THE EFFECTS OF HANDLING ON BEHAVIOURAL AND PHYSIOLOGICAL RESPONSES TO HOUSING IN TETHER-STALLS AMONG PREGNANT PIGS

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Twenty-four primiparous pregnant pigs were randomly assigned to three handling treatments: Minimal, Positive and Negative. The pigs were moved individually to indoor concrete-floored partial stalls with neck-tethers, 2 days before handling commenced. Positive (stroking and patting on approach to an experimenter) and Negative (brief electric shock of < 1s when failing to withdraw from the outstretched hand of an experimenter) handling was imposed for 3min day^{-1} and the amount of physical contact between handler and pig was recorded. The Minimal treatment group was subjected to routine husbandry practices only. After 3 weeks of the handling treatments and tether-housing, all pigs were catheterized under full surgical anaesthesia. The pigs were allowed 4 days of recovery before collecting the following data: daytime plasma cortisol concentration profiles, behavioural responses to a human in an arena test, cortisol responses to human proximity, cortisol responses to an ACTH-challenge and immunological responses to an injected mitogen.

In the Positive treatment, the amount of physical contact between pig and handler increased during the course of the experiment, while the amount of physical contact did not change in the Negative treatment. There were no effects of treatment on behavioural responses in the arena test. The average daytime concentration of free plasma cortisol was lower in the Positive treatment than in the Negative or Minimal treatments. The Positive treatment also showed lower total and free plasma cortisol concentrations pre- and post-human proximity when compared with the Negative treatment. No differences were found between treatments in total and free plasma cortisol concentrations following an ACTH challenge. The immunological response was greater in the Positive treatment compared with the Negative treatment and tended to be greater when compared with the Minimal treatment.

It was concluded that the nature of the human-animal relationship affected the physiological stress responses of pregnant pigs to tether-housing. Indications are that a positive human-animal relationship would obviate some of the negative effects of being kept *in tether-stalls by lowering the basal cortisol concentration and by increasing immunological responsiveness.*

Keywords: *animal welfare, behaviour, human-animal relationship, immunology, pigs, tether housing, stress*

Introduction

Both the nature of the human-animal relationship and the design of the housing system have a profound effect on the welfare of husbandry animals. A positive human-animal relationship improves their welfare on both behavioural and physiological levels compared with a negative relationship (Pedersen & Jeppesen 1990; Hemsworth *et al* 1993; Pedersen 1993, 1994). Reduced space allowance, a barren environment, and housing in tether-stalls result in impaired welfare (Barnett *et al* 1985; Ladewig & Smidt 1989; Jeppesen & Pedersen 1991). By combining these two areas of research, their relative importance for husbandry animals might be revealed. We considered the hypothesis that some husbandry species might benefit more from an improved human-animal relationship than from some form of enrichment of their physical environment, or vice versa. Improving both the human-animal relationship and the housing system might further improve welfare.

An approach which combined the two areas of research, and tested the hypothesis that a positive human-animal relationship might obviate previously demonstrated negative effects of a housing system, was adopted. It has been shown that housing pregnant pigs in tether-stalls of a specific design results in reduced welfare (Barnett *et al* 1985, 1987; Becker *et al* 1985; Cronin 1985). In addition, relative to regular aversive handling, regular pleasant handling or contact with humans improves the welfare of pigs (Hemsworth *et al* 1986; Patterson & Pearce 1992).

The aim of this study was to examine whether the nature of the human-animal relationship modified the behavioural and physiological responses of pregnant pigs to housing in tether-stalls. The hypotheses tested were that pigs which received regular positive handling during tethering would: i) show more approach behaviours to humans in an arena test; ii) have lower daytime cortisol concentration profiles; iii) have lower cortisol responses and a quicker recovery of the adrenocortical axis when exposed to human proximity; iv) would show less adrenocortical activity to an adrenocorticotropic hormone (ACTH) challenge; and v) would show greater immunological response to a mitogen injection (as a measure of cell-mediated immunity) than those pigs which received minimal human contact or regular negative handling during tethering. If the different handling procedures did not modify the behavioural or physiological responses of the pigs, it was hypothesized that the nature of the housing condition would have more impact on their welfare than the nature of the human-animal relationship. The importance of revealing whether the negative effects of some intensive housing conditions and management routines can be overridden or enhanced by the nature of the human-animal relationship is an important area of research as it impacts on the management of commercial pigs and, indeed, other intensively-handled animals.

