997) leading to activation of the sympathetic nervous system. Teeth resection constitutes a stressor due to the pain from the resection of the teeth (Hay *et al.*, 2004) but also due to the handling and restraint involved (Noonan *et al.*, 1994). The time taken to impose the teeth resection 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. Thus, it is likely that the pain associated with teeth resection was a more important factor in the reduction in skin temperature than handling and restraint. The reduction in skin temperature was short-lived and was no longer evident 10 minutes after resection. Therefore, teeth resection is a transient stressor. These findings also show that providing extra heat within the farrowing crate could ameliorate the possible consequences of the observed reduction in skin temperature after teeth resection.

Results from this study showed that the levels of CRP and SAA determined in 1 day-old piglets were low in comparison to 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). 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 had facial skin lesions at weaning (Lewis *et al.*, 2005a). Furthermore, piglets with resected teeth had more mouth lesions immediately prior to weaning (Lewis *et al.*, 2005a). Hence, the inflammatory and infectious processes were likely activated in 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, whereas pigs with clipped teeth presented 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). 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 HPA axis when these piglets were subjected to the subsequent stress of blood sampling (Kanitz *et al.*, 2004).

In conclusion, 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 is preferable to clipping since the latter method may have more negative long-term welfare implications.

Experiment 3. Effect of surgical castration on the behavioural and physiological responses of 5-day-old piglets

Introduction

In most countries male pigs are surgically castrated to avoid problems with boar taint. However, surgical castration is painful and consequently it constitutes an important welfare problem (SCV, 1997; EFSA, 2004). Higher frequency, intensity and duration of vocalisations have been reported during castration of pigs (Taylor and Weary, 2000; Taylor *et al.*, 2001). This is accompanied by increases in resistance movements and in heart rate (White *et al.*, 1995). Immediately after surgical castration, there is an activation of the hypothalamus-pituitary-adrenal (HPA) and sympathetic axes, which lead to increases in plasma adrenocorticotropic-releasing-hormone (ACTH) and cortisol (Prunier *et al.*, 2005). Alterations in the behaviour of castrated pigs show that pigs experience discomfort for up to 5 days after castration (McGlone and Hellman, 1988; McGlone *et al.*, 1993; Hay *et al.*, 2003). Less information is available on the physiological effects during the days following surgical castration (EFSA, 2004). Due to the surgical nature of castration, this practice inevitably causes an inflammatory reaction. Tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) are pro-inflammatory cytokines capable of triggering the acute phase response by inducing hepatocytes to synthesise acute phase proteins such as C-reactive protein (CRP), serum amyloid A (SAA) and haptoglobin (Hp) (Baumann and Gauldie, 1994). The objective of this study was to evaluate the behavioural and physiological responses of 5-day-old pigs to surgical castration, assessing the prevalence of these responses on the days following the procedure.

Materials and Methods

Litters of not less than 7 piglets and at least 4 males from 10 multiparous sows from the minimal disease Moorepark herd were used in each of two separate experiments. Litters were housed with their dams in identical farrowing rooms. Piglets could move freely around the pen and had access to a nipple water drinker. All piglets were subjected to ear notching, teeth clipping and tail docking within 24 h of birth. On day 5, four male piglets were selected in each litter on the basis of being nearest the average litter body weight (BW). These were randomly assigned to one of two treatments, where piglets were either surgically castrated or left intact. Selected piglets were individually removed from the farrowing house to an isolated but adjacent area where treatments were applied. During castration, piglets were restrained on a narrow wooden bench. Surgical castration was performed by removing the testes and epididymides through two incisions made on the previously disinfected scrotum (one over each testis) followed by the manual extraction of the testis. A topical disinfectant was applied on the ano-genital area before and after castration. Intact (handled) animals were restrained for the same amount of time and washed in the same way as their castrated littermates, in order to control for possible temporal effects of restraint and handling. All treatments were applied between 0900 and 1000 h.

In experiment 1, behaviour observations started at 1400 h on the day that treatments were imposed and lasted for 3 hours. During this time, behaviour was recorded by instantaneous scan sampling every three minutes, when posture, activity, location and body contact of all focal piglets were verified. Activities were classified as pain-related, originated by surgical castration, and non-specific. In experiment 2, the behaviour of experimental piglets was recorded on the day of castration and on three consecutive days thereafter. During these observations, behaviour of focal piglets was recorded by instantaneous scan sampling every

three minutes, when posture, activity, body contact and social cohesion parameters of experimental animals were recorded. Activities were classified as pain-related and non-specific. All observations were carried out by a single trained observer. Blood samples were taken by anterior vena cava puncture into lithium heparinised syringes [Vacutainer^{TM}, Unitech Ltd., Dublin 24, Ireland]. In experiment 1, blood samples were taken before (0 h) and 1, 2, 3 and 4 h after treatments were imposed. In experiment 2, blood samples were taken before (0 h) and 12, 24, 48 and 72 h after imposing the treatments. In both experiments, a total of 4 pigs per treatment (castrated or handled) were sampled at each of the sampling times. Plasma samples from experiment 1 were analysed for pro-inflammatory cytokines TNF-α and IL-1β using commercially available solid phase ELISA tests [Biosource International, Camarillo, CA, USA]. Plasma samples were also analysed for cortisol concentrations using an enzyme immunoassay [DRG-Diagnostics, Marburg, Germany]. Plasma samples from experiment 2 were analysed the acute phase proteins CRP, SAA and Hp using commercially available solid phase sandwich immunoassays [Tridelta Development Ltd., Maynooth, Co. Kildare, Ireland].

Behavioural data collected in experiment 1 were statistically analysed as a complete randomised designed using the GLM procedure [SAS®, 1999]. Data were subjected to ANOVA to test for the main effect of treatment. In experiment 2, behavioural data were analysed using PROC MIXED. The model included fixed effects of treatment and observation period and their interaction. Tukey's Test was used to establish pair-wise differences between treatment groups on each individual observation period. Data from plasma analysis were subjected to ANOVA to test for main effects of treatment and sampling time

