

EFFECTS OF DIFFERENT DEGREES OF SOCIAL ISOLATION ON THE BEHAVIOUR OF WEANED PIGLETS KEPT FOR EXPERIMENTAL PURPOSES

M S Herskin[†] and K H Jensen

Danish Institute of Agricultural Sciences, Department of Animal Health & Welfare, Research Centre Foulum, P O Box 50, DK-8830 Tjele, Denmark

[†] Contact for correspondence and requests for reprints

Final Acceptance: 21 December 1999

Abstract

Animal Welfare 2000, **9**: 237-249

The effect of degree of isolation on the behaviour of weaned piglets was investigated using eight replicates of littermates, weaned at 4 weeks and caged for 2 weeks in metabolism chambers under varying degrees of isolation: i) fully isolated without physical contact with littermates; ii) partly isolated with limited physical contact with littermates; or iii) grouped with three littermates. The behaviour of the piglets was video recorded from 0700h–2230h on days 1, 6 and 13 post-weaning. In addition, a test of behavioural reactivity towards a novel environment/object was performed on day 8.

Irrespective of degree, isolation initially increased the occurrence of behavioural indicators of stress such as the frequency of pawing and escape attempts as well as decreasing the frequency of play. In partly isolated piglets this initial response was more active than in fully isolated piglets, indicating an increased frustration with isolation. Within 2 weeks, however, the stress response in partly isolated piglets had almost completely waned, and they showed only a decreased frequency of play behaviour, whereas in fully isolated piglets an increased frequency of pawing as well as a more pronounced reduction of play behaviour were still evident on day 13. Both isolation treatments resulted in markedly lower reactivity in a novel environment, seen as fewer squares crossed and fewer vocalizations.

It is concluded that social isolation of newly weaned piglets is stressful. Although it may increase the initial stress response, provision of social contact with littermates, eg through wire mesh, might limit negative long-term effects of isolation in experiments where data collection requires individual housing.

Keywords: *animal welfare, behavioural reactivity, housing, metabolism chamber, novelty, stress*

Introduction

Experimental work with social species such as the pig often implies individual housing, eg caging of piglets in metabolism chambers. Only very few reports take the behavioural consequences of housing farm animals under experimental conditions into account. However, raising piglets in individual incubators from days 1–7 post-partum led to marked behavioural changes such as increased sitting and vocalization as well as decreased play behaviour

(Hutton & Wood-Gush 1983). Housing adult sheep in individual metabolism chambers produced both behavioural and physiological changes, which led Van Adrichem and Vogt (1993) to conclude that isolation of sheep constitutes a severe stressor. Data on the behavioural effects of social isolation of newly weaned piglets are not available.

However, in pig production, it is well established that early weaning is in itself stressful for piglets (eg Fraser [1978]; Dybkjær [1992]). Under these circumstances, weaning is a multifactorial stressor involving a change of diet, maternal deprivation, mixing and a change of environment as well as spatial restriction (eg Varley [1995]). Weaning piglets at 4 weeks of age involves behavioural changes such as belly-nosing, decreased play behaviour, increased chain manipulation, increased sitting behaviour, increased activity and increased aggression (eg Blackshaw [1981]; Metz & Gonyou [1990]; Worobec *et al* [1998]). Since weaned piglets in pig production are invariably kept in groups, these observations of changes in behaviour all come from experiments using group housing, and several of the behavioural elements can only be performed by at least two piglets.

Keeping newly weaned piglets in individual metabolism chambers changes several of the factors characterizing weaning in pig production. Instead of being stressed by mixing combined with spatial restriction, the piglets are socially isolated – a treatment known to result in chronic stress in older pigs (eg Barnett *et al* [1981]). Isolation (like tethering) is a complex stressor with diurnal variation in strength and mode of action depending on the motivational status of the animal, and interfering with several biological functions. As mentioned, the effects of isolation on the behaviour of weaned piglets, as well as the majority of farm animals, are only scantily documented, since these animals are rarely kept individually. In an examination of the effects of different degrees of isolation on the behavioural reactivity of veal calves, Veissier *et al* (1997) found that increasing the degree of isolation led to increased reactivity towards a water splash. During experiments requiring individual housing of newly weaned piglets, it is possible that provision of limited social contact might improve the adverse effects of the housing system. The aims of the present study were therefore: (i) to examine whether social isolation led to changed behaviour compared to group-housed piglets; and (ii) to examine whether provision of limited social contact through wire mesh affected the behaviour of newly weaned piglets.

Materials and methods

Animals and feeding

The experiment was carried out at the Research Centre Foulum, Danish Institute of Agricultural Sciences, Denmark. A total of eight litters of Danish Landrace x Yorkshire piglets from the resident herd were used. The piglets were raised in farrowing crates supplied with chopped straw. The experimental litters were initially selected to ensure litter sizes above eight and not subjected to standardization of litter sizes. Only barrows (castrated males) were used for behavioural observations. At 28 days of age (day 0) the piglets were weaned and transported in a trolley to the experimental room at 1000h.