Materials and methods

All procedures were approved by the Institute's Animal Experimentation Ethics Committee (Animal Experimentation Ethics Committees 1994).

Animals and housing

Sixty-six group-housed gilts (Large White x Landrace) were naturally mated at their second oestrus. When confirmed as pregnant, 24 were selected for the experiment on the basis of body weight and expected farrowing date. At 3-5 weeks of pregnancy the pigs were randomly assigned to three different handling treatments: Minimal, Positive and Negative. The pigs were placed in pairs, separated by a non-experimental pig of the same age and weight (taken from the pool of 66 pigs). The non-experimental pigs were used to equalize neighbour effects so that the first and last experimental pig in a row would have a neighbour on each side. Thus, all experimental pigs had one non-experimental pig and one experimental pig as a neighbour. Two rows with six treatment pairs in each were formed (a total of 19 pigs in each row). Each treatment was replicated twice per row.

The tethering arrangements consisted of concrete-floored partial stalls (0.5x1.1m) with neck-tethers (Ryon-snapper Tether, Fearing International, Northampton, UK), automatic drinkers and a 0.5m dunging channel at the rear. The pigs were floor-fed once daily (2-2.5kg of a dry sow wheat-based diet containing 14.3MJ kg^{-1} Digestible Energy and 16.7% crude protein). A black plastic blind that reached the ground was mounted in front of the stalls to minimize the pigs' contact with humans when not handled. An aperture in the blind (approximately 1x0.5m, starting 0.75m from the ground) was made in front of each pair of pigs to facilitate handling, although the aperture was covered by black plastic flaps except when feeding and handling. The pigs were only able to see the upper body (head, neck, shoulder and arms) of the human during these activities. The following three treatments were imposed:

1. Minimal handling. The pigs were subjected to routine husbandry practices: feeding once a day and cleaning once a week.
2. Positive handling. The pigs were subjected to routine husbandry practices as described above. In addition, they were exposed to human contact of a positive nature for 3min day^{-1} for 5 days $week^{-1}$. The handler squatted in front of the pig, talked to and patted the pig through the aperture (head, neck, shoulders and arms visible); patting occurred only if the pig approached the handler and did not withdraw or show other signs of discomfort. This allowed the pig to control the amount of physical contact received.
3. Negative handling. The pigs were subjected to routine husbandry practices as previously described and human contact of a negative nature for 3min day^{-1} for 5 days $week^{-1}$. The handler squatted in front of the pig, as in the Positive treatment. Then the handler stretched out her/his hand towards the pig at 30s intervals. The pig was briefly shocked (less than 1s) on the shoulder with a battery-operated prodder (11V, 2mA, Vaucluse Livestock Equipment, Maffra, Australia), if she failed to attempt to withdraw from the outstretched hand. Earlier studies (Gonyou *et al* 1986; Patterson and Pearce 1989) with this type of negative treatment indicated that, with minimal pain, it quickly established a negative association between the pig and handler.

Handling sessions commenced 48h after introduction to the tether-stalls and concluded 26 days later. Handling was imposed at random times (between 0900-1700h) on weekdays, by one male and one female handler, in a random order.

Behavioural observations

During each 3min handling session, any physical contact between each pig and the handler was recorded at t=0 and then at 5s (Positive treatment) or 30s (Negative treatment). A maximum of n=37 (Positive) or n=7 (Negative) physical interaction bouts could be recorded by one pig on any day.

Twenty-nine days after handling commenced (on the third test day, see *Physiological parameters*), the pigs were individually introduced into a 3x4m arena, with nine equal-sized squares marked on the floor. Each pig was allowed a 2min familiarization period in the arena during which an observer, who stood outside the arena, recorded the total number of squares entered. Then an experimenter, who was unfamiliar to the pigs, slowly entered the arena and stood still at the wall opposite the entrance. During the following 3min the activity of the pig was recorded as: i) latency to enter an area within 0.5m of the experimenter (area A); ii) total time spent in area A; iii) number of physical interactions with the experimenter (using a bout interval of 5s); and iv) latency to the first physical interaction with the experimenter.