Results

In experiment 1, castrated piglets spent significantly less time engaged in locomotory activities ($P < 0.05$), specifically walking ($P < 0.05$). Castrates tended to spend more time massaging the udder in comparison to handled piglets ($P = 0.075$). Castrated piglets were engaged in more exploratory activities such as chewing and/or licking ($P < 0.05$) and also showed a higher occurrence of scratching ($P < 0.05$). Castrated pigs exhibited significantly more pain-related activities ($P < 0.001$), in particular huddling ($P < 0.05$). No significant differences were found in time spent in different postures and the location of piglets that were studied ($P > 0.10$). However, castrated piglets spent significantly more time in contact with the sow in comparison with their handled littermates ($P < 0.05$). In experiment 2, behavioural data collected over a 4 day-period showed a significant treatment effect in the occurrence of dog-sitting. Castrated piglets spent less time in this posture compared with intact animals ($P < 0.01$). This trend was evident throughout the experiment, in particular on day 4 in the PM period ($P < 0.01$). No significant differences were found regarding any other posture ($P > 0.10$). Castrated pigs also showed a higher occurrence of pain-related activities ($P < 0.05$). A significant treatment by time interaction showed that pain-related activities were significantly higher on day 1 AM ($P < 0.001$). Castrated pigs huddled ($P < 0.001$), exhibited spasms ($P < 0.01$) and were trembling ($P < 0.05$). Handled animals showed none of these pain-related behaviours. A tendency towards a significant effect of treatment indicated that castrated pigs engaged less in playful activities ($P = 0.083$). Castrated piglets avoided social contacts ($P < 0.05$) and in particular tended to avoid contact with their littermates ($P = 0.061$). Throughout the duration of the experiment, castrated piglets tended to be more isolated ($P = 0.084$) and desynchronised ($P = 0.072$) than their handled littermates.

No significant effect of treatment was found in plasma concentrations of TNF-α ($P > 0.10$). A significant effect of sampling time showed that concentrations of this cytokine in plasma varied over the different sampling times ($P < 0.001$). Peak concentrations of TNF-α in plasma were

found 3 h after treatments were imposed, returning subsequently to basal levels. Concentrations of plasma IL-1β did not differ between treatment groups ($P > 0.10$). A significant time effect showed variation in the levels of IL-1β in plasma ($P < 0.01$). Peak plasma IL-1β concentrations were detected 2 and 3 h after treatments were imposed. Plasma levels of cortisol of castrated piglets tended to be higher than that of their handled littermates (means ± SEM handled vs. castrated: 111.96 ± 23.86 ng/ml vs 143.38 ± 27.38 ng/ml; $P = 0.093$). A significant effect of time was also found ($P < 0.001$). Plasma cortisol levels were elevated at 1 and 2 h, with peak concentrations occurring 3 h after treatments were imposed. Subsequent to this sampling time, concentrations of cortisol returned to basal levels. Analysis of plasma samples in experiment 2 showed no significant effect of treatment on plasma CRP ($P > 0.10$). A significant effect of sampling time ($P < 0.05$) showed that CRP concentration decreased 48 h after treatments were imposed. No significant effect of treatment or sampling time was found in plasma SAA ($P > 0.10$). Plasma Hp did not differ between handled and surgically castrated piglets ($P > 0.10$). A significant effect of sampling time ($P < 0.01$) showed that plasma Hp decreased subsequent to 24 h after treatments were imposed.

Conclusions

In agreement with previous studies, results from direct behavioural observations indicated that surgical castration causes pain (McGlone *et al.*, 1993; Taylor *et al.*, 2001; Hay *et al.*, 2003). Castrated piglets displayed pain-related activities throughout the experiment and specifically during the day of castration (Hay *et al.*, 2003). These included huddling, trembling and spasms. Nonetheless, as reported by several authors, surgical castration also altered other non-specific activities and postures that are normally displayed (McGlone *et al.*, 1993; Taylor *et al.*, 2001; Hay *et al.*, 2003). It is possible that certain activities such as walking and postures like dog-sitting were avoided by castrates in an effort to minimise pain. In addition, castrated piglets were less playful. Play, which is a behaviour characteristic of young, developing animals, is indicative of good welfare, since is said to occur only when all basic needs are satisfied. These behavioural adaptations can be described as protective, allowing animals to avoid or reduce the stimulation of painful tissues (Mellor *et al.*, 2000). In the present study, the occurrence of scratching was higher among castrated animals, which seems to reflect discomfort experienced after the surgical procedure (Hay *et al.*, 2003). Results from experiment 1 showed that castrated piglets were more inclined to massage the udder of the sow. Teat-seeking activities have been observed in piglets after being subjected to painful procedures (Taylor *et al.*, 2001). This behaviour is known to help animals to cope with stress (Noonan *et al.*, 1994) and it may constitute a way of indicating pain, since piglets of this age are still fully dependant on the sow (Taylor *et al.*, 2001). Furthermore, suckling has been reported to have analgesic effects in human infants and rat pups in response to surgical or heat pain (Blass, 1994). In experiment 2, castrated piglets spent more time alone (i.e. not in contact) and in particular, avoiding social contact with their littermates. Isolation is likely to be a behavioural adaptation with a protective role, which may be adopted in order to stop other animals from inflicting more pain (Mellor *et al.*, 2000; Hay *et al.*, 2003). Hence, results obtained in the present study indicate that pigs can adopt different behavioural strategies for coping with pain. Direct pain prevention may be achieved by avoiding postures and social contacts that could aggravate pain. In addition, pigs can also relieve the pain caused by surgical castration by adopting behaviours and/or activities with analgesic effects.

In the present study plasma cortisol in castrated pigs tended to be higher than in handled pigs. This indicates that castration activated the adrenal end of the HPA axis. However, results showed that handling also provoked an increase in the adrenal output of handled pigs. Thus,

although cortisol levels were numerically higher in castrated pigs 1, 2 and 3 h after treatments were imposed, possible treatment by time interactions may have been masked. This effect of handling would also explain the time effects in pro-inflammatory cytokine levels. Results from this study showed no treatment differences in plasma levels of CRP, SAA or Hp. However, concentrations of plasma CRP and Hp decreased at 48 h regardless of the treatment, reaching levels of these acute phase proteins that were previously described in pigs in normal conditions (Heegaard *et al.*, 1998). Thus, it is possible that CRP and Hp levels were elevated before treatments were imposed. Piglets used in the present study were subjected at 1 day of age to common husbandry practices including ear notching, teeth clipping and tail docking. Several authors have reported injuries and tissue damage as a result of these practices (Done *et al.*, 2003; Lewis *et al.*, 2005a). Hence, these managerial practices may have caused an inflammatory process prevalent for several days, which may have masked the acute phase response after surgical castration.

In conclusion, results from this study highlight the value of behavioural observations for assessing pain-induced distress after castration. Surgical castration caused specific pain-related behaviours, and also altered the occurrence of behaviours normally displayed by piglets. In general, these behavioural alterations were adopted to minimise stimulation of affected tissues, due to a specific activity or posture or by action of littermates. In contrast, pro-inflammatory cytokines and acute phase proteins were not relevant for monitoring the physiological consequences following surgical castration of piglets.

