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Consequences of weaning piglets at 21 and 28 days on growth, behaviour and hormonal responses

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Abstract

A recent European Directive raised the minimum weaning age of piglets to 28 days, although weaning at 21 days is still allowed (but not necessarily practiced) in batch breeding conditions. The aim of the present study was to determine the consequences of weaning at 3 and 4 weeks on animal weight gain, behaviour and stress neuro-endocrine responses, compared to sow-reared piglets. Three groups of Large-White piglets were compared: six litters of seven to eight piglets weaned at 21 days (W21 group), six litters of seven to eight piglets weaned at 28 days (W28 group) and six litters of eight suckling piglets reared by the sow for 40 days (C group). Piglets were regularly weighed from birth to 75 days. Each weaned group was observed for 2 h/day for 7 days (between d − 1 and d + 12 after weaning) at the same time as the control group. The specific behavioural parameters assessed by direct observation were position, activity and social interaction. Urine was collected to measure glucocorticoid (cortisol and cortisone) and catecholamine (adrenaline and noradrenaline) levels.

Compared to nursed piglets, weaning at 21 or 28 days induced a reduction in growth rate, as well as behavioural and hormonal changes. Early behavioural changes included an increase of vocalisations and of lying in litter cohesion; later changes included an increase of aggressive and nosing behaviour. Endocrine changes included a reduction in catecholamine (mainly noradrenaline) and cortisone levels in urine. Comparison of W21 and W28 showed that some of these changes were more intense and lasted longer with early weaning. Endocrine changes and certain behaviour (litter cohesion, vocalisations) could be related to food intake deficits, as measured by the greater reduction

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in growth rate in early-weaned animals. Changes in active behaviour (increase of aggressive and nosing behaviour) appeared earlier after weaning in the youngest animals.

This study shows that weaning at 21 days has more negative consequences on growth rate and stress endocrine responses than weaning at 28 days. However, piglets weaned at both 21 and 28 days showed behavioural disturbances, but often with different kinetics.

Keywords: Weaning; Piglet; Behaviour; Catecholamines; Glucocorticoids; Growth rate; Welfare

1. Introduction

In modern pig husbandry, weaning occurs abruptly and earlier than in natural conditions. The age of spontaneous weaning in domestic pigs in free-range conditions, which remains the sow's decision (Bøe, 1991), has been reported to be between 11–12.5 weeks (Bøe, 1991; Stolba and Wood-Gush, 1989) and 17.2 weeks (Jensen and Recén, 1989). Weaning management practices remove piglets from sows from 7 to 35 days depending on the country. In Europe, the only circumstance in which it is permitted to wean at 7 days of age is if this improves the health and welfare of the piglets or sows. In January 2003, the revised European Directive 91/630 raised the minimum weaning age from 21 to 28 days. Under the terms of this directive, weaning at 21 days is still allowed (but not necessarily practiced) in farms, where the sow herds are managed on a batch system (which applies to about 95% of French farms). This system has a specific hygiene procedure that involves total cleaning of all the compartments of the buildings between two subsequent batches of animals.

Despite these new governmental measures, the optimal weaning age with regard to piglet welfare is far from being accurately defined and is still disputed.

In addition to mother–young separation, weaning involves abrupt and profound modifications of the environment, feeding habits and social interactions when litters of piglets are mixed, all of which are considered to be stressful. These different factors could all contribute to a range of weaning-related disturbances, but it is difficult to distinguish their individual roles (see Mormède and Hay, 2003, for a review).

The transition from maternal milk to dry food results in a period of starvation, which in conjunction with the absence of the dam, impairs thermoregulation, as shown by behavioural and biological changes (Hay et al., 2001; Orgeur et al., 2001).

These responses are greater, when the earlier weaning occurs. It is evident that very early weaning (at 1 week), which aims to save supernumerary piglets born to hyperprolific sows, may cause adaptation problems. It has immediate negative effects on physiology, performance and behaviour. Very early weaning leads to elevated urinary cortisol levels the day after weaning and to prolonged suppression of the release of catecholamines (Hay et al., 2001). This may be caused by both emotional distress and acute food deprivation. The 1-week-old piglet's digestive apparatus is under-developed and low feed intake and diarrhoea are common when weaning occurs at this age. Compared to piglets weaned at a later age (d14 and d28), Worobec et al. (1999) reported that piglets weaned at 7 days spent only 1% of their time at the feeder during the first 2 days after weaning (3 and 5% for piglets weaned at 14 and 28 days, respectively). Orgeur et al. (2001) also found a starvation period ranging from 12 to 48 h after very early weaning. The growth of early-weaned piglets slowed down compared to that of nursed piglets, with differences in body weight between the two groups recorded up to day 74.