Catheterization

After 21 days of handling treatment and tether-housing all pigs had a catheter implanted via the cephalic vein (Takken & Williams 1981) under full surgical anaesthesia. The pigs were given a pre-medication of 5ml Stresnil™ (Janssen Pharmaceutica, Beerse, Belgium) as a tranquillizer 15min before induction of anaesthesia. They were individually guided into a crate and restrained by tightening the sides of the crate to minimize lateral movement. The crate was then inverted so that the pig rested on her back and a bar was placed above the pig's lower jaw to minimize head movements. Anaesthesia was induced with a Halothane (5%) and oxygen (3l min^{-1}) mixture. The level of anaesthesia was deepened by injection of 3-5ml barbiturate anaesthetic (Intraval™, May and Barker) before intubation. During surgery, anaesthesia was maintained with a Halothane (2.5-3.5%) and oxygen (1-1.5l min^{-1}) mixture. The shoulder and neck region were prepared for aseptic surgery (shaved, scrubbed with Savlon, sprayed with alcohol and coated with a iodine solution). Surgery was subsequently performed under aseptic conditions. A small incision (3-5cm) was made over the cephalic vein, below the shoulder. The vein was exposed and a catheter inserted. The incision was stitched close and the catheter exteriorized at the back of the neck. The catheter was held secure in a zippered pouch, attached to a collar of Elastoplast around the pig's neck.

The catheters were maintained by daily flushings; ie using a syringe, the contents of the catheter were removed, a new syringe coated with anti-coagulant was attached to the catheter and about 2ml of blood was withdrawn to make certain that the blood was flowing smoothly. The catheter was then refilled with anti-coagulant (1.25% ethylene-diamine-tetra-acetic acid [EDTA] in saline) and secured in the pouch. The pigs were allowed to recover in their individual tether-stalls for 4 days post-surgery but handling sessions were continued during this recovery period. Flushings of catheters took place after the handling session each day. Handling was not performed on the day of surgery.

Physiological parameters

On the first test day (4 days after catheterization), blood samples (5ml) were collected at hourly intervals between 0900h and 1700h. A catheter extension of 3m enabled the experimenter to bleed the pigs from behind the blind in order to minimize disturbance. The blood samples were centrifuged within 30min of extraction and the plasma stored at -18°C until analysed. Total plasma cortisol concentrations were determined by methods described in Barnett *et al* (1992). Free plasma cortisol concentrations were determined in a similar manner after centrifugation using a micropartitioning system (Amicon MPS-1: Amicon, Beverly, MA, USA). The mean value of the total and free plasma concentrations in the nine samples from the first test day was used as a 'daytime profile' for each pig, as described in Barnett *et al* (1981).

On the second test day, the total and free plasma cortisol response of the pigs to human proximity were determined. Blood samples were collected through the catheter extension from behind the blind at different time intervals prior to (-30, -15 and 0 min) and following (3, 10, 20, 40, 60, 90, and 120 min) an exposure to one of the two handlers who squatted in front of the pig and placed her/his hand on the bars.

On the fourth test day, each pig was given an intramuscular injection in the neck-region with 80iu synthetic ACTH (Synacthen®, Ciba-Geigy, Lane Cove, NSW, Australia) to stimulate cortisol secretion from the adrenals. One hour later a blood sample was collected from each pig to reveal total and free plasma cortisol responses to this ACTH challenge.

The pigs were left undisturbed for 3 days after the ACTH-challenge, except for flushing of the catheters and feeding. Then they were tested for immunological reactivity based on their cell-mediated response to an injection with 500μg leucoagglutin in 500μl saline (Sigma Chemical Company, Biochemicals, *Organic Compounds and Diagnostic Reagents Catalogue, Number L2769*) into the margin of the right ear. Before the injection the pigs were anaesthetized with a barbiturate anaesthetic (3-5ml^{-1} Intraval™, May and Barker) given via the catheter, and the double fold skin thickness at the margin of the right ear was measured using a pressure-sensitive digital micrometer. After 24h, the anaesthesia and skin thickness measurements were repeated. The percentage increase in the skin thickness was used to evaluate the cell-mediated immune response of the pigs.

Statistical Analysis

All data were subjected to a normality test (Shapiro Wilk statistics; Statistical Analysis Systems Institute Inc 1985) with the null hypothesis that the data were a random sample from a normal distribution. If the null hypothesis had to be rejected (values of $W < 0.05$), the data were transformed into log x or log $(x + 1)$. The data on 'amount of physical contact' between handler and pig during handling sessions were not normally distributed after transformation and therefore these data were subjected to a non-parametric test (Kruskal Wallis *t*-test for k-samples and Wilcoxon Mann Whitney *U*-tests for two-sample tests; Statistical Analysis Systems Inc 1985). All other data were subjected to a multiple analysis of variance (MANOVA) for unbalanced data for effects of treatment. When significant differences were revealed, a two-way ANOVA was performed to determine between-treatment effects (General Linear Models, GLM; Statistical Analysis Systems Institute Inc 1985). All statistics presented for between-treatment effects are from two-sample comparisons.