Experiment 4. Pro-inflammatory cytokine and acute phase protein responses to low-dose lipopolysaccharide (LPS) challenge in pigs

Introduction

Lipopolysaccharide (LPS), an intrinsic component of the outer membrane of gram-negative bacteria, has frequently been used as a model to study immune-neuroendocrine interactions in pigs (Wright *et al.*, 2000; Kanitz *et al.*, 2002; Tuchscherer *et al.*, 2004). LPS provokes the synthesis and release of cytokines by macrophages and neutrophils (Feghali and Wright, 1997; Johnson, 1997). The pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6) are capable of triggering the acute phase response by inducing hepatocytes to synthesise acute phase proteins such as C-reactive protein (CRP), serum amyloid A (SAA) and haptoglobin (Hp) (Baumann and Gauldie, 1994). LPS also activates the hypothalamus-pituitary-adrenal (HPA) axis via pro-inflammatory cytokine stimulation, resulting in increased secretions of glucocorticoids (Johnson *et al.*, 1996; Warren *et al.*, 1997). Low-dose endotoxin challenges have been successfully used to study the time response of TNF-α, IL-6 and cortisol in pigs (Warren *et al.*, 1997; Webel *et al.*, 1997). *In vivo* challenges with low doses of LPS can simulate sub-acute inflammation/infection and therefore give valuable information regarding the use of these acute phase response products as indicators of subtle conditions where the health and welfare of pigs may be also compromised. In most studies, determination of levels of pro-inflammatory cytokines and acute phase proteins is based upon blood collection (Webel *et al.*, 1997; Kanitz *et al.*, 2002; Frank *et al.*, 2005). However, blood sampling of pigs has several limitations. Hence, there is a growing interest in developing less-invasive techniques for the measurement of immune-related parameters. Dugué *et al.* (1996) determined levels of pro-inflammatory cytokines in saliva of humans after a heat stressor (sauna). Thus, it is possible that pro-inflammatory cytokines and acute phase proteins can also be detected in saliva of pigs after administration of a low dose of LPS.

Studies in rodents and humans have shown that gender differences exist at various levels of the HPA axis (Spinedi *et al.*, 1992; Gaillard and Spinedi, 1998; Da Silva, 1999). Furthermore, gender differences have also been established in both the humoral and cell-mediated immune responses (Olsen *et al.*, 1996). Gender differences in the immune response occur in response to direct action of gonadal steroids, but also indirectly, via the HPA axis (Gaillard and Spinedi, 1998). Thus, it is possible that gender differences may exist in the pro-inflammatory cytokine and acute phase protein responses, since pro-inflammatory cytokine synthesis is inhibited by the end products of the HPA axis (Sapolsky *et al.*, 2000).

The aim of this study was to determine the pro-inflammatory cytokine and acute phase protein responses to sub-acute inflammation/infection simulated by low-dose LPS challenge in pig plasma; and to evaluate whether these immune parameters could also be measured in saliva. The effect of gender on the acute phase reaction was also assessed.

Materials and Methods

Forty-eight crossbred [Duroc males x (LW x LR) females] pigs (24 males, 24 females) were selected from the minimal disease herd at Moorepark Research Centre. At one day of age, all piglets were subjected to ear notching, teeth clipping and tail docking. Male piglets were left

intact (i.e. not castrated). Selected pigs had an average body weight (BW) of 7.75 ± 0.88 kg at weaning at 4 weeks of age, and were housed in pairs in adjacent pens with a total of 0.94 m^2 of floor space (85 cm x 110 cm). Pairs consisted of two pigs of the same gender, and similar weight, originating from the same litter if possible. Pigs were maintained in an environmentally controlled building for 2 weeks prior to the experiment in order to allow adaptation to the new environment. The experiment took place when pigs were 6 weeks of age (average BW ± s.e.: 11.00 ± 1.05 kg). Each pen of two pigs was randomly assigned to one of two treatments and assigned to one of six sampling times (0, 2, 4, 8, 12 or 24 h after injection) when matched saliva and blood samples were collected. At 0900 h, following BW determination, pigs were injected intraperitoneally (i.p.) with 0.1 ml/kg of BW a solution of sterile saline containing 0 or 5 μg of LPS. At each sampling time a total of eight pigs were saliva and blood sampled, consisting of one male and one female pen (2 pigs/pen) per treatment (saline or LPS). Saliva samples were obtained by allowing the pig to chew a cotton bud [Salivette™, Sarstedt, Wexford, Ireland] until it was thoroughly soaked with saliva. The experimenter remained outside the pen, thereby minimising disturbance to the animals. In all cases, saliva was collected prior to blood sampling in order to eliminate the stressful effect of invasive blood collection. Blood samples were taken by anterior vena cava puncture, into lithium heparinised syringes [Vacutainer™, Unitech Ltd., Dublin, Ireland]. Levels of TNF-α and IL-1β in plasma and saliva were measured using a commercially available solid phase ELISA specific for these porcine pro-inflammatory cytokines [Biosource International, Camarillo, CA, USA]. Concentrations of CRP, SAA and Hp were determined using solid phase sandwich immunoassays [Tridelta Development Ltd., Maynooth, Co. Kildare, Ireland]. Plasma cortisol was determined by an enzyme immunoassay [DRG-Diagnostics, Marburg, Germany].

Data from plasma and saliva samples were analysed as a completely randomised design using the GLM procedure [SAS®, 1999]. All data were subjected to ANOVA to test for main effects of treatment, gender and sampling time, and for interactions between these factors. The significance of differences between c groups was analysed using the least significant differences from the combined analysis.

Results

There was an effect of treatment ($P < 0.001$), time ($P < 0.001$) and treatment by time interaction ($P < 0.001$) on plasma concentrations of TNF-α (Figure 5A). Two hours post-treatment, LPS-treated pigs had higher plasma concentrations of TNF-α than saline-treated pigs ($P < 0.001$), returning to basal levels by 12 h. In addition, the overall response of TNF-α differed between males and females ($P < 0.001$) irrespective of the treatment (Figure 5B). Whereas the mean plasma TNF-α concentration (± s.e.) of male pigs was 6.57 ± 0.51 pg/ml, the mean concentration in the plasma of female pigs was below the detection limit of the assay.