Before weaning, the piglets were not fed any supplementary food. Post-weaning, the piglets were fed and watered three times day⁻¹ (at 0800h, 1500h and 2200h) and fed a progressively increasing ration (50g piglet⁻¹ on the first day post-weaning increasing to 275g piglet⁻¹ on day 13 post-weaning) of concentrate (produced by the institute, composition [as % of dry matter] as follows: barley 33.2%, wheat 33.1%, soy meal 11%, fish meal 11%, skimmed milk powder 5%, animal fat 4%, vitamins and minerals 7.7%).

The temperature in the experimental room was kept at 23–25°C and the relative humidity above 30 per cent. Light from the outside was excluded and the room was lit artificially between 0700h and 2230h.

Design

There were eight replicates of six littermates in the experiment. Replication occurred over time as well as across litters of pigs. The three different treatments (Figure 1) were:

- (i) Fully isolated – individual metabolism chamber with transparent Perspex walls situated at least 2m from any other metabolism chamber allowing no physical contact with other piglets.
- (ii) Partly isolated – individual metabolism chamber with restricted physical contact with littermates through wire mesh (3x3 cm).
- (iii) Full social contact – a group of four littermates kept in one metabolism chamber. (One barrow was chosen at random as the focal animal and colour-marked on the back for visual identification, the remaining three piglets were not marked and their behaviour was not observed.)

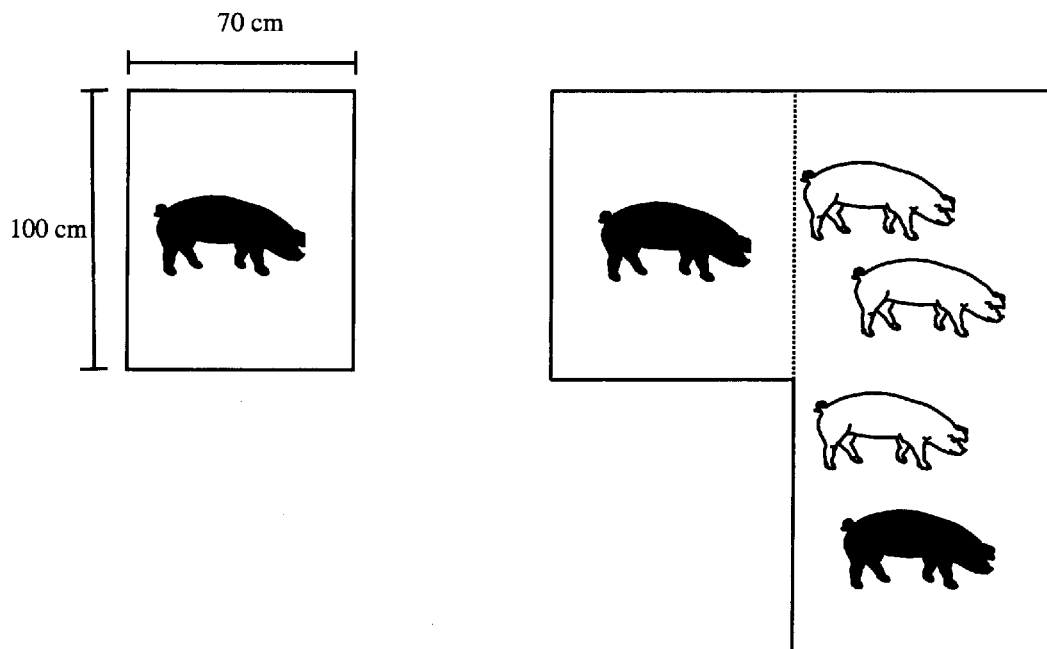


Figure 1 Schematic drawing of the experimental set-up, showing the three types of metabolism chamber.

All metabolism chambers contained slatted floors made of plastic, one feeding trough (two in the group treatment), one 40cm chain fixed to the wall (two in the group treatment). During replicates, the metabolism chambers were kept in the same room. The piglets were kept in the metabolism chambers until 14 days post-weaning.

Behavioural observations*Undisturbed behaviour*

Time-lapse video recordings (Panasonic TL700, Super VHS) were made from above the metabolism chambers during the light period on three occasions: from 0700h–2230h on days 1, 6 and 13 post-weaning. The videotapes were analysed by focal sampling and continuous recording (Martin & Bateson 1986). The ethogram of the behaviours recorded in the experiment is shown in Table 1.

Table 1 Behaviours recorded during the 15.5h observation periods. Events were observed during periods of activity.

	Behavioural variable	Definition
<i>States</i>	Lying	Lying down.
	Sitting	Sitting like a dog.
	Active	Standing or moving around.
<i>Events</i>	Manipulating chain	Touching the chain with the snout. Intervals of less than 30s between touching the chain were ignored.
	Pawing	Digging movements with the forelegs on the floor. Intervals of less than 30s between separate pawing movements were ignored.
	Escape attempt	Rearing on the hind legs. The next escape attempt could be initiated once the piglet had touched the floor with all four legs.
	Play	Scampering: running slowly with vertical and horizontal bouncy movements (Newberry <i>et al</i> 1988). Intervals of less than 30s between bouts of play behaviour were ignored.
	Snout contact with wall	Directing the snout at the wall facing the other metabolism chambers. Maximum distance: the width of the snout.