Behavioural responses to early weaning have also been commonly observed, such as increased aggressive behaviour and the development of abnormal behaviour resembling the motor pattern used when massaging the sow's udder (Fraser, 1978). This behaviour, described as belly-nosing, is related to weaning, since it has been observed both during the first 2 weeks after weaning (Orgeur et al., 2001) and after 3–4 weeks (Van Putten and Dammers, 1976), but is rarely exhibited by sow-reared piglets. Morever, some studies have shown greater disturbances with earlier weaning, e.g. Worobec et al. (1999) reported that piglets weaned at 7 days displayed a higher frequency of massaging and nibbling other piglets than those weaned at 28 and 14 days. Metz and Gonyou (1990) showed that some belly-nosing developed after weaning at 14 days, while it was rare in 28-day-old weaned piglets. Dybkjaer (1992) observed redirected oral behaviour towards littermates in piglets weaned at 28 days, while Bøe (1993) found that massaging and sucking pen-mates was less frequent amongst piglets weaned at 6 weeks than those weaned at 4 weeks. In extreme cases, piglets reared under semi-natural conditions increased their level of chewing other pigs between 8 and 10 weeks of age, which coincides with the beginning of the natural weaning period and the consequent competition for solid food starting at this time (Newberry and Wood-Gush, 1988).

Most studies have compared different weaning ages without any comparison with a control group, which could be useful for assessing the animal weight gain effects of industrial weaning. Between 21 and 28 days of age, a piglet's degree of maturity is two-thirds of the final digestive ability (Sève, 1986). Landrain et al. (1997) found no difference between days 21 and 76 in the average daily gain of piglets weaned at 21 and 28 days, but the difference with sow-reared piglets was not reported.

The aim of the present study was to compare the immediate effects (during the first 2 weeks) of the two disputed weaning ages (21 and 28 days) on piglets' performance, behaviour and stress biology in the post-weaning pens, compared also to an unweaned group maintained with the sow in the farrowing pens.

2. Material and methods

2.1. Animals and accommodation

This work was undertaken in the experimental pig farm at the INRA unit of Reproduction and Behavioural Physiology at Nouzilly (France). The experiment involved 18 litters of 8 Large-White piglets (4 castrated males and 4 females). Litters consisted of piglets with homogeneous weights, chosen 48 h post-partum, when fostering occurred. Canine teeth clipping and tail docking were carried out on the day of birth and all males were castrated by incision on day 13.

Six litters were assigned to each of the three treatment conditions: (i) piglets weaned at 21 days of age (W21) (average weight = 6.8 kg); (ii) piglets weaned at 28 days (W28) (AW = 9.4 kg); (iii) control piglets, sow-reared until weaning at 40 days (C) (AW at 21 days = 6.6 kg and at 28 days = 8.7 kg). Of the 144 experimental piglets, 3 died before weaning, 2 in the W21 condition (batches 2 and 3) and 1 in the W28 condition (batch 5). Farrowing was induced on the 112th day of pregnancy with an intramuscular injection of prostaglandin F2α (Alfabédyl® alfaprostol).

The sow and her offspring were kept in a 4.25 m^2 farrowing pen (0.33 m^2/piglet, excluding sow space allowance), with an infrared lamp providing heat (maintained at 29–30 °C from birth to weaning for W21 and W28 conditions and until d40 for C condition) above a nest-site equipped with a rubber mat. The pen had a partially slatted-floor. The average temperature in the piggery was maintained at 25–28 °C. In addition to daylight from the windows, artificial light was provided between 07:30 and 17:00 h.