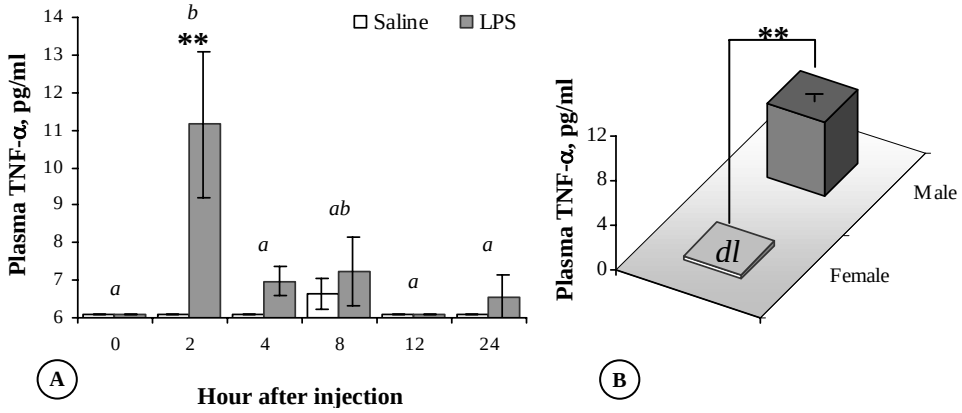

Figure 5. Plasma concentration of tumor necrosis factor-alpha (TNF-α) in pigs following lipopolysaccharide (LPS) challenge, where pigs were injected i.p. with 0.1 ml/kg of BW of a solution of sterile saline containing 0 or 5 μg of LPS from *E. coli*. [A] Time-response curve of plasma TNF-α. [B] Effect of gender on plasma TNF-α levels.

Plasma concentrations of IL-1β were not affected by the administration of LPS ($P > 0.10$). No significant effect of time or any of the interactions between main effects were found ($P > 0.1$; Figure 6A). However, plasma IL-1β concentrations differed between genders ($P < 0.05$; Figure 6B).

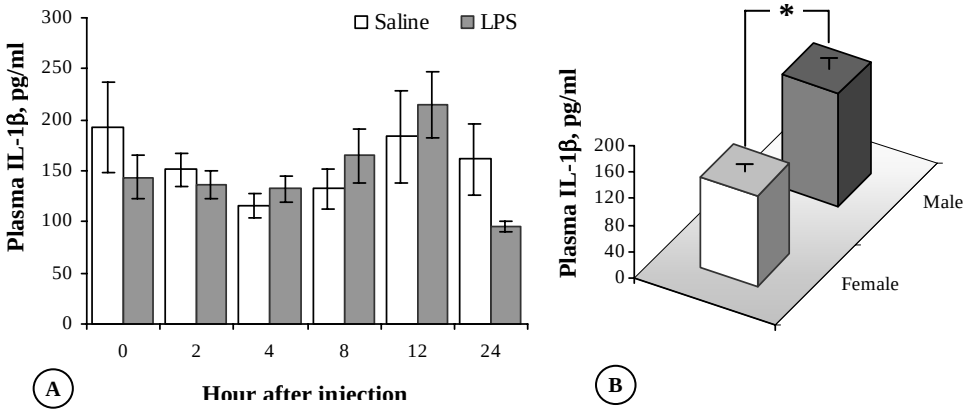

Figure 6. Plasma concentration of interleukin-1beta (IL-1β) in pigs following lipopolysaccharide (LPS) challenge, where pigs were injected i.p. with 0.1 ml/kg of BW of a solution of sterile saline containing 0 or 5 μg of LPS from *E. coli*. [A] Time-response curve of plasma IL-1β. [B] Effect of gender on plasma IL-1β levels.

A significant effect of treatment ($P < 0.05$), time ($P < 0.05$) and their interaction ($P < 0.05$) was found in plasma concentrations of CRP (Figure 7). Plasma CRP concentrations of LPS-treated pigs were higher than levels determined in saline-treated animals. Plasma CRP levels of saline-treated pigs did not vary over the 24 h period ($P > 0.10$). However, a 3-fold increase in CRP levels in the plasma of LPS challenged animals resulted in a difference between the two treatments at 12 h ($P < 0.01$). Concentrations of CRP in plasma of LPS-treated pigs were still elevated 24 h after LPS administration. No gender effect was found ($P > 0.10$).

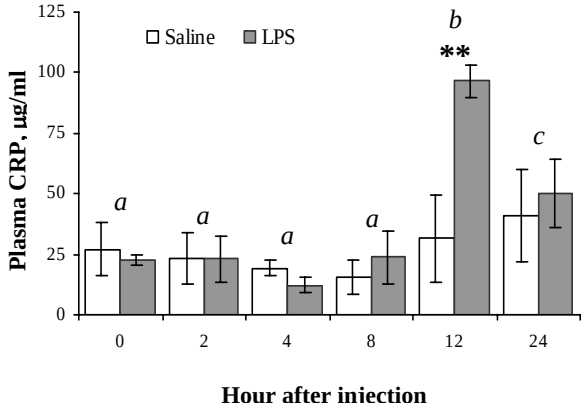

Figure 7. Plasma C-reactive protein (CRP) levels in pigs following lipopolysaccharide (LPS) challenge. Pigs were injected i.p. with 0.1 ml/kg of BW of a solution of sterile saline containing 0 or 5 µg of LPS from *E. coli*.

Plasma concentrations of SAA differed between treatments ($P < 0.05$). LPS-treated pigs had higher values than saline-treated pigs. No gender effects were found ($P > 0.10$). Administration of LPS did not increase plasma Hp ($P > 0.10$). No effect of gender was found ($P > 0.10$).

Treatment tended to have an effect on plasma cortisol ($P = 0.056$) and an effect of time was also found ($P < 0.01$; Figure 8A). Pigs on the LPS treatment had higher plasma cortisol than pigs on the saline. There was also a significant treatment by gender interaction ($P < 0.05$; Figure 8B). Plasma cortisol concentrations in response to the LPS challenge were higher in females than in males ($P < 0.05$, whereas basal concentrations did not differ between genders ($P > 0.10$).

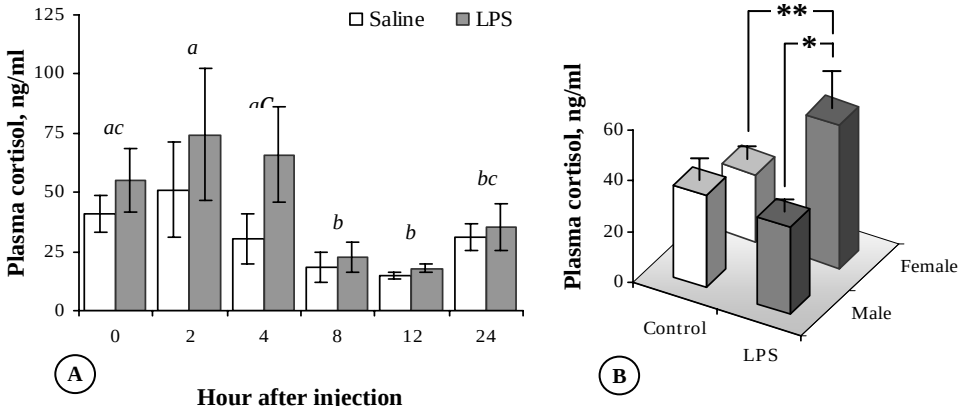

Figure 8. Plasma concentration of cortisol in pigs following lipopolysaccharide (LPS) challenge, where pigs were injected i.p. with 0.1 ml/kg of BW of a solution of sterile saline containing 0 or 5 µg of LPS from *E. coli*. [A] Time-response curve of plasma cortisol. [B] Effect of treatment by gender interaction on plasma cortisol levels.

