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Effect of surgical castration on the behavioural and acute phase responses of 5-day-old piglets

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Abstract

Pain and discomfort provoked by surgical castration of male pigs causes behavioural alterations that are particularly evident in the immediate days following this procedure. Less information is available in relation to whether the physiological consequences of surgical castration also persist with time. The objective of this study was to assess the behavioural response to this procedure; and evaluate its effects on levels of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β), as well as cortisol [Exp. 1]; and the acute phase proteins C-reactive protein (CRP), serum amyloid A (SAA) and haptoglobin (Hp) [Exp. 2]. Forty male piglets were used in each experiment. At 5 days-of-age, pigs were randomly assigned to undergo surgical castration or to being handled identically but not castrated (treatments were imposed in the morning period). Behaviour was assessed by scan sampling every 3 min for 3 h in the afternoon period on the day of castration [Exp. 1]; and for intervals of 2.5 h on the day of castration (morning and afternoon periods) and 2 h-intervals on three consecutive days thereafter (afternoon period) [Exp. 2]. Pigs were assigned to one of five sampling times, where blood was collected by venipuncture before (0 h), 1, 2, 3 or 4 h [Exp. 1] and 0, 12, 24, 48 or 72 h after treatments [Exp. 2]. Castration provoked specific pain-related behaviours ($P < 0.001$) throughout the duration of both experiments, but particularly immediately after castration [Exp. 2]. These included behaviour such as huddling up, spasms and trembling. Castrated pigs walked less ($P < 0.05$) and avoided dog-sitting postures ($P < 0.01$). In Exp. 1, castrates tended to spend more time at the udder ($P < 0.1$) and in contact with the sow ($P < 0.05$). In Exp. 2, castrated pigs spent more time alone (i.e. not in contact with sow or siblings) ($P < 0.05$). Castrated pigs tended to be more isolated and desynchronised ($P < 0.1$). Castration had no effect on plasma levels of TNF-α, IL-1β, CRP, SAA and Hp ($P > 0.1$). Castrated piglets tended to have higher cortisol levels than handled pigs ($P < 0.1$). Hence, behavioural observations were useful for evaluating the consequences of surgical

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castration on the welfare of pigs, indicating that animals undergoing this surgical procedure experience pain and discomfort that is persistent for up to 4 days. However, pro-inflammatory cytokines and acute phase proteins did not provide relevant information on the physiological consequences of this husbandry practice.

Keywords: Surgical castration; Behaviour; Cytokines; Acute phase proteins; Cortisol; Pig

1. Introduction

In most EU countries, with the exception of Ireland and the United Kingdom, male pigs are surgically castrated to avoid problems with boar taint when higher slaughter weights are reached (EFSA, 2004). The problem of boar taint is illustrated by current European Community legislation (Directive 64/433/EEC) indicating that male carcases over 80 kg may be allowed for human consumption provided that they bear a special mark and undergo treatment (i.e. processing) before entering the food chain. However, surgical castration is painful and consequently it constitutes an important welfare problem (SVC, 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 hormone (ACTH) and cortisol (Prunier et al., 2005). Alterations in the behaviour of castrated pigs shows 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, particularly the acute phase response, during the days following surgical castration of pigs (EFSA, 2004). In relation to this, Hay et al. (2003) did not find differences in urinary levels of corticosteroids and catecholamines on the days following surgical castration of piglets, most likely due to the short-lived activity of the adrenal and sympathetic axes (Prunier et al., 2005). Nonetheless, due to the surgical nature of castration, this practice seems to elicit an inflammatory reaction (Fisher et al., 1997; Ting et al., 2003). Inflammation and infectious processes can result in the activation of the acute phase response (Baumann and Gauldie, 1994). 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) (Kushner, 1993). Hence, determination of levels of pro-inflammatory cytokines and acute phase proteins might give valuable information of the physiological consequences of surgical castration of young male pigs. Thus, the objective of this study was to evaluate the behavioural and acute phase responses of 5-day-old pigs to surgical castration, assessing the prevalence of these responses on the days following the procedure.