2.2. Weaning procedure

At weaning (21 or 28 days), the piglets from each litter were moved at 09:00 h from the farrowing section to a 1.34 m × 1.60 m post-weaning pen (0.23 m^2/piglet). This flat-deck had an expanded polypropylene slatted-floor and solid PVC walls. The pen was provided with two self-feeders and an automatic water cup. An infrared lamp supplied heat (29–30 °C) on one-third of the total surface. This area was considered as the nest-site even if no rubber mat was provided. Pens in all systems were thoroughly cleaned between replicates. No daylight could filter into the pen, which was illuminated from 07:30 to 17:00 h. Ventilation and heat were adjusted daily in order to maintain a stable temperature (25–28 °C).

In the farrowing pen, piglets began eating dry food (a 30% milk product, 22% protein starter diet) from days 4 to 21. Between days 21 and 25, this food was mixed with pellets (an 18% protein, cereal-based growth diet) and gradually phased out between days 25 and 40.

2.3. Animal weight gain measures

All piglets were regularly weighed on days 0, 7, 14, 21, 23, 28, 30, 35 and 40. Females were subsequently weighed every week until day 75. Average daily weight gain (ADG) was calculated for the following relevant growth periods: d0/d21, d21/d23, d23/d28, d28/d30, d30/d35, d35/d47 and d47/d75.

2.4. Behavioural measures

Each litter was directly observed for 2 h per day (1 h between 09:00 and 11:00 h, and 1 h between 14:00 and 16:00 h) on d − 1 (the day before weaning), d0 (the day of weaning), d + 1, d + 4, d + 6, d + 8 and d + 12. The control groups were observed on these same days: the W21 control (C21) from d20 to d33 and the W28 control (C28) from d27 to d40.

Each piglet was numbered on its back with permanent ink. Trained observers sitting outside the pen collected the data. When animals had been handled before an observational sequence (weaning, marking or urine collection), there was a minimum interval of 15 min before beginning the observation.

Two recording methods were used to collect the behavioural data.

Continuous recording of active behaviour:

- *Aggressive behaviour*: The number of fights between piglets (regardless of duration), a fight involving two piglets and characterised by pushing, head-thrusting, biting and chasing.
- *Nosing*: The number of massages, butting, chewing and sucking bouts by each piglet towards littermates' belly, ears, tail, paws or snout.
- *Vocalisations*: The duration of collective grunts and squeals was measured when at least three piglets were vocalising (in minutes). We voluntarily ignored the specific squeals emitted by piglets to initiate suckling, considering that these could not be compared with the vocalisations emitted in weaned groups.
- *Feeding behaviour*: The number of feeding bouts.

Scan samples were performed every 10 min to assess the piglets' positions:

- percentage of piglets lying down;
- litter cohesion (percentage of resting piglets in direct body contact and forming the largest group).

2.5. Hormonal measures

2.5.1. Urine samples

Three litters per experimental group were sampled. Urine was collected between 07:00 and 09:00 h on days 21, 22 and 26 for groups W21 and C21 and on days 27, 29 and 33 for groups W28 and C28. One person was assigned per pen (two people were needed every sampling day). Urine sampling consisted first of waiting for spontaneous urination from the eight piglets per group. Urine was collected in a beaker fixed on a 50 cm length stem to avoid proximity between the person and the piglet, which could disturb the piglet during urination. If all piglets were not sampled at 08:00, they were gently caught and placed in a container filled with 2–3 cm water. Contact of the feet with water usually provoked urination and urine was collected in the beaker. The samples were acidified using 6 M HCl (1% of urine volume) and immediately frozen at −20 °C.

2.5.2. Hormonal analyses

Urinary catecholamines (adrenaline (AD) and noradrenaline (NA)) were assayed using an ion-exchange purification procedure followed by liquid chromatography with electrochemical detection, as previously described (Hay and Mormède, 1997a). In brief, urine samples were loaded on cationic columns and the catecholamines were eluted with boric acid. The eluates were then assayed by high-pressure liquid chromatography (HPLC) with electrochemical detection, using an oxidising potential of +0.65 V. The intra- and inter-assay coefficients of variation (%) were 7.0 and 7.1 for AD and 6.5 and 11.6 for NA, respectively.

Urinary cortisol and cortisone (the oxidised metabolite of cortisol) were assayed using a solid phase extraction procedure followed by HPLC with UV absorbency detection (254 nm), as previously described (Hay and Mormède, 1997b). In brief, the filtered urine samples were loaded onto C18 cartridges mounted on a vacuum processing station.