Administration of LPS reduced salivary output, making saliva collection impossible in some cases. In addition, it proved difficult to collect sufficient volumes of saliva from each pig to facilitate the measurement of all the physiological parameters. Nonetheless, TNF-α and IL-1β levels were consistently detectable in all of the saliva samples. Furthermore, concentrations in saliva of both cytokines were significantly higher than in plasma ($P < 0.001$). In contrast, determination of CRP in saliva was not possible due to inconsistencies in its detection. This was reflected by the fact that out of 38 undiluted saliva samples analysed, only 6 could be numerically quantified. Similarly, only a few undiluted saliva samples had detectable SAA levels despite the large number of samples tested.

Conclusions

Results from our experiment showed increases in plasma TNF-α levels that were comparable temporally, although not in magnitude, to the increases reported by other authors (Warren *et al.*, 1997; Webel *et al.*, 1997). The results of this experiment also showed that low-dose LPS challenge was insufficient to induce increases in plasma levels of IL-1β, despite the increased plasma TNF-α production. Burrell (1990) described LPS as the most potent stimulant of TNF-α synthesis. However, it is also possible that the low dose of LPS may account for the lack of increases in plasma IL-1β. In the current study, administration of a low dose of LPS induced the hepatic acute phase response, subsequent to the production of pro-inflammatory cytokines. Hence, significant increases in the concentration of plasma CRP and SAA were observed. CRP production was more prominent as indicated by the peak recorded 10 h after the highest plasma TNF-α levels. The delay in time between TNF-α and CRP peaks is a reflection of the time required for the synthesis and release of TNF-α, its interaction with hepatocyte receptors, the production of CRP by hepatocytes and its accumulation in the plasma compartment (Richards, 1998). SAA concentrations in plasma increased after LPS administration but did not show any marked peak. Although Hp is one of the most studied acute phase protein in pigs (Petersen *et al.*, 2004), plasma levels were not altered after aseptic inflammation with a low dose of LPS. Administration of a low dose of LPS also activated the HPA axis, as indicated by a strong tendency for plasma cortisol to increase in LPS-treated pigs. Hence, results from this study indicate that plasma levels of CRP and SAA, but not Hp, were altered in response to low-dose LPS challenge and therefore they may be useful as indicators of sub-acute inflammation/infection in pigs.

In agreement with Dugué *et al.* (1996), our results showed consistency in the detection of TNF-α and IL-1β in saliva of pigs. Results showed that salivary concentrations of both pro-inflammatory cytokines were higher than those determined in plasma. TNF-α and IL-1β have a molecular size of approximately 17 kDa and they are lipophobic molecules. Although transportation from the blood stream into the saliva may have occurred, it is known that salivary gland epithelial cells can synthesise a wide variety of cytokines (Sugawara *et al.*, 2002). In contrast, determination of CRP and SAA in pigs' saliva was not successful. It is likely that the molecular size of both porcine CRP and SAA, 115 and 180 kDa respectively (Petersen *et al.*, 2004), impedes transport into the salivary stream (Vining *et al.*, 1983).

Gender differences reported in the present study are likely to reflect the interaction between the HPA axis, the hypothalamus-pituitary-gonadal (HPG) axis and the immune system (Da Silva *et al.*, 1999). Sex steroids regulate HPA axis activity, with androgens inhibiting and oestrogens enhancing its responsiveness to stress (Da Silva *et al.*, 1999). Thus, female pigs presented higher levels of cortisol in response to low-dose LPS challenge. Although several publications have reported the absence of gender effects in HPA axis activity in pigs after LPS administration (Webel *et al.*, 1997) or other noxious stimuli (de Jong *et al.*, 1998; de Groot *et al.*, 2001), castrated male pigs were used by these authors. As a consequence of the removal of the testes, castration results in the lack of male sexual hormones. Therefore, castrates were reported to have a more responsive HPA axis similar to that of females (de Groot *et al.*, 2001). The development of the male gonad in the pig is characterised by a high degree of differentiation of Leydig cells, which is associated with an increased production of steroids, from the late foetal life to a peak at 3-5 weeks after birth (Schwarzenberger *et al.*, 1993). Thus, it is possible that male pigs used in the present study were under the influence of relatively high levels of male sexual hormones. In addition, gender differences were found in plasma concentrations of both TNF-α and IL-1β. Taking into account that the HPA axis activity was higher in females, it seems that the inhibitory actions of glucocorticoids on pro-inflammatory cytokine production

(Sapolsky *et al.*, 2000) were more pronounced in this gender group, and therefore higher concentrations of both cytokines were found in males.

In conclusion, results from this study indicate that TNF-α, CRP and SAA are useful indicators of sub-acute inflammation/infection in pigs as simulated by low-dose LPS challenge. The current study also shows that pro-inflammatory cytokines are detectable in saliva of pigs, although further work is necessary in other to validate their use as less-invasive welfare indicators. Finally, gender differences exist in the pro-inflammatory cytokine response, probably due to the differential adrenal response in males and female pigs to the low dose of LPS used in this study.

Experiment 5. Surgical castration of pigs affects the behavioural but not the physiological responses to a low-dose lipopolysaccharide (LPS) challenge after weaning

Introduction

In the majority of countries, male pigs are routinely castrated to avoid problems with boar taint (EFSA, 2004). However, surgical castration constitutes a severe stressor as indicated by alterations in the vocalisation pattern during castration (Taylor and Weary, 2000), significant rises in cortisol synthesis (Prunier *et al.*, 2005) and changes in the behaviour of pigs (McGlone and Hellman, 1988; McGlone *et al.*, 1993; Hay *et al.*, 2003). It is possible that subjecting piglets to this acute stressor early in their life may affect their ability to respond and cope with subsequent stressors. In this regard, surgical castration of male pigs has been shown to have long term effects. For example, De Kruijf and Welling (1988) showed an increased incidence of chronic inflammation in castrates at slaughter in comparison with gilts. Furthermore, Van Erp-Van der Kooij *et al.* (2000) reported differences between castrates and female pigs in their response to a backtest (measurement of escaping behaviour). Lessard *et al.* (2002) showed that castration of 10-day-old or older pigs depresses antibody responses and modifies lymphocyte mitogenic reactions 21 days after castration. Finally, Frank *et al.* (2005) reported higher mortality following an endotoxin challenge at 8 weeks of age in castrates in comparison with female pigs. Hence, this suggests that castration may impair health and welfare.