On the second test day, when the adrenal response of the pigs to human proximity was measured, no differences within each treatment in total or free plasma cortisol concentrations were found at the different time intervals (-30, -15, 0, 3, 10, 20, 40, 60, 90 and 120 min; $P > 0.1$). Means were therefore calculated for the samples drawn prior to the human exposure (Pre-sample, -30, -15, 0 min) and after the human exposure (Post-sample, 3, 10, 20, 40, 60, 90, 120 min). The Pre-sample was used as a covariate when analysing post-sample effects between treatments with the analysis of co-variance (ANCOVA) procedure (Statistical Analysis Systems Institute Inc 1985).

Results

Behavioural observations

Figure 1 illustrates the average amount of daily physical contact between handler and pig during the course of the experiment, expressed as the mean percentage of total daily contact possible for each treatment. The amount of daily physical contact between handler and pigs in the Positive treatment increased during the course of the experiment ($P < 0.05$; Figure 1). In the Negative treatment, the average daily number of administered shocks showed no change ($P > 0.1$; Figure 1). The average amount of daily physical contact for the entire handling period was 30.4 ± 10.5 bouts per pig in the Positive treatment (bouts of 5s; $37 = 100\%$) and 1.3 ± 1.4 shocks per pig (shocks < 1s; $7 = 100\%$) in the Negative treatment.

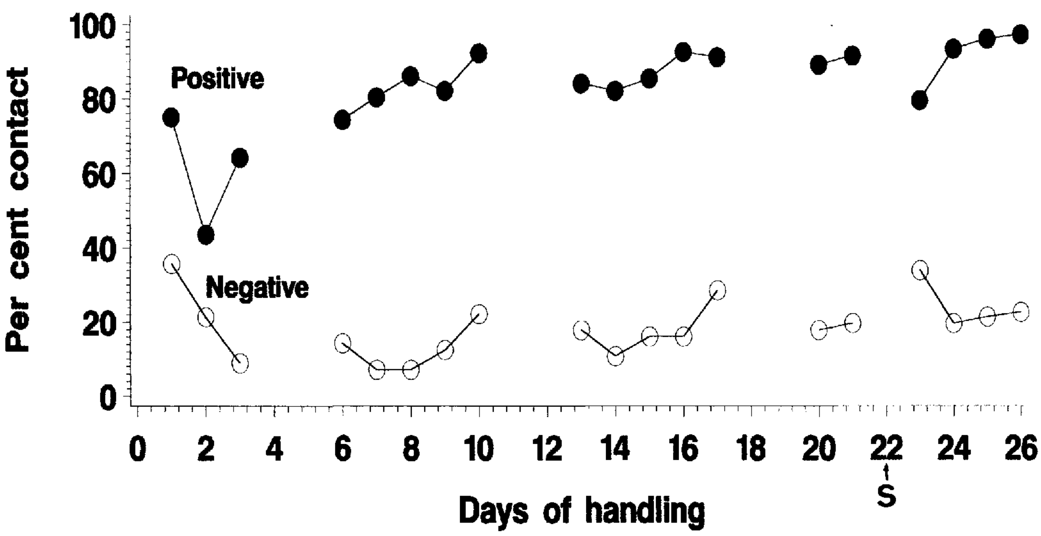

Figure 1 **The amount of daily physical contact between handler and pig during the 26 days of the experiment. Handling took place on weekdays only. The amount of daily physical contact is expressed as the mean percentage of total contact possible per treatment each day. The Positive treatment is indicated by solid circles and the Negative treatment by open circles. 'S' indicates the day of surgery.**

In the arena test, there were no significant differences between treatments in the number of squares crossed during the 2min familiarization period ($P > 0.1$; Table 1); in latency to reach area A close to the experimenter ($P > 0.1$); in time spent in area A ($P > 0.1$); in latency to interact with the experimenter ($P > 0.1$); or in the number of interactions with the experimenter ($P > 0.1$).