Corticosteroids were eluted using absolute ethanol. After evaporation of ethanol, the dried residues containing corticosteroids were redissolved in mobile phase and injected in the HPLC system. The intra- and inter-assay coefficients of variation (%) were 7.4 and 10.6 for cortisol and 5.4 and 10.9 for cortisone, respectively.

Creatinine levels were determined using a colorimetric quantitative reaction (Procedure 500, Sigma Diagnostics, Saint-Quentin-Fallavier, France). This method is based on the bleaching of the colour derived from the reaction between creatinine and alkaline picrate (Jaffe's reaction) when the mixture is acidified. Thus, the difference in colour intensity measured at 500 nm before and after acidification of the mixture is proportional to creatinine concentration. The catecholamine and glucocorticoid levels are expressed as ng/mg of creatinine to take into account urine dilution.

2.6. Statistical analyses

The total frequencies of active behaviour for the entire litter were measured. For measures of position (with scan sampling), we calculated the percentage of the total number of piglets observed in a pen (seven or eight) every 10 min for 2 h/day (12 times), i.e. 84 or 96 piglets. For all conditions, the behavioural data are presented as a function of the time from weaning.

All the statistical analyses were conducted using SYSTAT statistical software, with the litter as the experimental unit. Because the behavioural and weight gain data lacked normality, we used non-parametric tests. Intra-group comparisons (over time) were analysed with Friedman and Wilcoxon tests. For inter-group comparisons, the Kruskall–Wallis test was used followed by the Mann–Whitney test when significant Kruskall–Wallis values ($p < 0.05$) were obtained. Behavioural and weight gain data are presented as median ($\pm$IQ) values calculated on $n = 6$ litters in each experimental group.

For hormonal measures, data were transformed into their logarithmic scores for normalisation. In a number of samples, levels of cortisol were undetectable and were given the value of the limit of detection (1.9 ng/mg creatinine). Statistical analysis was performed by GLM Procedure of SAS (1992). Experimental groups, day of sampling, their interaction and litter were assumed to have fixed effects. Least square means were generated by LSMEANS statement.

3. Results

3.1. Weight gain data

Body weights in W21 and W28 did not differ significantly from the control group during the experimental period (Fig. 1A). However, comparisons between weaned groups showed that W28 piglets were heavier than the W21 group on d28 (9.41 and 7.94 kg, respectively, $p < 0.05$) and a tendency persisted on d30 (9.86 kg versus 8.85 kg, $p = 0.055$).

In W21, daily weight gain decreased for 1 week (between d21 and d28) and was lower than that of groups W28 and C ($p \leq 0.01$) (Fig. 1B). In W28, weight gain decreased