2. Materials and methods

2.1. Animals and management

Litters of not less than 7 piglets and at least 4 males from 10 multiparous sows (parity 1–7) from the minimal disease Moorepark herd were used in each of two separate experiments. Litters were housed with

their dams in similar farrowing rooms consisting on 10 individual pens ($L = 235$ cm $\times$ $W = 155$ cm). The floor was fully slatted (Tribar®, Nooyen Roosters B.V., Deurne, The Netherlands) with a continuous heat pad (142 cm $\times$ 43 cm) at both sides of the centrally positioned farrowing crate. The dimensions of the farrowing crate were 235 cm $\times$ 48 cm. Piglets could move freely around the pen and had access to a nipple drinker fitted in each pen. All piglets were subjected to ear notching, teeth clipping and tail docking within 24 h of birth. These practices constitute part of the routine management of the piglets in order to individually identify animals, reduce the occurrence and severity of facial injuries, as well as the incidence of tail-biting. Previous research has shown that teeth clipping does not influence the levels of acute phase proteins in 1-day-old piglets (Llamas Moya et al., 2006a).

2.2. Experimental design

On day 5, all piglets within each litter were individually identified by ink marks according to the number recorded by ear notching. Subsequently, four male piglets were selected in each litter on the basis of being nearest the average litter body weight (BW) (2.04 ± 0.36 kg and 2.49 ± 0.40 kg in experiments 1 and 2, respectively). These piglets 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. Hence, treatments were imposed without disturbing other experimental piglets. Treatments were imposed on individual piglets in a period of time no longer than 4 min. 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 each testis. Special care was taken to avoid the tearing of tissues as indicated in current European welfare legislation [2001/93/EC]. Nonetheless, straight cuts were also avoided in order to reduce bleeding (EFSA, 2004). A topical disinfectant, consisting of a solution at 10% of povidone iodine, 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. This was conducted in order to control for possible temporal effects of restraint and handling. Due to the age of the pigs, the use of anaesthetics was not required to perform the surgical castration of the animals (Directive 2001/93/EC). All treatments were applied between 09:00 and 10:00 h by the same trained technician in order to ensure similarity in the application of the experimental treatments.

2.3. Behavioural response to surgical castration

In experiment 1, behaviour observations started at 14:00 h on the day that treatments were imposed and lasted for a 3-h-period. The afternoon had been previously identified as the most active period for pigs of all ages (Beattie, 1994). During this period, behaviour was recorded by instantaneous scan sampling every 3 min. Therefore, every 3 min the posture, activity, location (whether on the heat pad or not) and body contact (with the sow, littermate or no contact) of all piglets were recorded (Table 1). This interval between scans allowed the observer to monitor the behaviour of all experimental piglets, while maximising the frequency of observations. Activities were classified as pain-related, likely originated by surgical castration, and non-specific activities (Hay et al., 2003). A single trained observer executed all observations. The observer was not blind to treatments.

In experiment 2, the behaviour of experimental piglets was recorded on the day of castration and on three consecutive days thereafter: day 1 morning (10:30–13:00 h), day 1 afternoon (14:00–16:30 h), day 2 afternoon (14:00–16:30), day 3 afternoon (14:00–16:00 h) and day 4 afternoon (14:00–16:00 h). During these observations, the behaviour of piglets was recorded by instantaneous scan sampling every 3 min (Table 1). Thus, the posture, activity, body contact (with the sow, littermate or no contact) and social cohesion parameters of experimental animals were recorded every 3 min, as described for the previous experiment. Activities were also classified as pain-related and non-specific activities. All observations were carried-out by the same single trained observer.

Table 1
Description of postures, activities and social cohesion parameters (modified from Hay et al., 2003)