Open-field/novel object test

Each experimental piglet was tested once between 1000h and 1300h on day 8 post-weaning for behavioural reactivity in an open-field/novel object test. The test took place in the same room as the metabolism chambers in a 2.4x2.4 m arena with solid wooden walls 0.9m high, and 25 equal squares marked on the floor. The test piglet was carried to the arena and the test began as it was released from a starting position in one corner. The behaviour of the piglet was videotaped for 5min, while the number of vocalizations were counted by direct observation at 1-min intervals. After 5min, a red bucket was lowered quietly from the ceiling and recording of behaviour and vocalizations continued for a further 5min. The ethogram of the behaviours recorded during the open-field/novel object test is shown in Table 2. The testing order of the piglets was randomized.

Variables*Undisturbed behaviour*

Means of the following variables were calculated per hour and day of observation: i) time spent lying; ii) time spent sitting; iii) time spent active; iv) frequency of sitting; v) number of postural changes hour⁻¹, calculated as frequency of [lying + sitting + active - 1]; vi) frequency of chain manipulation; vii) frequency of pawing; viii) frequency of escape attempts; ix) frequency of play behaviour; x) frequency of snout contact with the transparent wall facing the littermates.

Table 2 Behaviours recorded during the open-field/novel object test.

Behavioural variable		Definition
<i>Ambulation Behaviour</i>	Squares crossed	At least one foreleg has entered the square.
	Lying	Lying down.
	Sitting	Sitting like a dog.
	Standing	Standing, neither moving forwards or backwards.
	Walking	Slowly moving forward with one leg at a time. Intervals of less than 1 s between walking bouts were ignored.
	Running	Trotting or galloping without sudden changes in direction/speed.
<i>Event</i>	Escape attempt	Rearing on the hind legs. The next escape attempt could be initiated once the piglet had touched the floor with all four legs.
<i>Position in the arena during the 5 min with the bucket</i>	Periphery	Situated in one of the squares along the wall.
	Close to bucket	Situated within 0.5 m of the bucket.
	Touching the bucket	Touching the bucket or being closer to the bucket than the width of the snout.

Open-field/novel object test, 5 min without an object in the arena

The mean values of the following variables were calculated: i) number of vocalizations min^{-1} ; ii) number of squares crossed; iii) time spent standing; iv) time spent walking; v) time spent running; vi) behavioural transitions, calculated as the frequency of [standing + walking + running - 1]; vii) frequency of escape attempts.

Open-field/novel object test, 5 min with the novel object in the arena

The mean values of the following variables were calculated: i) number of vocalizations min^{-1} ; ii) number of squares crossed; iii) time spent standing; iv) time spent walking; v) time spent running; vi) behavioural transitions, calculated as the frequency of [standing + walking + running - 1]; vii) frequency of escape attempts; viii) latency to touch the novel object; ix) latency to come close to (< 0.5 m) the novel object; x) time spent touching the novel object; xi) time spent close to (< 0.5 m) the novel object; xii) frequency of touching the novel object.

Statistical analyses

Due to technical difficulties, the number of observations within each treatment varied from 5–8 animals (Tables 3 and 4). A probability of $P < 0.05$ was chosen as the level of significance, with $0.05 < P < 0.1$ reported as a tendency.

Continuous variables

Data were subjected to an analysis of variance according to the Mixed Procedure of SAS® (Littell *et al* 1996). Each piglet/pen was used as an experimental unit and the results expressed as least squares means \pm SEMs and associated probability values. The basic model included treatment as a general fixed effect. Replicate was treated as a random factor in all analyses. In order to test for linear as well as non-linear relationships, the body weight at weaning as well as the square of the body weight at weaning were included as covariates if $P < 0.20$. Furthermore, the testing order of the individual piglets was included as a fixed effect in the analysis of data from the open-field/novel object test if $P < 0.20$. Due to the

Table 3 Comparison of undisturbed behaviour (mean ± SEM per hour) for the piglets on days 1, 6 and 13 post-weaning, based on observations during the light period (15.5 h per day), and associated probabilities.