Weaning is a critical time period in pig production, in which animals are suddenly separated from the dam and mixed with unfamiliar pigs in a new environment. Thus, weaning constitutes a severe nutritional, physical and psychological stressor (Pluske and Williams, 1996), which affects the behaviour, as well as the endocrine and immune responses (Blecha *et al.*, 1983; de Groot *et al.*, 2001; Kanitz *et al.*, 2002). An important aspect to consider is the increase in disease susceptibility associated with weaning (Kanitz *et al.*, 2002), which may be associated with a reduced pro-inflammatory cytokine response (Carstensen *et al.*, 2005). Immune challenges with lipopolysaccharides (LPS) are widely used to study the immune-neuroendocrine interactions in pigs (Kanitz *et al.*, 2002; Tuchscherer *et al.*, 2004). Thus, subjecting weaned pigs to aseptic inflammation with LPS can give valuable information on their immune status and their susceptibility to suffer from infection (Carstensen *et al.*, 2005). The present study was conducted in order to assess the effect of surgical castration on post-weaning behaviour, and to evaluate the effect of this surgical procedure on the subsequent behavioural, endocrine and immune responses elicited by a low-dose bacterial endotoxin challenge in weaned pigs.

Materials and Methods

Animals were selected from the litters of 28 multiparous sows from the minimal disease herd at Moorepark Research Centre (Fermoy, Co. Cork, Ireland) over a 4-week period. The litters were housed with their dams in identical farrowing rooms consisting of 10 individual pens. The floor was fully slatted [Tribar®, Nooyen Roosters B.V., Deurne, The Netherlands] with a continuous heat pad at both sides of the centrally positioned farrowing crate. Piglets could move freely around the pen and had access to the nipple drinker fitted in each pen. At one day of age, all piglets were subjected to ear notching, teeth clipping and tail docking. At five days of age, after being weighed individually, 4 male piglets were selected within each litter. Piglets that were closest in weight to the litter average were selected. Subsequently, two male piglets were

randomly assigned to undergo surgical castration. The other two males and all other males in the litter were left entire. At this time, pigs were also assigned at random to the administration of saline or LPS and to sampling time groups one day post-weaning. During castration, piglets were restrained on a narrow wooden bench. Surgical castration was performed by removing the testes and epididymes through two incisions made on the previously disinfected scrotum (one over each testis) followed by the manual extraction of each testis. A topical disinfectant was applied on the ano-genital area before and after castration. At 28 days of age, all piglets were weaned. Experimental piglets were mixed on a treatment basis. Thus, one group of entire males and one group of castrates (14 pigs per group) were formed in each replicate of the experiment. Groups were housed in adjacent pens of 3.58 m^2 (275 cm x 130 cm). Animals had free access to food and water during the experiment.

The behaviour of weaned pigs in each treatment group was studied in all 4 replicates on the day of weaning. The frequency of agonistic interactions was studied by recording all the aggressive events (i.e. threat/knock, bite, fights, chase and flee) during a one-hour period, which started immediately after the group of 14 weaned pigs of the same treatment was constituted. If agonistic encounters between the same animals occurred repeatedly in a short period of time but not as part of a fight, they were considered as part of the same agonistic bout.

Saliva samples were collected from pigs assigned at 5 days of age to the saline-treated group for the challenge. A total of 8 pigs from each treatment group (entire male or castrate) were sampled at 3, 4, 5, 6 and 8 weeks of age. Saliva samples were obtained by allowing the pig to chew a cotton bud [Salivette™, Sarstedt, Wexford, Ireland] until it was thoroughly soaked. Salivary concentrations of testosterone were determined by an enzyme immunoassay [DRG-Diagnostics, Marburg, Germany].

In each treatment group of weaned pigs (entire male or castrate), eight animals previously selected on day 5 and randomly assigned to one of two challenge groups (saline or LPS) and one of four sampling times (0, 2, 12 or 24 h) were used. At 0900 h on the day after weaning, pigs were injected intraperitoneally (i.p.) with 0.1 ml/kg of BW of a solution of sterile saline containing 0 or 5 μg of LPS. The behaviour of experimental pigs was assessed subsequent to the administration of the challenge. Only experimental pigs in each treatment group were observed. Thus, the behaviour of 4 pigs per challenge group in each treatment was studied. The behaviour of experimental animals was recorded by instantaneous scan sampling every 5 minutes for a 45-minute period. Hence, postures, activities and social cohesion parameters were recorded every 5 minutes for all experimental pigs. Each observation period started at 0, 1, 2, 3, 4, 6 and 8 h after administration of the LPS challenge. During the bacterial endotoxin challenge, blood samples were taken by anterior *vena cava* puncture into lithium heparinised syringes [Vacutainer™, Unitech Ltd., Dublin 24, Ireland] before (0 h), 2, 12 and 24 h after challenge injection. At each sampling time a total of four pigs were sampled consisting of one entire male and one castrate per challenge group. Plasma levels of TNF-α and IL-1β were measured using a commercially available solid phase ELISA specific for these porcine pro-inflammatory cytokines [Biosource International, Camarillo, CA, USA]. Concentrations of CRP, SAA and Hp were determined using solid phase sandwich immunoassays [Tridelta Development Ltd., Maynooth, Co. Kildare, Ireland]. Plasma cortisol was determined by an enzyme immunoassay [DRG-Diagnostics, Marburg, Germany].

Salivary testosterone concentrations were analysed using the PROC MIXED [SAS®, 1999]. The model included fixed effects of treatment, age and their interaction. Tukey's Test was used to establish pair-wise differences between treatment groups on each individual age. Data from behavioural observations 1-hour post-mixing (agonistic interactions) were subjected to ANOVA

using the GLM procedure, and were tested for the main effect of treatment. Sickness behaviour data were not normally distributed. Hence, these data were analysed by the non-parametric Kruskal-Wallis test using the NPAR1WAY procedure. Sickness behaviour data were analysed for treatment, challenge and time, and all possible interactions. Concentrations of TNF-α, IL-1β, CRP, SAA and cortisol were analysed after log transformation using the GLM procedure. Data were subjected to ANOVA to test for the main effect of treatment, challenge and sampling time, and for any interactions between these factors. The significance of differences between specific groups was analysed using the least significant differences from the combined analysis.

Results

A significant effect of treatment was found in the total number of agonistic interactions during the 1-hour period after mixing ($P < 0.05$). In particular, entire males tended to be engaged in two-sided fights more than castrated pigs ($P < 0.10$). Furthermore, the total number of agonistic bouts tended to be higher among the group of entire males than castrates ($P < 0.10$).