Posture	
Standing	Body weight supported by the four legs
Kneeling	Body weight supported by front carpal joints and hind legs
Dog-sitting	Body weight supported by hind-quarters and front legs
Ventral lying	Body weight supported by belly. Sternum in contact with the floor
Lateral lying	Body weight supported by side. Shoulder in contact with floor
Activity: non-specific	
Walking	Slowly moving forward with one leg at a time
Running	Trot or gallop without sudden change in direction or speed
Teat-seeking	Attempts to find a teat by walking and pushing other piglets while most of the others are suckling
Udder massage	Nose in contact with the udder, leaning against it. Ample and rhythmic up and down head movements
Suckling	Teat in the mouth. Vigorous rhythmic suckling movements
Nosing	The snout is close to or in contact with a substrate or a pen-mate. Snout movement may be observed
Chewing	Nibbling at littermates (ears, tail or foot,. . .) or substrates
Licking	Rubbing the tongue over littermates, floor or pen walls
Playing	Head shaking, springing (sudden jumping or leaping), running with vertical and horizontal bouncy movements. Can involve a partner (gentle nudging or pushing, mounting, chasing,. . .)
Scratching	Scratching the rump by rubbing it against the floor or the pen walls
Awake inactive	Eyes open but doing nothing
Sleeping	Eyes closed while lying
Activity: pain-related	
Huddled up	Lying with at least three legs tucked under the body
Spasms	Quick sudden involuntary contractions of the muscles under the skin, mainly affecting the limbs
Trembling	General shivering as if cold
Social cohesion	
Isolation	Away from other pigs, alone or with one pen-mate at the most. A distance of at least 40 cm (about the width of two piglets) separates the animal from the closest group of pigs
Desynchronisation	Engaged in different behaviours to that of the majority of the group (at least 75%)

2.4. Acute phase response to surgical castration

Blood samples were taken in a supine position by anterior *vena cava* puncture into lithium heparinised syringes [Vacutainer™, Unitech Ltd., Dublin 24, Ireland]. Venipuncture required less than 1 min per animal. 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. Sampling times were selected according to the expected time-responses of these pro-inflammatory cytokines and acute phase proteins following results from previous studies by the present authors (Llamas Moya et al., 2006b). In both experiments, a total of four pigs per treatment (castrated or handled) were sampled at each of the sampling times. Thus, each animal was only sampled once, eliminating the effect of prior blood sampling. This experimental design was adopted in order to avoid the influence of repeated blood sampling on the acute phase response, while using the minimum number of experimental units necessary to detect significant differences (Webel et al., 1997; Llamas Moya et al., 2006b). Subsequently blood samples were immediately centrifuged at 2000 × g for 10 min at 5 °C. Plasma was

stored at −20 °C (Tuchscherer et al., 2004) until analysis for concentrations of tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β) and cortisol in experiment 1; and C-reactive protein (CRP), serum amyloid A (SAA) and haptoglobin (Hp) in experiment 2, using validated assays.

2.5. Analysis of plasma samples

Plasma samples from experiment 1 were analysed for their concentration of the pro-inflammatory cytokines TNF-α and IL-1β using commercially available solid phase Enzyme Linked Immuno Sorbent Assay (ELISA)-specific for these cytokines in pigs [Biosource International, Camarillo, CA, USA]. Plasma samples were analysed in duplicate at 1:2 dilution for both cytokines. The minimum detectable dose of porcine TNF-α by the assay was 6.0 pg/ml. The intra- and inter-assay coefficients of variation (CV) were <4.2% and <8.7%, respectively. The minimum detectable dose of porcine IL-1β by the assay was 15 pg/ml. The intra- and inter-assay CV were <3.2% and <4.8%, respectively. Plasma samples were also analysed for cortisol concentrations using an enzyme immunoassay [DRG-Diagnostics, Marburg, Germany]. The lowest detectable level of cortisol that could be distinguished from the zero standard was 1.14 ng/ml. The intra- and inter-assay CV were <2.3% and <3.6%, respectively. Plasma samples from experiment 2 were analysed for their concentration of the acute phase proteins CRP, SAA and Hp using commercially available solid phase sandwich immunoassays [Tridelta Development Ltd., Maynooth, Co. Kildare, Ireland]. Plasma samples were analysed in duplicate at 1:100 and 1:200 dilution for CRP concentrations; 1:250 and 1:500 dilution for SAA levels and undiluted samples for Hp concentrations. The sensitivity of the CRP assay was 20 ng/ml. The assay had an intra- and inter-assay CV of <1.9% and <4.6%, respectively. The sensitivity of the SAA assay was 1.9 μg/ml. The intra- and inter-assay CV were <4.1% and <6.6%, respectively. The sensitivity of the Hp assay was determined as 0.05 ng/ml. The intra- and inter-assay CV were <1.3% and <6.9%, respectively.

All procedures were carried out under licence in accordance with the European Communities (Amendment of Cruelty to Animals Act, 1876) Regulations 2002.