Day post weaning	Fully isolated									Partly isolated									Grouped			P-value
	1	6	13	1	6	13	1	6	13	1	6	13	1	6	13	1	6	13				
<i>Time lying (min)</i>	47.8 ± 2.8 n = 7	46.6 ± 1.5 ^b n = 5	43.9 ± 2.3 n = 6	40.8 ± 2.6 n = 6	42.0 ± 1.2 ^a n = 7	39.4 ± 2.1 n = 7	41.5 ± 2.9 n = 5	47.4 ± 1.6 ^b n = 5	42.1 ± 2.4 n = 5	day 6: P < 0.05												
<i>Time sitting (s)</i>	61 ± 25 ^a	23 ± 14	18 ± 7	24 ± 16 ^b	24 ± 13	8 ± 5	10 ± 11 ^b	4 ± 7	22 ± 9	day 1: P = 0.09												
<i>Time active (min)</i>	9.6 ± 2.1 ^a	9.7 ± 1.1 ^a	12.2 ± 2.1	17.9 ± 2.9 ^b	15.4 ± 1.2 ^b	17.4 ± 2.4	14.5 ± 2.8	11.2 ± 1.2 ^a	15.6 ± 2.5	day 1,6: P < 0.05												
<i>Freq of sitting</i>	2.2 ± 0.6 ^a	0.8 ± 0.2	0.6 ± 0.1	1.5 ± 0.5 ^a	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.3 ^b	0.5 ± 0.1	0.7 ± 0.1	day 1: P = 0.08												
<i>Postural changes (n)</i>	22.5 ± 5.4 ^b	11.3 ± 2.7 ^a	10.8 ± 2.1	40.2 ± 7.3 ^a	17.6 ± 3.1 ^b	13.7 ± 2.3	21.6 ± 5.6 ^b	16.7 ± 3.4 ^b	13.3 ± 2.4	day 1: P < 0.01												
<i>Freq of chain manipulation</i>	2.1 ± 0.5 ^a	2.1 ± 0.5 ^a	3.0 ± 0.5	5.4 ± 0.9 ^b	4.6 ± 0.7 ^b	4.0 ± 0.5	3.1 ± 0.7 ^a	2.2 ± 0.1 ^a	2.7 ± 0.5	day 6: P = 0.08												
<i>Freq of pawing</i>	0.9 ± 0.2 ^a	1.1 ± 0.3 ^a	1.6 ± 0.3 ^a	1.1 ± 0.3 ^a	1.0 ± 0.2 ^a	0.8 ± 0.2 ^b	0.1 ± 0.1 ^b	0.2 ± 0.1 ^b	0.2 ± 0.1 ^b	day 1, 6: P = 0.07												
<i>Freq of escape</i>	3.6 ± 1.2 ^a	0.1 ± 0.02	0.4 ± 0.1	1.8 ± 0.9 ^a	0.2 ± 0.02	0.9 ± 0.2	0.1 ± 0.2 ^b	0.03 ± 0.01	0.2 ± 0.1	day 13: P < 0.05												
<i>Freq of play behaviour</i>	0 ^a	0.02 ± 0.02 ^a	0.2 ± 0.1 ^a	0.01 ± 0.02 ^a	0.2 ± 0.1 ^a	0.4 ± 0.1 ^b	1.4 ± 0.3 ^b	1.3 ± 0.2 ^b	1.4 ± 0.2 ^c	day 1: P < 0.05												
<i>Freq of snout contact at wall</i>	4.7 ± 2.1 ^a	2.0 ± 0.9	2.6 ± 0.9	11.8 ± 3.6 ^b	4.8 ± 1.2	4.3 ± 1.9	3.0 ± 1.9 ^a	2.7 ± 1.0	3.3 ± 1.1	day 6: P < 0.01												
										day 13: P < 0.001												
										day 1: P < 0.05												

Different lettered superscripts within a row indicate differences between treatments (P < 0.1).

Table 4 Comparison of the behaviour of the piglets during the open-field/novel object test performed at day 8 post-weaning. Data are expressed as means \pm SEM unless otherwise indicated, probabilities are as shown.

Behavioural parameter	Smin alone in arena			Smin with novel object			P-value
	Fully isolated	Partly isolated	Group	Fully isolated	Partly isolated	Group	
<i>Vocalization, min 1</i>	n = 7 1.4 \pm 1.6 ^a	n = 8 10.6 \pm 4.4 ^b	n = 8 9.8 \pm 4.0 ^b	n = 7 3.2 \pm 2.9 ^a	n = 8 16.5 \pm 6.3 ^b	n = 8 37.9 \pm 9.5 ^c	P < 0.001
<i>Vocalization, min 2</i>	2.3 \pm 1.7 ^a	13.8 \pm 4.2 ^b	22.1 \pm 5.2 ^b	3.8 \pm 2.6 ^a	18.8 \pm 5.6 ^b	33.8 \pm 7.5 ^c	P < 0.001
<i>Vocalization, min 3</i>	2.4 \pm 1.9 ^a	14.0 \pm 4.4 ^b	34.0 \pm 6.9 ^c	2.9 \pm 2.3 ^a	25.0 \pm 6.4 ^b	29.2 \pm 7.3 ^b	P < 0.001
<i>Vocalization, min 4</i>	4.9 \pm 2.0 ^a	17.1 \pm 3.6 ^b	39.7 \pm 5.4 ^c	3.9 \pm 2.6 ^a	20.5 \pm 5.6 ^b	36.2 \pm 7.9 ^c	P < 0.001
<i>Vocalization, min 5</i>	3.3 \pm 2.0 ^a	22.1 \pm 4.9 ^b	33.1 \pm 5.8 ^b	3.8 \pm 2.8 ^a	16.8 \pm 5.5 ^b	36.1 \pm 8.5 ^c	P < 0.01
<i>Squares crossed</i>	25.5 \pm 7.3 ^b	33.7 \pm 8.5 ^b	60.9 \pm 11.7 ^a	25.3 \pm 7.2 ^a	33.2 \pm 8.6	62.4 \pm 13.2 ^b	P = 0.09
<i>Time standing (s)</i>	207 \pm 15	202 \pm 15	185 \pm 14	213 \pm 16 ^a	226 \pm 17 ^a	161 \pm 14 ^b	P < 0.01
<i>Time walking (s)</i>	84 \pm 11 ^b	82 \pm 11 ^b	118 \pm 13 ^a	68 \pm 14	78 \pm 16	97 \pm 20	ns
<i>Time running (s)</i>	0 (0-0)	1 (0-3)	2 (0-7)	1 \pm 1	1 \pm 1	1 \pm 1	ns
<i>Escape attempts</i>	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)	ns ¹
<i>Behavioural transitions</i>	33.4 \pm 5.9 ^a	52.4 \pm 7.1 ^b	51.1 \pm 7.0 ^b	40.7 \pm 7.3	42.2 \pm 7.9	68.5 \pm 11.2	ns
<i>Latency, close to object (s)</i>				29 (4-90)	39 (5-128) ^a	5 (1-7) ^b	P < 0.05 ¹
<i>Latency, touch object (s)</i>				96 (9-255)	100 (18-218)	17 (5-80)	ns ¹
<i>Time close to object (s)</i>				26 \pm 8 ^a	38 \pm 10 ^a	64 \pm 12 ^b	P < 0.05
<i>Time touching object (s)</i>				3 \pm 2 ^a	12 \pm 5 ^b	12 \pm 5 ^b	P < 0.05
<i>Freq touching object</i>				2.4 \pm 0.8 ^a	3.1 \pm 0.9 ^a	5.4 \pm 1.2 ^b	P = 0.06