Concentrations of testosterone in saliva were significantly different between treatment groups ($P < 0.001$). Significantly higher salivary concentrations of testosterone were detected in entire males (0.63 ± 0.04 ng/ml) in comparison with castrates (0.29 ± 0.04 ng/ml). Significant age effects showed that the highest concentrations of testosterone were found in pigs at 3 and 4 weeks of age, decreasing subsequently ($P < 0.01$; Figure 9). A tendency towards a significant treatment by age interaction ($P = 0.088$) showed that concentrations of testosterone in saliva were significantly higher in entire males in comparison with castrated pigs at 4 weeks of age ($P < 0.001$; Figure 9).

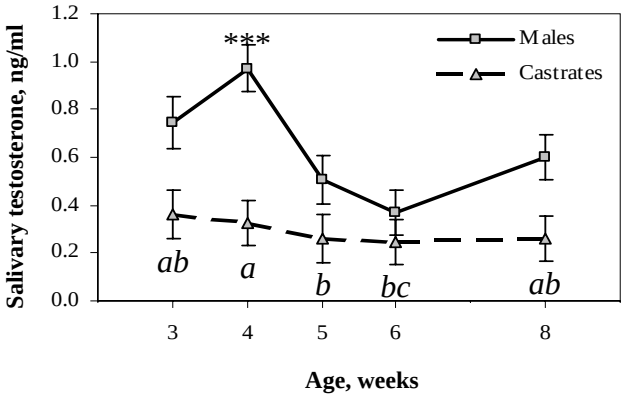

Figure 9. Salivary concentrations of testosterone in entire male and castrated pigs aged 3 to 8 weeks.

LPS administration provoked a significant reduction in activity ($P < 0.05$), in particular eating ($P < 0.05$) and exploring ($P < 0.05$), as well as aggressive interactions ($P < 0.05$). LPS also produced alterations in the postures displayed by the animals, with LPS-treated pigs tending to stand less ($P = 0.053$) and lie laterally more ($P < 0.05$). A significant effect of time was found for many activities and postures, with the exception of drinking, eliminatory activities, and the occurrence of ventral lying and isolation ($P > 0.10$). A significant challenge by time interaction showed that LPS-treated pigs were less active 3 h and 4 h after the administration of the challenge ($P < 0.05$). LPS-treated pigs explored less ($P < 0.01$) and tended to display fewer ingestive activities ($P = 0.065$) than saline-treated animals 3 h after injection. In particular, LPS-treated pigs showed lower occurrences of chewing and/or licking ($P < 0.001$) and eating ($P < 0.01$). The reduction in eating activities after LPS administration was also evident at 1 h ($P < 0.05$). One hour post-challenge, LPS-treated pigs exhibited less agonistic interactions than

saline-treated animals ($P < 0.05$). LPS-treated pigs spent significantly more time lying laterally 2 and 3 h after injection ($P < 0.05$) and tended to spend less time lying in a ventral position ($P = 0.058$). During this period, LPS-treated animals tended to sleep more than saline-treated animals ($P = 0.058$). Regardless of the challenge group, entire male pigs displayed more ingestive activities ($P < 0.05$), in particular they spent more time eating ($P < 0.01$). Furthermore, entire males spent more time awake while inactive ($P < 0.01$) and less time sleeping ($P < 0.05$) than castrated pigs. The effects of the challenge also differed between treatment groups. LPS administration caused a significant reduction in ingestive activities ($P < 0.05$), decreasing the occurrence of eating in the group of entire males ($P < 0.01$). In addition, entire males that were treated with LPS explored less ($P < 0.05$), showing a reduction in the occurrence of chewing/licking behaviours ($P < 0.05$). Entire males injected with LPS were more inactive ($P < 0.05$), in particular while awake ($P < 0.01$). In contrast, LPS administration caused a significant reduction in levels of aggression in the castrated group ($P < 0.01$) and increased the occurrence of lateral lying ($P = 0.052$) and isolation in these animals ($P < 0.05$).

There was a significant effect of challenge ($P < 0.05$), time ($P < 0.001$) and a challenge by sampling time interaction ($P < 0.01$) on plasma concentrations of TNF-α (Figure 10). Plasma TNF-α concentrations of LPS-treated pigs were significantly elevated in comparison to saline-treated animals, in particular at 2 h post-treatment ($P < 0.001$; Figure 10). Concentrations of TNF-α during the challenge did not differ between entire males and castrated pigs ($P > 0.10$).

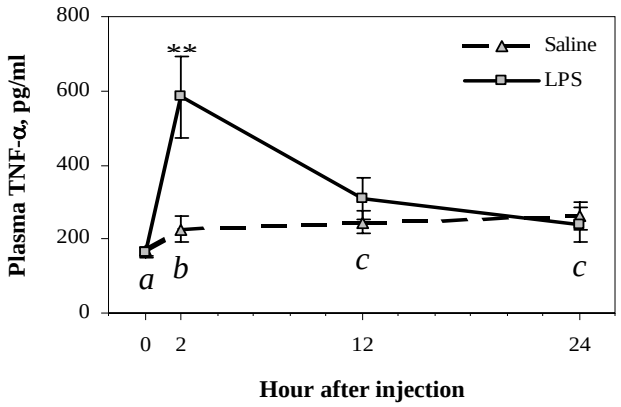

Figure 10. Plasma concentrations of tumor necrosis factor-alpha (TNF-α) in pigs following lipopolysaccharide (LPS) challenge, where pigs were injected i.p. with 0.1 ml/kg of BW of a solution of sterile saline containing 0 or 5 μg of LPS from *E. coli*.

No significant effect of challenge or time was found in plasma CRP ($P > 0.10$). However, a tendency towards a significant interaction between challenge and time ($P = 0.088$) showed that plasma CRP of LPS-treated pigs was higher in comparison with saline-treated animals 12 h after the onset of the challenge ($P < 0.05$; Figure 11). Plasma CRP levels did not differ between entire males and castrated pigs ($P > 0.10$).

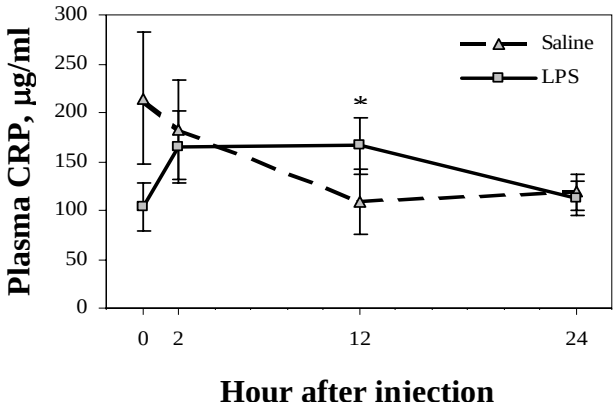

Figure 11. Plasma concentrations of C-reactive protein (CRP) in pigs following lipopolysaccharide (LPS) challenge, where pigs were injected i.p. with 0.1 ml/kg of BW of a solution of sterile saline containing 0 or 5 µg of LPS from *E. coli*.