2.6. Statistical analysis

Behavioural data collected in experiment 1 were statistically analysed as a complete randomised design using the general linear model (GLM) procedure of SAS® [Statistical Analysis System (SAS) V8 Institute Inc., Cary, NC, USA (1999)]. Data were subjected to analysis of variance (ANOVA) to test for the main effect of treatment. Data were normally distributed ($P > 0.1$) and had equal variance ($P > 0.1$). Data are presented as means ± S.E.M. In experiment 2, behavioural data were analysed using the mixed procedure (PROC MIXED) of SAS®. The model included fixed effects of treatment and observation period (d1 morning, d1 afternoon, d2 afternoon, d3 afternoon and d4 afternoon) and their interaction. The repeated statement was used to take into account repeated measures for each individual animal. Where significant effects were found, Tukey's test was used to establish pair-wise differences between treatment groups on each individual observation period. Data are presented as lsmeans ± S.E. Data from plasma analysis were subjected to ANOVA to test for main effects of treatment and sampling time. Data were also normally distributed ($P > 0.1$) and had equal variance ($P > 0.1$). The procedures PROC UNIVARIATE and PROC INSIGHT of SAS® were used to test these assumptions. Data are presented as means ± S.E.M. Individual animals were used as the experimental units.

3. Results

3.1. Behavioural response to surgical castration

In experiment 1, castrated piglets spent significantly less time engaged in locomotory activities ($P < 0.05$; Table 2), specifically walking ($P < 0.05$; Table 2). Castrates tended to spend more time massaging the udder in comparison to handled piglets ($P = 0.075$; Table 2). Castrated

Table 2
Occurrence (%) of non-specific activities, pain-related activities and social contacts ($N = 20$) in handled and castrated 5-day-old piglets (Experiment 1) during 3-h-period in the afternoon, started at 1400 h, on the day that treatments were imposed (at 0900–1000 h). Data are presented as means ± S.E.M.

Non-specific activities	Handled	Castrated	P
Active	31.8 ± 2.5	35.8 ± 2.5	0.28
Locomotor	6.0 ± 0.8	3.5 ± 0.6	0.02
Walking	5.8 ± 0.7	3.5 ± 0.6	0.02
Running	0.2 ± 0.1	0.0 ± 0.0	0.15
Feeding	17.8 ± 1.7	21.8 ± 1.2	0.13
Teat-seeking	1.2 ± 0.4	1.1 ± 0.4	0.91
Udder massage	13.3 ± 1.3	17.6 ± 1.9	0.07
Suckling	3.3 ± 0.5	3.1 ± 0.6	0.81
Exploratory	7.4 ± 1.0	9.9 ± 1.2	0.11
Nosing	6.3 ± 0.9	6.8 ± 0.8	0.67
Chewing and/or licking	1.1 ± 0.4	3.0 ± 0.8	0.04
Playing	0.6 ± 0.3	0.6 ± 0.3	0.82
Scratching	0.3 ± 0.2	1.1 ± 0.3	0.04
Inactive	68.2 ± 2.5	64.2 ± 2.5	0.28
Awake inactive	13.1 ± 1.5	12.2 ± 1.3	0.66
Sleeping	55.1 ± 2.7	52.0 ± 2.8	0.44
Pain-related activities			
Total	0.0 ± 0.0	0.7 ± 0.3	0.02
Huddled-up	0.0 ± 0.0	0.4 ± 0.2	0.03
Spasms	0.0 ± 0.0	0.0 ± 0.0	1.00
Trembling	0.0 ± 0.0	0.2 ± 0.1	0.15
Social contacts			
Sow	2.8 ± 0.4	2.3 ± 0.4	0.02
Littermates	83.0 ± 1.1	80.1 ± 1.1	0.39
No contact	14.2 ± 1.1	17.6 ± 1.1	0.65

piglets were engaged in more exploratory activities such as chewing and/or licking ($P < 0.05$; Table 2) and also showed a higher occurrence of scratching ($P < 0.05$; Table 2). Castrated pigs exhibited significantly more pain-related activities ($P < 0.001$; Table 2), in particular huddling up ($P < 0.05$; Table 2). Nine castrated piglets showed more than one pain-related activity during the observation period, in comparison to only one piglet in the handling treatment group (data not shown). Time spent in different postures and the location of piglets did not differ between treatments ($P > 0.1$). However, castrated piglets did spend significantly more time in contact with the sow in comparison with their handled littermates ($P < 0.05$; Table 2).