Different lettered superscripts within a row indicate significant differences between treatments (P < 0.1).

¹ Median (25-75% quartiles).

distribution of data, either a square root or a logarithmic transformation of the dependent variables was implemented to meet the assumptions of normality and homogeneous variances.

In case of non-fulfilled assumptions, data were analysed using the non-parametric Kruskal-Wallis ANOVA on ranks (SigmaStat; Siegel & Castellan 1988). These results are presented as medians, 25–75 per cent quartiles and the associated probability value.

To describe the within-treatment development over time, data from day 1, 6 and 13 post-weaning were analysed using a one-way repeated-measures ANOVA (SigmaStat; Siegel & Castellan 1988). The probability values for these results are presented.

Censored variables

The latencies to come close to and touch the novel object were regarded as censored variables (Kleinbaum 1996) and compared between treatments using the non-parametric Lifestest® for survival data (SAS Institute Inc 1996). These results are presented as medians, 25–75 per cent quartiles, the chi-square value and associated *P*-value.

Results

Undisturbed behaviour

Table 3 shows the effects of different degrees of isolation on the undisturbed behaviour of the piglets on days 1, 6 and 13 post-weaning.

Day 1

Irrespective of degree, isolation tended to increase the frequency of sitting compared with the group-housed piglets ($F_{2,6} = 3.90$, $P = 0.08$). Time spent sitting was very limited, and varied considerably between individuals (range 2–195 s h⁻¹). The fully isolated piglets, however, tended to spend more time sitting than the partly isolated and the grouped piglets ($F_{2,6} = 3.82$, $P = 0.09$). Furthermore, the full isolation decreased time spent active compared with partial isolation ($F_{2,6} = 5.44$, $P < 0.05$).

In addition, both isolation treatments increased the frequency of escape attempts ($F_{2,8} = 7.59$, $P < 0.05$), decreased the frequency of play behaviour ($F_{2,8} = 7.28$, $P < 0.05$) and tended to increase the frequency of pawing behaviour ($F_{2,8} = 3.97$, $P = 0.07$) compared with group housing.

Partly isolated piglets differed from the two other treatments in that they performed an increased number of postural changes ($F_{2,7} = 10.57$, $P < 0.01$), a higher frequency of chain manipulation ($F_{2,7} = 8.49$, $P < 0.05$) and an increased frequency of snout contact with the transparent wall facing the littermates ($F_{2,8} = 5.39$, $P < 0.05$).

Day 6

A reduced frequency of play behaviour ($F_{2,8} = 15.94$, $P < 0.01$) and the tendency to perform more pawing behaviour ($F_{2,8} = 3.66$, $P = 0.07$) were still evident in the two isolation treatments when compared with group-housed piglets.

The fully isolated piglets tended to perform fewer postural changes than the two other treatments ($F_{2,7} = 3.65$, $P = 0.08$), whereas the partly isolated piglets spent less time lying per hour ($F_{2,7} = 4.80$, $P < 0.05$) and more time active ($F_{2,8} = 7.41$, $P < 0.05$) than the two other treatments. Furthermore, the partly isolated piglets showed a higher frequency of chain manipulation than the two other treatments ($F_{2,8} = 5.01$; $P < 0.05$).

Day 13

Most of the behavioural variables were at comparable levels in isolated and group-housed piglets at this time. However, the frequency of play behaviour was still significantly lower in the fully and partly isolated piglets compared with their group-housed littermates, and this difference was most evident in fully isolated piglets ($F_{2,7} = 26.47$, $P < 0.001$). Furthermore, the frequency of pawing was still higher in fully isolated piglets ($F_{2,8} = 5.91$, $P < 0.05$).

Development over the 13 days post-weaning

The data are presented in Table 3. No significant changes were found for: duration of lying h^{-1} ; duration of sitting h^{-1} ; duration of activity h^{-1} ; or frequency of chain manipulation h^{-1} .