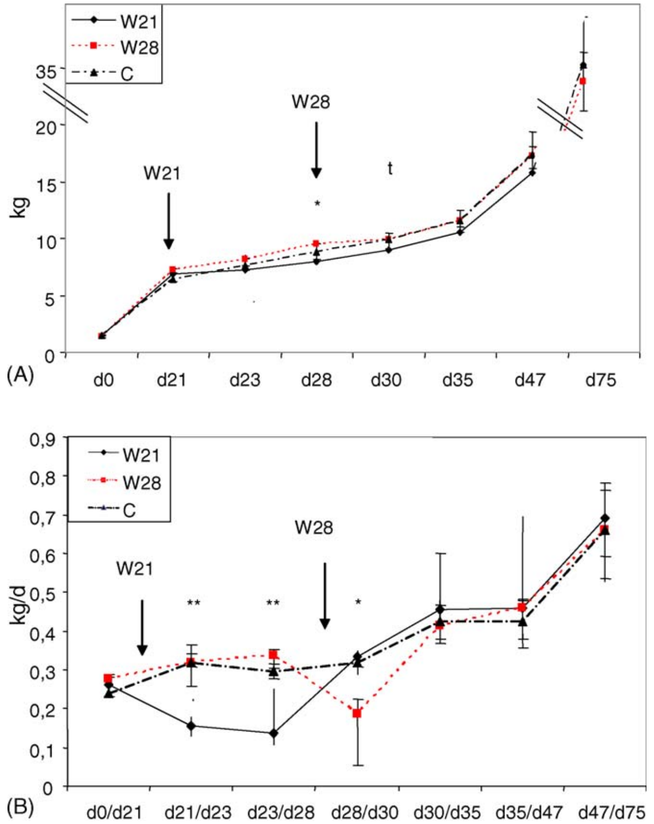

Fig. 1. Effect of weaning at 21 and 28 days (groups W21 and W28 compared to suckling group C) on median (quartiles: 25 and 75%) weight (kg) (A) and daily weight gain (kg/d) (B). Weaning occurred on d21 and d28. (A) *W28 different from W21 ($p < 0.05$); t: W28 tends to differ from W21 ($0.05 < p < 0.1$). (B) *W28 different from the other two groups ($p < 0.05$); **W21 different from the other two groups ($p < 0.01$) (Mann–Whitney U-tests).

sharply between d28 and d31 and was lower than the other two groups for these 2 days ($p < 0.05$). It then increased from d31 ($p < 0.05$).

No further difference between groups appeared from d31 until the end of the post-weaning period.

3.2. Behavioural data

3.2.1. Feeding behaviour (Fig. 2)

The median number of eating bouts increased significantly over time in both weaned groups ($p < 0.01$). Feeding behaviour was more frequent in W21 than in C21, with 101 (59–159) versus 15 (7–33) eating bouts, respectively ($p < 0.01$), at d + 1, to 68 (52–76) versus 22 (13–35), respectively ($p < 0.01$), at d + 12. The median number (quartiles:

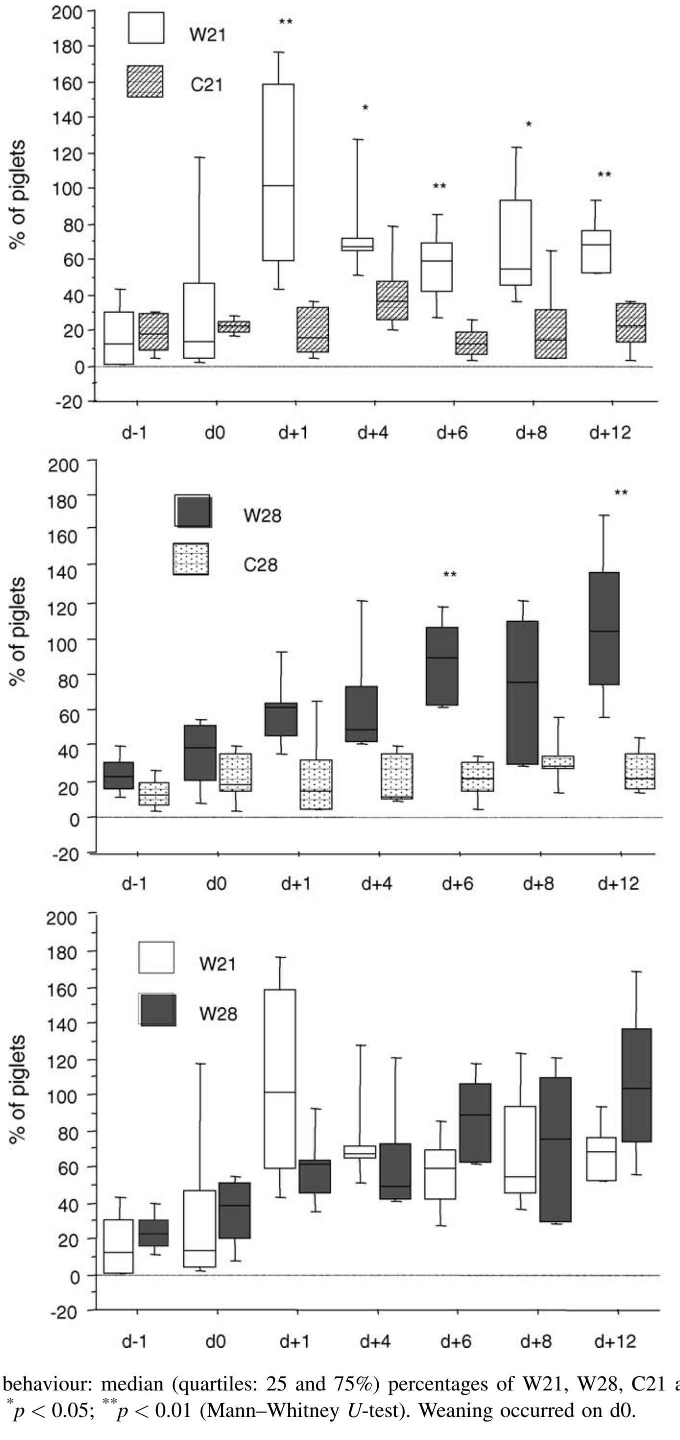

Fig. 2. Feeding behaviour: median (quartiles: 25 and 75%) percentages of W21, W28, C21 and C28 piglets observed eating; $^{*}p < 0.05$; $^{**}p < 0.01$ (Mann–Whitney *U*-test). Weaning occurred on d0.