In experiment 2, behavioural data collected over a 4-day period showed a significant treatment effect in the occurrence of dog-sitting posture. Castrated piglets spent less time in this posture in comparison with intact animals ($P < 0.01$; Table 3). This trend was evident throughout the experiment, in particular on d4 in the afternoon period ($P < 0.01$; Table 3). No significant differences were found regarding any other posture ($P > 0.1$; Table 3). Castrated pigs also showed a higher occurrence of pain-related activities throughout the experiment ($P < 0.05$; Table 4). A significant treatment by time interaction showed that pain-related activities were significantly higher on d1 in the morning period ($P < 0.001$; Table 4). During this particular period, nine castrated piglets showed more than one pain-related activity in comparison to only one piglet in the handling treatment group (data not shown). Castrated pigs huddled up ($P < 0.001$; Table 4), exhibited spasms ($P < 0.01$; Table 4) and were trembling ($P < 0.05$;

Table 3
Occurrence (%) of postures ($N = 20$) in handled (H) and castrated (C) 5-day-old piglets (Experiment 2) on the day of castration (d1 morning [AM] and d1 afternoon [PM]) and three consecutive days thereafter (d2 afternoon, d3 afternoon and d4 afternoon) [**($P < 0.01$) indicates significant differences between treatment groups for the each period of observation]. Data are presented as lsmeans ± S.E.

Postures	d1 AM	d1 PM	d2 PM	d3 PM	d4 PM	Total	S.E.	*P*	
								Trt	Trt × time
Standing									
C	19.5	27.1	36.0	24.2	30.6	27.5	1.7	0.70	0.75
H	18.2	27.6	34.7	26.1	35.2	28.4	1.6		
Dog-sitting									
C	1.4	2.0	2.5	1.7	1.6**	1.9	0.5	0.006	0.03
H	4.1	3.6	3.4	1.9	6.0	3.8	0.5		
Kneeling									
C	0.3	0.3	0.1	0.3	0.6	0.3	0.1	0.23	0.92
H	0.6	0.4	0.4	0.3	0.7	0.5	0.1		
Lateral lying									
C	32.5	24.7	24.2	28.0	21.5	26.2	1.6	0.92	0.79
H	33.34	28.4	23.0	26.8	18.0	25.9	1.5		
Ventral lying									
C	46.2	45.8	36.9	45.5	45.7	44.0	1.3	0.16	0.48
H	43.6	39.9	38.4	44.8	40.1	41.4	1.3		

Table 4). Handled animals showed none of these pain-related behaviours (Table 4). A tendency towards a significant effect of treatment indicated that castrated pigs engaged less in playful activities (lsmeans ± S.E. castrated versus handled: 0.72 ± 0.16% versus 1.12 ± 0.16%; $P = 0.083$). Castrated piglets avoided social contacts ($P < 0.05$; Table 5) and in particular tended

Table 4
Occurrence (%) of pain-related activities ($N = 20$) in handled (H) and castrated (C) 5-day-old piglets (Experiment 2) on the day of castration (d1 morning [AM] and d1 afternoon [PM]) and three consecutive days thereafter (d2 afternoon, d3 afternoon and d4 afternoon) [***($P < 0.001$), **($P < 0.01$) and *($P < 0.05$) indicate significant differences between treatment groups for the each period of observation]. Data are presented as lsmeans ± S.E.

Pain-related activities	d1 AM	d1 PM	d2 PM	d3 PM	d4 PM	Total	S.E.	*P*	
								Trt	Trt × time
Total									
C	2.3***	0.1	0.0	0.0	0.0	0.5	0.1	0.02	<0.001
H	0.0	0.0	0.0	0.0	0.0	0.0	0.1		
Huddled-up									
C	1.5***	0.0	0.0	0.0	0.0	0.3	0.1	0.05	0.003
H	0.0	0.0	0.0	0.0	0.1	0.0	0.1		
Spasms									
C	0.3**	0.0	0.0	0.0	0.0	0.1	0.0	0.08	0.01
H	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Trembling									
C	0.4*	0.1	0.0	0.0	0.0	0.1	0.0	0.04	0.06
H	0.0	0.0	0.0	0.0	0.0	0.0	0.0		

Table 5
Occurrence (%) of social contacts and social cohesion indicators ($N = 20$) in handled and castrated 5-day-old piglets (Experiment 2) over the experimental period. Data are presented as lsmeans ± S.E.