The frequency of postural changes, however, decreased significantly for the fully isolated as well as the partly isolated piglets from day 1 until days 6 and 13 post-weaning ($F_{2,8} = 11.54$, $P < 0.01$ and $F_{2,11} = 10.80$, $P < 0.01$, respectively), whereas play behaviour was more frequent on day 13 than on day 1 ($F_{2,8} = 3.80$, $P = 0.06$ and $F_{2,11} = 5.94$, $P < 0.05$ for fully isolated and partly isolated piglets, respectively). In addition, the frequency of sitting ($F_{2,8} = 3.48$, $P = 0.08$) and of escape attempts ($F_{2,8} = 4.11$, $P < 0.06$) tended to decrease from day 1 to days 6 and 13 in fully isolated piglets.

In addition, in the partly isolated piglets the frequency of snout contact with the transparent wall facing the littermates decreased from day 1 to days 6 and 13 ($F_{2,11} = 4.24$, $P < 0.05$).

Behaviour during the open-field/novel object test

Table 4 shows the effects of different degrees of isolation on the behaviour during the open-field/novel object test performed at 8 days post-weaning.

Behaviour during the 5min without the object in the arena

Irrespective of degree of isolation, the isolated piglets crossed fewer squares ($F_{2,11} = 3.95$, $P = 0.05$) and spent less time walking ($F_{2,7} = 5.82$, $P < 0.05$) than the group-housed ones. In addition, the fully isolated piglets performed fewer behavioural transitions ($F_{2,13} = 6.10$, $P < 0.05$) and vocalized less during each of the 5min periods than the two other treatments (first minute – $F_{2,8} = 3.72$, $P = 0.07$; second minute – $F_{2,12} = 14.79$, $P < 0.001$; third minute – $F_{2,13} = 36.83$, $P < 0.001$; fourth minute – $F_{2,9} = 21.05$, $P < 0.001$; and fifth minute – $F_{2,11} = 3.95$, $P < 0.001$). Partly isolated piglets only vocalized less than their group-housed littermates during the third and fourth minutes.

Behaviour during the 5min with the novel object

The number of squares crossed after introduction of the novel object tended to be lower for the isolated piglets compared with their group-housed littermates ($F_{2,10} = 3.11$, $P = 0.09$), whereas the time spent standing was increased ($F_{2,11} = 9.11$, $P < 0.01$).

The frequency of vocalization during the 5min with the novel object was very similar to the frequency of vocalization before introduction of the novel object. During each of the 5min the fully isolated piglets vocalized less than their partly isolated littermates, which vocalized less than the group-housed piglets in all but the third minute (first minute – $F_{2,13} = 14.60$, $P < 0.001$; second minute – $F_{2,13} = 16.37$, $P < 0.001$; third minute – $F_{2,12} = 17.49$, $P < 0.001$; fourth minute – $F_{2,12} = 14.18$, $P < 0.001$; and fifth minute – $F_{2,8} = 10.28$, $P < 0.01$).

Treatments affected the behaviour toward the novel object as well. Irrespective of degree of isolation, the isolated piglets spent less time close to the object ($F_{2,8} = 5.91$, $P < 0.05$) and

tended to show a lower frequency of touching the object ($F_{2,13} = 3.48$, $P = 0.06$) than their group-housed littermates. Furthermore, in the fully isolated piglets the time spent touching the object ($F_{2,13} = 5.18$, $P < 0.05$) was significantly shorter than in the two other treatments. The latency to come close to the object was only significantly increased in partly isolated, as compared with group-housed, piglets (chi-square = 4.24, $P < 0.05$).

Discussion

The results presented for undisturbed behaviour indicate that isolation, irrespective of its degree, constituted a stressor in weaned piglets, but the long-term effects of isolation may be ameliorated by provision of limited contact with littermates through a wire mesh. In both treatments of isolation, several variables considered indicative of stress, such as escape attempts, pawing behaviour and the frequency of sitting, were initially increased, and the frequency of play behaviour was decreased. On day 13, however, partly isolated piglets only differed from group-housed by a decreased frequency of play, whereas fully isolated piglets showed a more pronounced decrease in play behaviour as well as an increased frequency of pawing.

Behavioural responses similar to those initially shown by isolated piglets have been reported for piglets kept under intensive housing conditions in pig production (eg Fraser [1978]; Dybkjær [1992]) and are considered indicative of stress. In addition, investigations of long-term housing in different intensive systems as well as of subjecting animals to intermittent electric shocks have shown that sitting behaviour, which is occasionally observed in semi-natural conditions (Petersen 1994) and in the wild boar, *Sus scrofa* (Gundlach 1968), is often increased under such circumstances (eg Pearce & Paterson [1993]; Jensen *et al* [1996]). Jensen *et al* (1996) suggested that sitting behaviour might be an unspecific behavioural indicator of stress in pigs. Similarly, Fraser (1974) suggested that pawing behaviour, which was not observed until the day of weaning, is a sign of the so-called 'I-cannot-get-comfortable-syndrome' and can, therefore, be characterized as another behavioural indicator of stress. Furthermore, decreased occurrence of play behaviour has been reported as a result of intensive housing (Metz & Osterlee 1981) as well as under-nutrition (Barnes *et al* 1976), and Dybkjær (1992) suggested that lack of play behaviour can be used as a behavioural indicator of stress in newly weaned piglets.