25–75%) of eating bouts was significantly higher in the W28 than in the C28 piglets, with 88.7 (62–106) versus 21 (14–30), respectively ($p < 0.01$), on d + 6, and 103.5 (74.3–137) versus 21.5 (16–35), respectively ($p < 0.01$), on d + 12. W28 and W21 did not differ. We did not observe any starvation (Fig. 2).

3.2.2. Active behaviour

3.2.2.1. Aggressive behaviour (Fig. 3). The median frequency of aggressive behaviour varied significantly over time in both weaned groups, increasing between d0 and d + 4, d + 6 and d + 12 in W28 ($p < 0.05$), and between d0 and all subsequent days in W21 ($p < 0.05$) (Fig. 3).

Inter-group comparisons showed no significant differences between W21 and C21, but the W28 piglets were more involved in fights than C28 on d + 4 and d + 6 ($p < 0.01$). On d + 6, group W28 had a higher level of aggressive behaviour (23 fights/2 h) than group W21 (6 fights/2 h) ($p < 0.05$). There was no other significant difference between W21 and W28.

3.2.2.2. Nosing (Fig. 4). In group W21, the total number of nosing behaviour displayed by piglets towards their littermates was lower on d − 1 than on d + 6, d + 8 and d + 12 ($p < 0.05$). Differences were also found between d0 and d + 4, d + 8, d + 12 ($p < 0.05$). In W28, weaning halved the frequency of nosing behaviour towards littermates (d − 1 versus d + 1; $p < 0.05$), but nosing increased on subsequent days, reaching a peak on d + 8 (78 events/2 h). Nosing was more frequent in W21 than in C21 on d + 8 and d + 12 ($p < 0.01$ for both days) and was observed more frequently in W28 than in C28 on d + 6, d + 8 and d + 12 ($p < 0.05$, <0.01 and 0.055, respectively) (Fig. 4).

The level of nosing in W28 was observed to be higher than in C28 and W21 just before weaning on d − 1 ($p < 0.05$ and <0.01, respectively). We did not observe any difference between W21 and W28 after weaning.

3.2.2.3. Vocalisations (data not shown). W21 and W28 piglets emitted grunts and distress squeals at weaning and on the following day, while control piglets never emitted that kind of vocalisation during the experimental period, only vocalising with squeals to initiate suckling. The peak of vocalisations occurred on d0 with similar median lengths for W21 and W28 groups (49 and 52 min/2 h, respectively), and were higher than in their respective control groups ($p < 0.01$). While W28 piglets vocalised frequently on d + 1 (21 min/2 h), they stopped entirely on d + 4, whereas W21 piglets continued to vocalise (0 min/2 h versus 2 min/2 h, $p = 0.055$).

3.2.3. Position

3.2.3.1. Lying (Fig. 5). In W21 and W28 groups, the median percentage of piglets lying down (either in sternal recumbency or on their side) increased significantly between d − 1 and the 2 following days ($p < 0.05$). W21/C21 comparisons showed a greater percentage of W21 piglets lying down on d + 6 ($p < 0.05$). On d0 and d + 1, W28 piglets were lying down more frequently than the C28 group ($p < 0.05$). On d + 6, resting positions were observed more frequently in W21 than in W28 ($p < 0.05$) (Fig. 5).