	Handled	Castrated	S.E.	P
Social contacts				
Sow	2.8	2.3	0.4	0.40
Littermates	83.0	80.1	1.1	0.06
No contact	14.2	17.6	1.1	0.04
Social cohesion				
Isolation	7.1	9.6	1.0	0.08
Desynchronisation	3.0	4.3	0.5	0.07

to avoid contact with their littermates ($P = 0.061$; Table 5). Throughout the duration of the experiment, castrated piglets tended to be more isolated ($P = 0.084$; Table 5) and desynchronised ($P = 0.072$; Table 5) than their handled littermates.

3.2. Acute phase response to surgical castration

No significant effect of treatment was found in plasma concentrations of TNF-α ($P > 0.1$). A significant effect of sampling time showed that concentrations of this cytokine in plasma varied over the different sampling times ($P < 0.001$; Fig. 1A). Peak concentrations of TNF-α in plasma were found 3 h after treatments were imposed, returning subsequently to basal levels (Fig. 1A). The treatment by sampling time interaction was found to be not significant ($P > 0.1$; Fig. 1A). Concentrations of plasma IL-1β did not differ between treatment groups ($P > 0.1$). No significant treatment by time interaction was found ($P > 0.1$; Fig. 1B). A significant time effect showed variation in the levels of IL-1β in plasma ($P < 0.01$; Fig. 1B). Peak plasma IL-1β concentrations were detected 2 and 3 h after treatments were imposed (Fig. 1B). Plasma levels of cortisol of castrated piglets tended to be higher than that of their handled littermates (means ± S.E.M. handled versus castrated: 111.96 ± 23.86 ng/ml versus 143.38 ± 27.38 ng/ml; $P = 0.093$). A significant effect of time was also found ($P < 0.001$; Fig. 1C). Plasma cortisol levels were elevated at 1 and 2 h, with peak concentrations occurring 3 h after treatments were imposed (Fig. 1C). Subsequent to this sampling time, concentrations of cortisol returned to basal levels (Fig. 1C). Nonetheless, no significant treatment by time interaction was found ($P > 0.1$; Fig. 1C).

Analysis of plasma samples taken in experiment 2 showed no significant effect of treatment on plasma concentrations of CRP ($P > 0.1$). A significant effect of sampling time ($P < 0.05$) showed that plasma CRP concentrations decreased 48 h after treatments were imposed (Fig. 2A). No significant treatment by sampling time interaction was found ($P < 0.1$; Fig. 2A). No significant effect of treatment, sampling time or treatment by sampling time interaction was found regarding plasma concentrations of SAA ($P > 0.1$; data not shown). Plasma Hp concentrations did not differ between handled and surgically castrated piglets ($P > 0.1$). Plasma Hp levels decreased subsequent to 24 h after treatments were imposed (Fig. 2A). No significant treatment by sampling time interaction was found ($P < 0.1$; Fig. 2A).

4. Discussion

In agreement with previous studies, results obtained from direct behavioural observations indicated that surgical castration causes pain (McGlone et al., 1993; Taylor et al., 2001; Hay