Taken together, the present observations of differences between isolated and group-housed piglets indicate that isolation in metabolism chambers is stressful for newly weaned piglets and that traditional behavioural indicators of stress in pigs, which have so far been used primarily in pig production, can also be applied to experimentally kept animals. Furthermore, the present results confirm previous results on pigs (Jensen *et al* 1996) as well as mink, *Mustela vison* (Heller & Jeppesen 1985) and rodents (eg Sudha & Pradhan [1993]) showing time-dependent changes from behavioural activation to behavioural depression as a characteristic of the stress response. The initial changes in the undisturbed behaviour of the isolated piglets involved an increase in, for example, escape attempts indicating behavioural activation, whereas the response after nearly 2 weeks was mainly characterized by depressed play behaviour, which may be interpreted as a more passive response. Furthermore, the frequencies of escape attempts and postural changes were significantly reduced at day 13 in comparison with the initial response.

The response of the partly isolated piglets reveals that their initial behavioural activation was more pronounced than in fully isolated piglets. On day 1, access to limited contact with littermates resulted in a longer duration of activity, increased frequency of postural changes

and chain manipulation as well as a tendency to sit for a shorter time compared with full isolation. After 6 days of partial isolation, there were still large effects of the housing system, which were similar to day 1, in that the partly isolated piglets were more active, and performed more chain manipulation as well as more postural changes than their isolated littermates. The only significant change between the first two observation days was a lower frequency of postural changes on day 6. The behavioural activation from day 1 was, therefore, apparently still present on day 6. The initially increased activity in partly isolated pigs may be interpreted as a stress-induced behavioural activation due to increased frustration when having access to limited social contact. This apparently increased the tendency for social interaction, as indicated by the increased frequency of snout contact with the transparent wall facing the littermates.

Although the initial response to isolation seemed to be enhanced in partly isolated piglets, the long-term behavioural effects of partial isolation were apparently less pronounced than the effects of full isolation. After almost 2 weeks in the metabolism chambers, the partly isolated piglets only differed from their group-housed littermates by a slightly decreased frequency of play behaviour, and compared with the fully isolated piglets they played more frequently and performed less pawing behaviour. Thus, at this stage, the behaviour of the partly isolated piglets had almost returned to a state comparable with that of the group-housed piglets.

In the present study the response in undisturbed behaviour in the isolated piglets was accompanied by a response in the open-field/novel object test, which was characterized by a decrease in the number of squares crossed, decreased walking and decreased vocalization in the novel environment; and by less vocalization, increased standing, less time spent close to the object, and less frequent contact with the novel object. According to the interpretations of various authors working on pigs at different ages and open-field testing (eg Fraser [1978]; Von Borell & Hurnik [1991]; Von Borell & Ladewig [1992]; Jensen *et al* [1995a]; Jensen *et al* [1995b]; Thodberg *et al* [1999]) increased locomotion, vocalization and escape attempts are signs of increased emotional reactivity. Thus, the response of the isolated piglets can be interpreted as decreased emotional reactivity. Based on previous studies on the effects of intermittent stress on the reactivity in open-field/novel object testing in pigs (eg Jensen *et al* [1995a]), either an increased centre location (equating to the variable 'close to object' in the present study) in early stages of stress or an increased reactivity in later stages of stress would have been expected. In addition to stress, the responses in the present study may, however, reflect the obvious differences between treatments in the discrepancy in social environment between the home pen and the test condition. The open-field test provokes a complex behavioural response in animals involving, for instance, exploration, active and passive fear, and a response toward social isolation (see review by Munksgaard & Jensen [1996]).

Measurements of reactivity in novel environments have been used in several experiments examining the effects of individual housing. Generally, individual housing results in changes in the behavioural reactivity toward novel environments although there are large differences between species (eg calves – Veissier *et al* [1997]; male Wistar rats – Vanderschuren *et al* [1995]).

Using the level of behavioural reactivity of the group-housed piglets as a reference, it is clear that the reactivity of the partly isolated piglets is intermediate between the two other treatments. Assuming that the differences in response in the test were not solely due to

different perceptions of the test condition, access to limited social contact was less compromising for the reactivity than full isolation.

In conclusion, this study has shown that, irrespective of the degree of isolation, keeping newly weaned piglets isolated in metabolism chambers provokes behavioural stress responses. Although it may increase the initial behavioural stress response, provision of limited social contact with littermates through wire mesh might be one way to limit negative long-term effects of isolation in experiments where data collection requires individual housing.

Animal welfare implications

The welfare implications of the effects of housing pigs as experimental animals have only been scantily investigated. In experiments where individual housing of newly weaned piglets is warranted, this housing must be as limited as possible since it results in the occurrence of behavioural stress indicators. Attention should be focused on the provision of social contact, where snout contact through a wire mesh might make it easier for the piglets to cope with the housing system, particularly during longer experiments.