Table 1 **Behavioural responses in the arena test expressed as mean (± standard deviation) number of squares crossed during the 2min familiarization period; mean latency (s) to enter area A; duration of time (s) in area A; mean latency (s) to interact with the experimenter; and mean number of interactions with the experimenter.**

Treatments	Minimal	Positive	Negative
Number of squares crossed	14.6±4.5	17.9±4.5	16.0±4.0
Latency to enter area A	41.9±60.4	70.1±60.2	24.6±25.1
Time spent in area A	43.0±19.6	27.1±19.1	38.0±20.1
Latency to interaction with experimenter	49.0±58.2	88.3±73.8	90.0±78.0
Number of interactions	5.4±3.4	3.3±2.4	4.6±3.6

Physiological measurements

The Positive handling group showed a lower daytime average of both total ($P < 0.01$; Figure 2a) and free plasma cortisol concentrations ($P < 0.05$; Figure 2b) compared to the Negative handling group. The free plasma cortisol concentration was lower in the Positive treatment than in the Minimal treatment ($P < 0.05$), whereas only a tendency in the same direction was revealed between these treatments for total plasma cortisol ($P = 0.09$).

Total and free plasma cortisol concentrations pre- and post- exposure to human proximity did not differ significantly within each treatment ($P > 0.1$; Figure 3). In the Positive treatment, a lower concentration of total and free plasma cortisol was revealed in the Pre-sample ($P < 0.01$, $P < 0.05$, respectively; Figure 3a,b) compared with the Negative treatment. Pigs in the Minimal treatment also showed a lower total and free plasma cortisol level in the Pre-sample compared with pigs in the Negative treatment ($P < 0.01$, $P < 0.05$, respectively; Figure 3a,b). In the Post-sample, total plasma cortisol concentrations were significantly lower in the Minimal treatment, than in the Negative treatment ($P < 0.05$); and the Positive treatment showed a tendency in the same direction ($P = 0.08$) when compared to the Negative treatment. No differences in total or plasma free cortisol concentrations between the Minimal and Positive treatments were revealed ($P > 0.1$).

Total and free mean plasma cortisol concentrations 1h after an ACTH-challenge did not differ between treatments (mean ± SD: Min total = 238.3±49.3 nmol^{-1}, free = 59.6±11.6 nmol l^{-1}, n = 8; Pos total = 225.0± 16.1 nmol^{-1}, free = 56.9±6.3 nmol^{-1}, n = 4; Neg total = 277.1±70.1 nmol l^{-1}, free = 60.6±25.1 nmol^{-1}, n = 8; $P > 0.1$).

There was no difference between treatments in skin fold thickness at the margin of the ear before injection (mean ± SD: Min = 477.5±7 7.5 μm; Pos = 427.0±4 8.2 μm; Neg = 476.1±62.3 μm; $P > 0.1$). The percentage increase in skin thickness 24h after the injection with the mitogen was smaller in pigs from the Negative treatment compared with pigs from the Minimal ($P < 0.05$) and Positive treatments ($P < 0.01$; Figure 4). When comparing Minimal and Positive treatments, a tendency for a larger increase in the Positive treatment was found ($P = 0.08$).