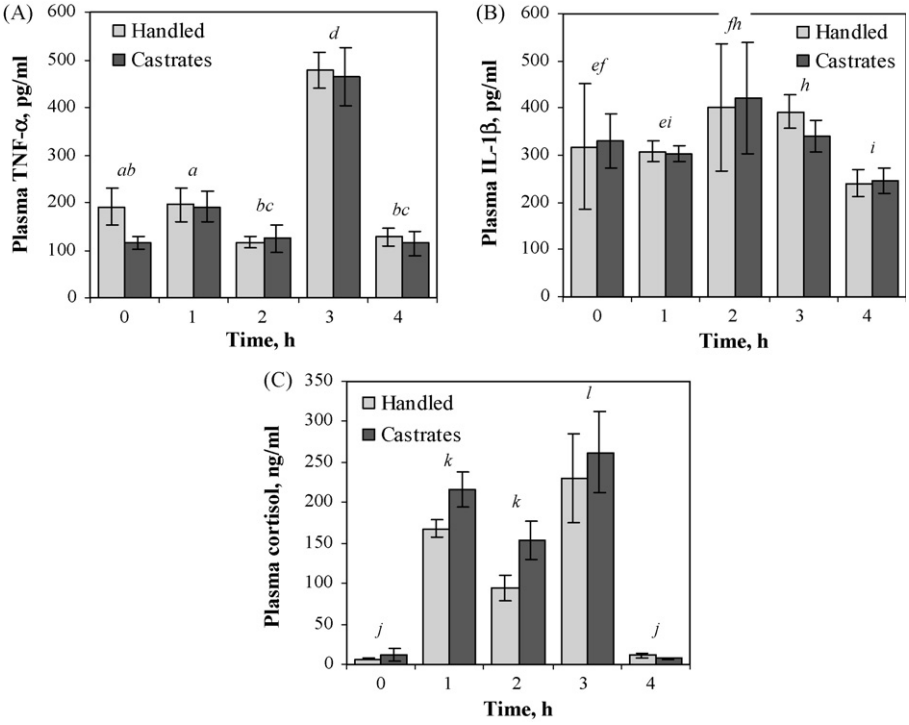

Fig. 1. Plasma concentrations of [A] tumor necrosis factor-alpha (TNF-α), [B] interleukin-1beta (IL-1β), and [C] cortisol in handled and surgically castrated 5-day-old piglets at different sampling times after treatments were imposed ($N = 4$). Different letters indicate significant differences between sampling times [A] a,bc: $P < 0.05$; ab,d: $P < 0.001$; bc,d: $P < 0.001$. [B] ef,h: $P < 0.05$; ef,i: $P < 0.1$; ei,fh: $P < 0.05$; ei,h: $P < 0.01$; fh,i: $P < 0.01$; h,i: $P < 0.001$. [C] j,k: $P < 0.001$; j,l: $P < 0.001$; k,l: $P < 0.05$. Data are presented as means ± S.E.M.

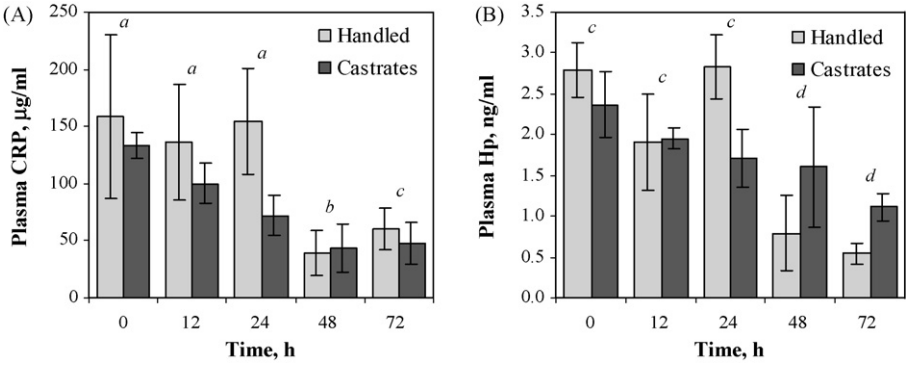

Fig. 2. Plasma concentrations of [A] C-reactive protein (CRP) and [B] haptoglobin (Hp) in handled and surgically castrated 5-day-old piglets at different sampling times after treatments were imposed ($N = 4$). Different letters indicate significant differences between sampling times [A] a,b: $P < 0.05$. [B] c,d: $P < 0.05$. Data are presented as means ± S.E.M.