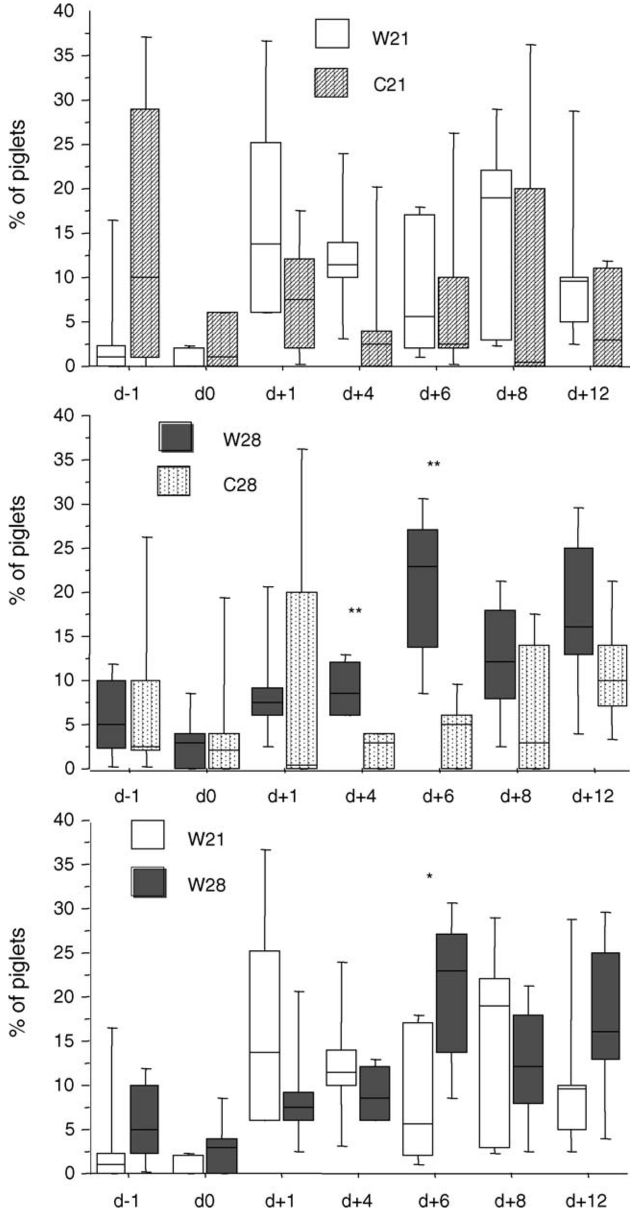

Fig. 3. Aggressive behaviour: median (quartiles: 25 and 75%) percentages of W21, W28, C21 and C28 piglets observed fighting; $^{*}p < 0.05$; $^{**}p < 0.01$ (Mann–Whitney *U*-test). Weaning occurred on d0.

3.2.3.2. Litter cohesion (Fig. 6). Litter cohesion varied over time only in group W28 ($p < 0.05$). In W28, weaning had an increasing effect on cohesion between d − 1 and d0 ($p < 0.05$) followed by a decrease from d + 1 to d + 6 ($p < 0.05$). In W21, piglets were resting in cohesion up to d + 6, followed by a decrease ($p < 0.05$). Inter-group