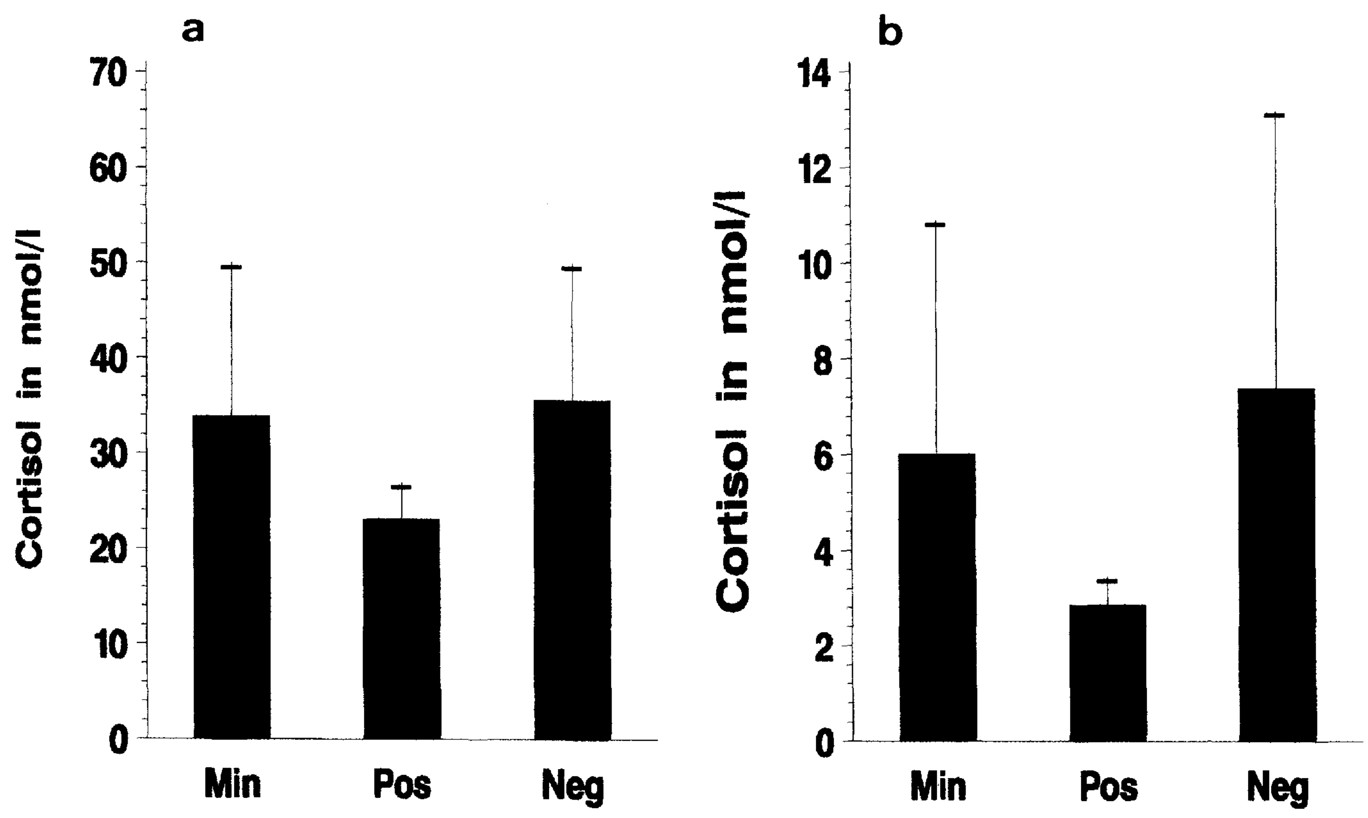

Figure 2 Mean (+standard deviation) daytime plasma cortisol concentrations (nmol l^{-1}) in three treatments, Minimal (Min), Positive (Pos) and Negative (Neg). The left figure (a) shows total plasma cortisol concentration, and the right figure (b) shows free plasma cortisol concentration.