Plasma SAA concentrations tended to be higher in LPS-treated pigs than in saline-treated (P = 0.098). SAA in plasma did not vary with time (P > 0.10) and did not differ between entire males and castrated pigs (P > 0.10). Plasma concentrations of cortisol did not differ between challenge and treatment groups (P > 0.10). Plasma cortisol varied with time (P < 0.01). Plasma IL-1β was not affected by treatment, challenge or time (P > 0.10).

Conclusions

Results from this study showed that entire male pigs were more aggressive than castrates during a 1-h period post-mixing. In particular, the occurrence of two-sided fights was higher among animals from the entire male group. It is well recognised that entire male pig production is associated with social stress and fighting during the growing and fattening stages and also with management difficulties due to the aggressiveness of these animals (EFSA, 2004). However, this is to our knowledge, the first study reporting differences in aggression levels between entire and castrated male pigs at weaning. Gonadal steroids influence the behaviour, in particular social and aggressive behaviours in pigs (Giersing *et al.*, 2000; EFSA, 2004). Analysis of saliva samples collected in the present study showed that salivary testosterone levels were higher in entire male pigs throughout the experiment, but in particular on week 4, which coincided with weaning. In addition, these results agree with previous studies where elevated concentrations of male gonadal steroids were found in plasma of male pigs in early postnatal life (Schwarzenberger *et al.*, 1993). Thus, it is possible that peak levels of testosterone detected on 4-week-old pigs were in part responsible for the higher frequency of agonistic encounters among entire male pigs 1-h post-mixing. In addition, results from this study confirm that age-related variations in testosterone are also detectable in saliva. Thus, salivary testosterone determination can be used as a non-invasive method of monitoring testicular function in the pig. In addition, castration of male piglets also caused a significant decrease in salivary testosterone concentrations relative to those of entire males.

During the present challenge, LPS administration reduced general activity of pigs, in particular eating and exploring, as well as levels of aggression between animals. Furthermore, LPS increased the time spent lying at the expense of standing. Thus, in agreement with other authors, LPS administration depressed activity levels and provoked anorexia and lethargy in the pigs (Johnson and von Borell, 1994; Warren *et al.*, 1997). Data collected in this study showed that the behavioural consequences of LPS administration were most pronounced 3 h after the

administration of the challenge. It is possible that the synchronicity in the appearance of these behaviours may be related to the synthesis of pro-inflammatory cytokines, such as TNF-α, in response to the administration of bacterial endotoxin (Dantzer, 2001; Kelley *et al.*, 2003). In the present study, entire males exhibited behavioural differences in comparison with castrated pigs at weaning. Furthermore, administration of LPS increased the exhibition of sickness behaviours by entire male pigs in comparison to castrates. Entire males injected with LPS showed a significant reduction in activity levels, in particular ingestive and exploratory behaviours. Entire males treated with LPS were also more idle, which may reflect lethargy. In contrast, LPS administration only provoked a reduction in levels of aggression and caused isolation among the group of castrated pigs, increasing the time spent lying laterally. These results suggest that castrated pigs did not display general behavioural symptoms of sickness, such as anorexia or lethargy, after administration of an endotoxin challenge. Hence, castration may have affected the coping mechanisms of these animals in response to low-dose LPS administration. Alternatively, it is possible that entire males were more efficient in overcoming the bacterial endotoxin challenge, taking into consideration the beneficial effects of sickness behaviours in the recovery of homeostasis (Hart, 1988; Kelley *et al.*, 2003).

Results from this study showed that the pro-inflammatory cytokine and hepatic acute phase response elicited by a low-dose of LPS did not differ between treatment groups. This indicates that castration did not affect the responsiveness of the pro-inflammatory cytokine reaction and therefore, it may not impair disease susceptibility as simulated by a low dose of endotoxin (Carstensen *et al.*, 2005). However, higher doses of LPS could have resulted in a different outcome. According to Lessard *et al.* (2002), age at which castration is performed may be an important factor to consider. Hence, it is possible that castrating pigs at an early age does not alter subsequent endocrine or immune responses to a low-dose endotoxin challenge.

In conclusion, results from the present study indicate that surgical castration of 5-day-old piglets reduces aggression levels after weaning. This may be associated with significantly lower testosterone levels in the saliva of castrated pigs at weaning age. Furthermore, castration affects the behavioural but not the endocrine or immune responses to a low-dose endotoxin challenge subsequent to weaning. Results show that castrated pigs did not exhibited general sickness behaviour symptoms after LPS administration. It is possible that behavioural alterations are the most biologically cost-effective response to the low-dose of LPS that was used in the present study. Nonetheless, these results indicate that surgical castration of young pigs can affect the mechanisms available for coping with subsequent stressors.

General conclusions

The results from these experiments give relevant information on the effect of husbandry and low-dose lipopolysaccharide (LPS) on the acute phase response of young pigs. The main findings from these experiments are:

- Age-related changes exist in the concentration of the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) and the acute phase proteins serum amyloid A (SAA) and haptoglobin (Hp) during the first week of life of neonatal pigs.
- Hp levels in plasma are sensitive indicators of the impact of managerial practices in newborn piglets, such as ear notching, teeth clipping and tail docking.
- Teeth resection by clipping and grinding causes a significant but transient reduction in the skin temperature of newborn piglets.
- Grinding piglets' teeth reduces the levels of plasma C-reactive protein (CRP) one-day post weaning in comparison with piglets with clipped teeth, suggesting a lower activation of the acute phase response.
- Behaviour alterations are indicative of the pain-induced distress caused by the surgical castration of 5-day-old piglets.
- Plasma TNF-α, CRP, SAA and cortisol are useful indicators of sub-acute inflammation/infection in pigs as simulated by low-dose lipopolysaccharide (LPS) challenge.
- Pro-inflammatory cytokines TNF-α and interleukin-1beta (IL-1β) are detectable in saliva of pigs
- Gender differences exist in the pro-inflammatory cytokine response, which may be associated with differential hypothalamus-pituitary-adrenal (HPA) axis activity in male and female pigs.
- Surgical castration of 5-day-old piglets reduces aggression in groups of newly-weaned pigs.
- High levels of testosterone exist in the saliva of entire male pigs during the early postnatal life.
- Surgical castration of 5-day-old piglets affects the behavioural response elicited by a low-dose LPS challenge, indicating that this procedure reduces the occurrence of sickness behaviours induced by the bacterial endotoxin challenge.

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