References

- Barnes R H, Levitsky D A, Pond W G and Moore U** 1976 Effect of postnatal dietary protein and energy restriction on exploratory behaviour in young pigs. *Developmental Psychobiology* 9: 425-435
- Barnett J L, Cronin G M and Winfield C G** 1981 The effects of individual and group penning on total and free plasma corticosteroids and the maximum corticosteroid binding capacity. *General and Comparative Endocrinology* 44: 219-225
- Blackshaw J K** 1981 Some behavioural deviations in weaned domestic pigs: persistent inguinal nose thrusting, and tail and ear biting. *Animal Production* 33: 325-332
- Dybkjær L** 1992 The identification of behavioural indicators of 'stress' in early weaned piglets. *Applied Animal Behaviour Science* 35: 135-147
- Fraser D** 1978 Observations on the behavioural development of suckling and early-weaned piglets during the first six weeks after birth. *Animal Behaviour* 26: 22-30
- Gundlach H** 1968 Brutfürsorge, Brutpflege, Verhaltensontogenese und Tagesperiodik beim europäischen Wildschwein. *Zeitschrift für Tierpsychologie* 25: 955-995
- Heller K E and Jeppesen L L** 1985 Behaviour and eosinophil leukocyte responses to single and repeated immobility stress in mink. *Scientific* 9: 174-178
- Hutton R C and Wood-Gush D G M** 1983 Abnormal behaviour of piglets reared in individual incubators. *Applied Animal Behaviour Science* 11: 83-84
- Jensen K H, Pedersen L J, Giersing Hagelsø A M, Heller K E, Jørgensen E and Ladewig J** 1995a Intermittent stress in pigs: behavioural and pituitary-adrenocortical reactivity. *Acta Agriculturae Scandinavica. Section A, Animal Science* 45: 276-285
- Jensen K H, Pedersen L J, Nielsen E K, Heller K E, Ladewig J and Jørgensen E** 1996 Intermittent stress in the pig: effects on behaviour, HPA-axis, growth and gastric ulceration. *Physiology and Behaviour* 59: 741-748
- Jensen P, Forkman B, Thodberg K and Köster E** 1995b Individual variation and consistency in piglet behaviour. *Applied Animal Behaviour Science* 45: 43-52
- Kleinbaum D G** 1996 *Survival Analysis - A Self-learning Text*. Springer Verlag: New York, USA
- Littell R C, Milliken G A, Stroup W W and Wolfinger R S** 1996 *SAS® System for Mixed Models*. SAS Institute Inc: Cary, USA
- Martin P and Bateson P P G** 1986 *Measuring Behaviour - An Introductory Guide*. Cambridge University Press: Cambridge, UK

- Metz J H M and Gonyou H W** 1990 Effect of age and housing conditions on the behavioural and haemolytic reaction of piglets to weaning. *Applied Animal Behaviour Science* 27: 299-309
- Metz J H M and Osterlee C C** 1981 Immunologische und etologische Kriterien für die artgemässe Haltung von Sauen und Ferkeln. *KTBL-Schrift* 264: 39-50
- Munksgaard L and Jensen M B** 1996 The use of open-field tests in the assessment of welfare of cattle. *Acta Agriculturae Scandinavica. Section A, Animal Science* 27: 82-85
- Newberry R, Wood-Gush D G M and Hall J W** 1988 Playful behaviour of piglets. *Behavioural Processes* 17: 205-216
- Pearce G P and Paterson A M** 1993 The effect of space restriction and provision of toys during rearing on the behaviour, productivity and physiology of male pigs. *Applied Animal Behaviour Science* 36: 11-28
- Petersen V** 1994 The development of feeding and investigatory behaviour in free-ranging domestic pigs during their first 18 weeks of life. *Applied Animal Behaviour Science* 42: 87-98
- SAS Institute Inc** 1996 *SAS/STAT User Guide, Version 6.12*. SAS Institute Inc: Cary, USA
- Siegel S and Castellan N J** 1988 *Nonparametric Statistics for the Behavioural Sciences*. McGraw-Hill: Singapore
- Sudha S and Pradhan N** 1993 Behavioural consequences of predictable vs. unpredictable shocks in rats. *Physiology and Behaviour* 54: 243-247
- Thodberg K, Jensen K H and Herskin M S** 1999 A general reaction pattern across situations in prepubertal gilts. *Applied Animal Behaviour Science* 63: 103-119
- Van Adrichem P W M and Vogt J E** 1993 The effect of isolation and separation on the metabolism of sheep. *Livestock Production Science* 33: 151-159
- Vanderschuren L J M J, Stein E A, Wiegant V M and Van Ree J M** 1995 Social isolation and social interaction alter regional brain opioid receptor binding in rats. *European Neuropsychopharmacology* 5: 119-127
- Varley M A** 1995 Behavioural patterns of the weaned piglet. *Pig Journal* 34: 71-97
- Veissier I, Chazal P, Pradel P and Le Neindre P** 1997 Providing social contact and objects for nibbling moderates reactivity and oral behaviours in veal calves. *Journal of Animal Science* 75: 356-365
- Von Borell E and Hurnik J F** 1991 Stereotypic behaviour, adrenocortical function, and open-field behaviour of individually confined gestating sows. *Physiology & Behavior* 49: 709-713
- Von Borell E and Ladewig J** 1992 Relationship between behaviour and adrenocortical response pattern in domestic pigs. *Applied Animal Behaviour Science* 34: 195-206
- Worobec E K, Duncan I J H and Widowski T M** 1998 The behaviour and performance of piglets weaned at three ages. *Journal of Animal Science* 76, Suppl. 1: 385