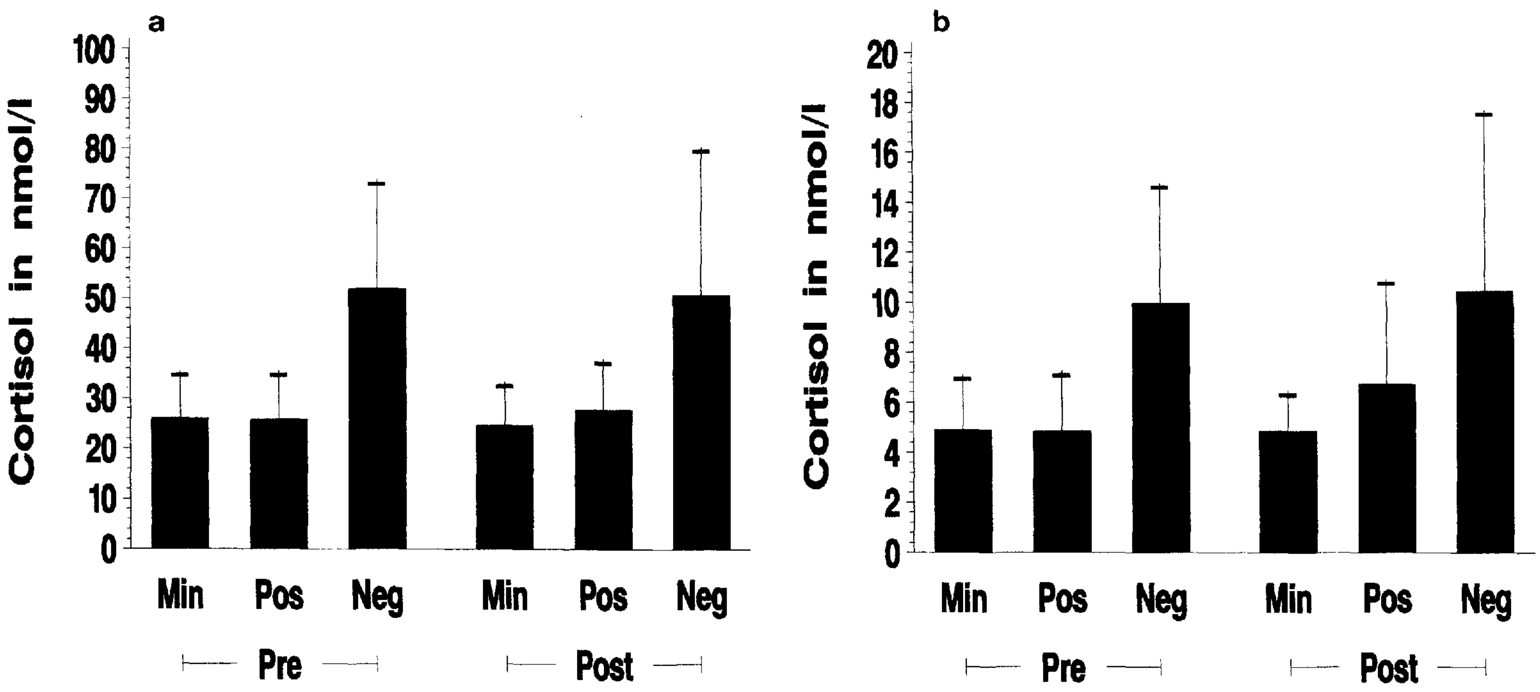

Figure 3 Mean (+standard deviation) plasma cortisol concentrations (nmol l^{-1}) in three treatments, Minimal (Min), Positive (Pos) and Negative (Neg), prior to (Pre) and after (Post) exposure to human proximity. The left figure (a) shows total plasma cortisol concentration and the right figure (b) shows free plasma cortisol concentration.

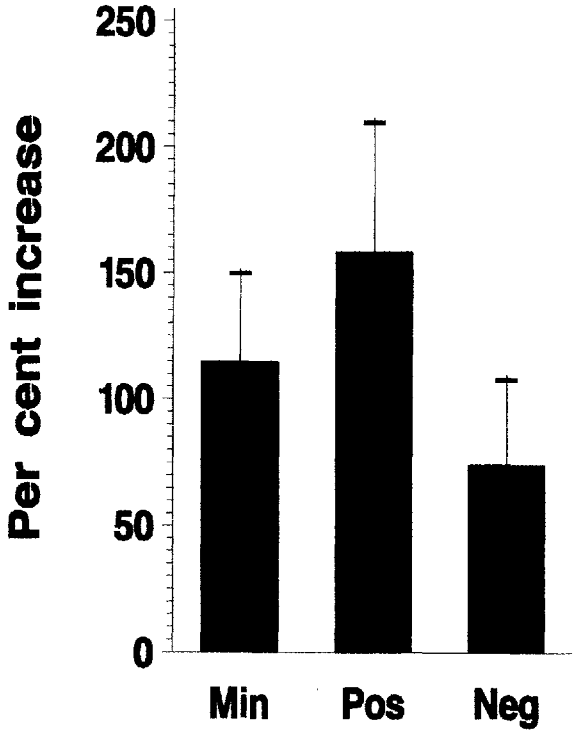

Figure 4 **Immunological responsiveness in three treatments, Minimal (Min), Positive (Pos), and Negative (Neg), expressed as the mean (+standard deviation) percentage increase of the skin thickness at the margin of the ear 24h after an injection with a mitogen.**

Discussion

The increased amount of daily physical contact between handler and pigs in the Positive treatment indicated that the pigs experienced handling as pleasant, since they actively sought contact with the handler during the handling sessions. On the other hand, the pigs in the Negative treatment generally avoided contact with the handler. These pigs quickly learned to show an appropriate withdrawal response due to the unpleasant electric shock. The fact that the number of administered shocks remained more or less constant, and that the pigs regularly withdrew from the handler, suggests that the procedure had caused negative conditioning of the pigs. It appears that the different handling procedures accomplished a positive human-animal relationship (ie approach) in one group of pigs; and a negative human-animal relationship (ie avoidance) in another.

On the basis of earlier studies (Hemsworth *et al* 1981, 1986) we hypothesized that the positively handled pigs would explore more and be quicker to interact with the human in the arena test. However, the present study failed to demonstrate any effects of treatments on behavioural responses in the arena test. This result could indicate that the avoidance response of pigs in the Negative treatment was situational or context-specific. Alternatively, and perhaps more likely, the pigs in this treatment may not have recognized the human imposing the treatment (only head, arms and shoulders visible to the pigs) as similar to the erect, stationary human in the arena test.