et al., 2003). Castrated piglets displayed pain-related activities throughout the experiment and specifically during the day of castration (Hay et al., 2003). These pain-specific behaviours included huddling-up, trembling and spasms. Nonetheless, as reported by several authors, surgical castration also altered other non-specific activities and postures that are normally displayed by piglets (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, results from experiment 2 showed that castrated piglets were less playful, which could be indicative of poor welfare (Fagen, 1981). These behavioural adaptations can be described as protective, allowing animals to avoid or reduce the stimulation of painful tissues (Mellor et al., 2000). As previously reported, the occurrence of scratching was higher among castrated animals, which seems to reflect discomfort experienced after the surgical procedure (Hay et al., 2003). In agreement with several authors, castration of pigs did not modify the location of the animals within the farrowing crate (McGlone and Hellman, 1988; Taylor et al., 2001; 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, such as teeth resection (Noonan et al., 1994). Taylor et al. (2001) also reported that castrated piglets spent more time at the udder. This behaviour is known to help animals to cope with stressful situations, which may constitute a way of pain signalling towards the dam (Noonan et al., 1994; Taylor et al., 2001). Suckling has also been reported to have analgesic effects in human infants and rat pups in response to pain, which can be exerted via gustatory and/or tactile activities (Blass, 1994). In the present study, this corresponds with the observed increases in udder massage and social contact with the sow by castrated piglets. In experiment 2, castrated piglets spent more time alone (i.e. not in contact) and in particular, avoiding social contact with their littermates. In accordance to Hay et al. (2003), results from this study showed that castrated piglets also tended to be more isolated and desynchronised than their handled 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). It is likely that pigs can adopt different behavioural strategies for coping with pain. Results from this study showed that direct pain prevention may be achieved by avoiding postures and social contacts that could aggravate pain as a result of the stimulation of affected tissues. Pigs may also relieve the pain caused by surgical castration by adopting behaviours and/or activities with analgesic effects. Therefore, behavioural alterations in response to pain are closely associated to the particular sensations experienced by the animals (Mellor et al., 2000).

Surgical castration did not elicit significant differences in the levels of plasma TNF-α or IL-1β determined in this study. However, concentrations of these pro-inflammatory cytokines varied over time. A prompt and acute peak in TNF-α levels was observed 3 h after treatments were imposed. Time effects in plasma levels of IL-1β were less pronounced and were sustained over time. Differences in the kinetics of these cytokines have been described by other authors (Frank et al., 2003). Previous research has shown that psychological stress, such as early social isolation, influences the pro-inflammatory cytokine response of pigs (Kanitz et al., 2004; Tuchscherer et al., 2004). Thus, it is possible that handling may have contributed to the lack of an effect of castration on the pro-inflammatory cytokine levels determined in this study. Plasma cortisol levels of castrated pigs tended to be higher than that of handled pigs. 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. Prunier et al. (2005) showed significant differences in plasma cortisol concentrations between castrated and handled piglets, which were observable as early as 15 min and up to 90 min after surgical castration. Although these authors reported no differences in cortisol responses between sham-castrated and not handled animals, a limited stress response to handling was ensured by subjecting all animals to repeated handling before castration (Prunier et al., 2005). Hence, the lack of an absolute statistical difference between castrated and handled pigs and the elongated cortisol response found in the present study may have resulted from using pigs unaccustomed to handling.

Results from this study showed no treatment differences in plasma levels of CRP, SAA or Hp. In a previous study, Lackner et al. (2002) reported increases in CRP and Hp levels in 4-day-old piglets 24 h after surgical castration that were no longer evident 72 h after the surgical procedure. Fisher et al. (1997) and Ting et al. (2003) reported increases in Hp and fibrinogen, 3 days after burdizzo castration of bull calves. On the other hand, rubber ring castration of lambs failed to increase Hp levels (Price and Nolan, 2001). This variability in the response to the same practice may indicate that surgical, mechanical (burdizzo) or ischaemic (rubber ring) damage can lead to a differential response of the acute phase reaction. In the present study, 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 plasma 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; Hay et al., 2004; Lewis et al., 2005). Although it has been previously shown that teeth clipping does not influence CRP and SAA levels 24 h after teeth resection (Llamas Moya et al., 2006a,b), stressors have an additive effect (Hyun et al., 1998). Furthermore, acute phase protein concentrations can remain elevated for up to weeks following stimulation (Petersen et al., 2004). Hence, the combination of these managerial practices may have caused an inflammatory process prevalent for several days, which may have masked the acute phase response after surgical castration. This potential interference must be avoided when assessing the influence of specific management practices in the behavioural and physiological responses of young pigs.

In conclusion, results from this study highlight the value of behavioural observations for assessing pain-induced distress after castration of young male pigs. 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 direct action of littermates. In contrast, under the conditions of this study, pro-inflammatory cytokines and acute phase proteins were not relevant for monitoring the physiological consequences following surgical castration of piglets.

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