The physiological data strongly indicated that the Positive treatment obviated some of the negative consequences of being kept in tether-stalls. The average daytime levels of free plasma cortisol in the Minimal and Negative treatments closely resembled those of pregnant pigs kept in tether-stalls (6.6 nmol l^{-1}) in the study of Barnett *et al* (1991), whereas the average daytime level of free plasma cortisol in the Positive treatment was even lower than for group-housed pregnant pigs (4.4 nmol l^{-1}) in the same study. This sustained elevation of free plasma cortisol indicated an impaired welfare in the Minimal and Negative treatments relative to the Positive treatment. The daytime profiles of cortisol which were obtained in the present study obviously did not resemble absolute base levels of cortisol. However, the daytime profile of free plasma cortisol in the Minimal treatment most probably reflected the physiological effect of being in tethers and having minimal human contact. Taking the Minimal treatment as a reference level, the daytime profile of free plasma cortisol in the Positive treatment showed a reduction of 52 per cent, contrasting with an elevation of 23 per cent in the Negative treatment. This indicated that the established, positive human-animal relationship reduced the physiological stress experienced by pregnant pigs kept in tethers, while the established, negative human-animal relationship to some extent enhanced the physiological stress they experienced.

Contrary to our hypothesis, pigs in the Negative (and Minimal) treatments did not show elevated plasma cortisol concentrations in response to human proximity. However, the pre- and post-exposure concentrations of plasma cortisol, reflect sustained elevation of plasma cortisol in these pigs as found in the daytime average data. The withdrawal responses shown by the pigs during negative handling also indicated high levels of fear of humans, which have previously been associated with elevations of plasma cortisol (Hemsworth & Barnett 1987). Additional behavioural data during the pre-/post- human proximity test would have been useful in interpreting the lack of a cortisol response to a simulated handling session. However, these data were not collected in this preliminary study.

The ACTH challenge was performed to determine if the adrenal capacity to secrete cortisol differed between differently handled pigs given an ACTH dose that provoked maximum stimulation of the adrenals. It has been shown that chronically stressed animals which show an elevated basal cortisol concentration respond faster, and to a greater extent, to an ACTH-challenge (Friend *et al* 1979, 1985). However, no significant effect of treatment on the cortisol response to the ACTH challenge was found in the present study. Rampacek *et al* (1984) took repeated blood samples after an ACTH injection in confined and non-confined gilts and found that plasma cortisol concentrations peaked 2h after injection. In the present study, only one sample was taken 1h post ACTH challenge. Thus, the cortisol levels might not have peaked at this point, although a study by Baldi *et al* (1989) and review by Stephens (1980) indicate that in pigs the maximal response to an ACTH challenge is reached approximately 1h after injection. Another explanation for lack of differences between treatments in adrenocortical reaction to the ACTH administration found in this study was that, due to failure of the catheters, only four of the eight pigs in the Positive treatment could be sampled.

The immunological data support the animal welfare implications of the daytime cortisol data, with evidence of a suppressed cell-mediated response in the Negative treatment and a tendency in the same direction in the Minimal treatment. Immunosuppression in pigs housed in tether-stalls was found in the study of Barnett *et al* (1987); while reduced space allowance caused higher concentrations of free corticosteroids and immunosuppression in pregnant pigs (Barnett *et al* 1992). Since both hormonal data and immunological data point in the same direction in the present study, there is consistent evidence that negatively handled, and to some extent the minimally handled, pigs suffered from reduced welfare.

Conclusion

This should be viewed as a preliminary study with no firm conclusions possible on the basis of the small sample size. However, the results support the hypothesis that the nature of the human-animal relationship affects the physiology of tethered pregnant pigs and that a positive human-animal relationship obviates some of the negative effects of being tethered, by lowering the physiological stress level and by strengthening the immunological reactivity of pregnant pigs. In further studies on a larger scale, a combination of different housing systems and different levels of human contact should be included in order to evaluate the importance of these two parameters for the animals.

Animal welfare implications

In the light of the results of the present study it should be emphasized that welfare in husbandry animals might not be a question about unidirectional solutions (housing *or* human-animal relationships) but rather about multidirectional ones (housing *and* human-animal relationships). The importance of the nature of the human-animal relationship should not be overlooked in the search for the optimal physical environment.

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