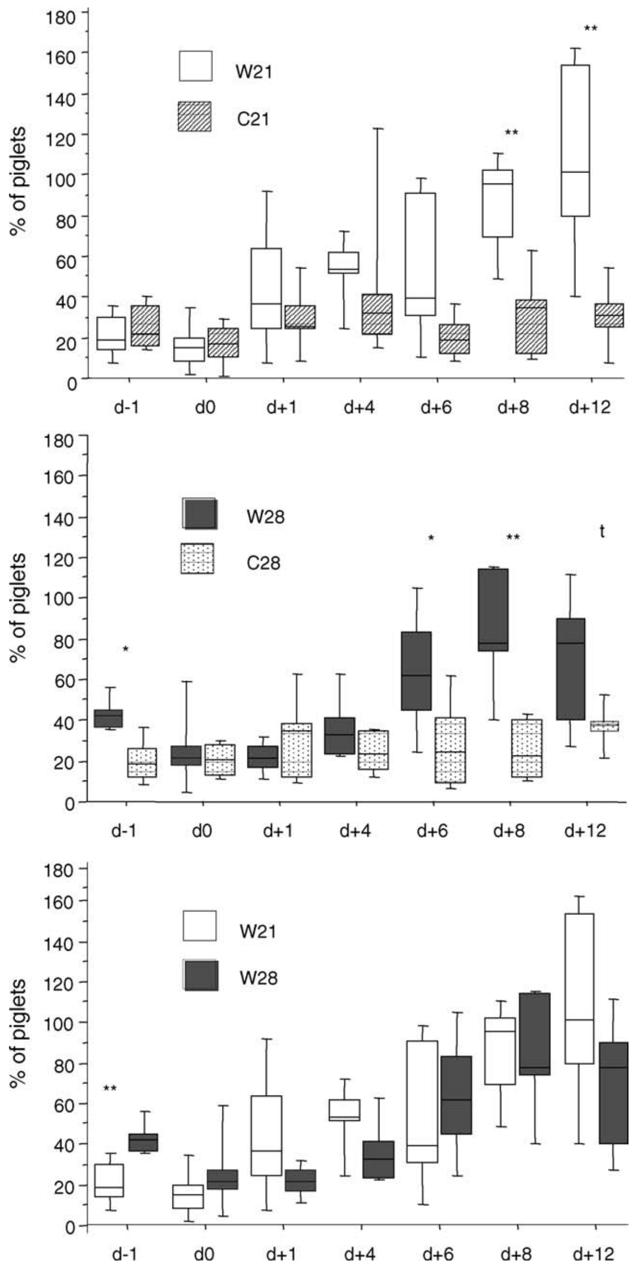

Fig. 4. Nosing behaviour: median (quartiles: 25 and 75%) percentages of W21, W28, C21 and C28 piglets observed nosing or chewing penmates; $^{*}p < 0.05$; $^{**}p < 0.01$, t: $0.05 < p < 0.1$ (Mann–Whitney *U*-test). Weaning occurred on d0.

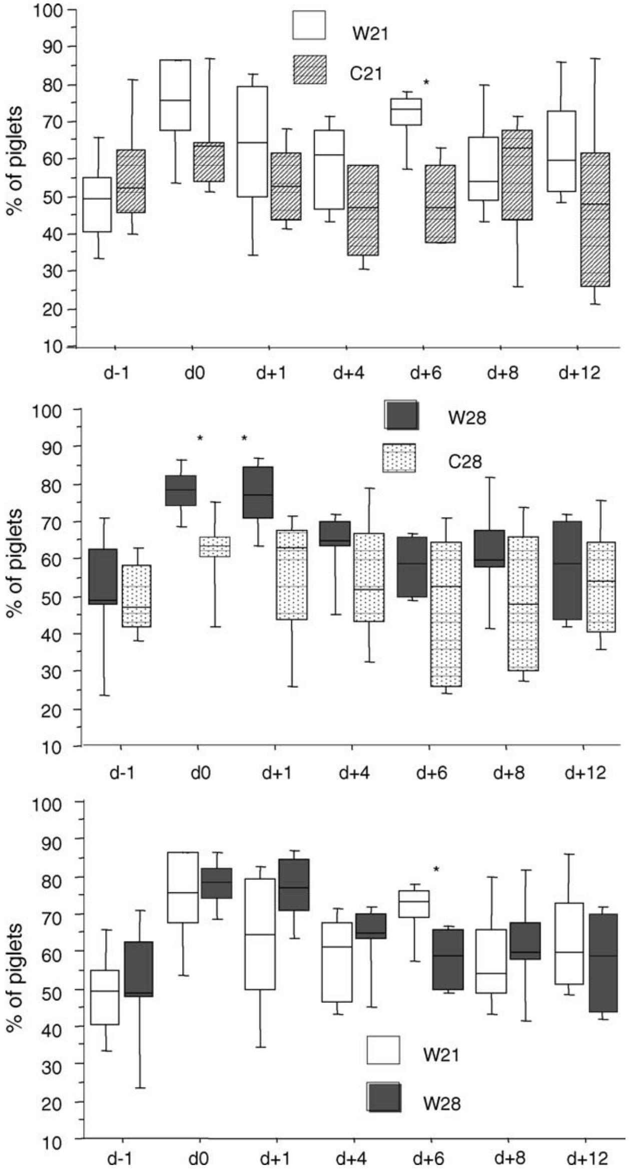

Fig. 5. Lying: median (quartiles: 25 and 75%) percentages of W21, W28, C21 and C28 piglets observed lying; $^{*}p < 0.05$; $^{**}p < 0.01$ (Mann–Whitney *U*-test). Weaning occurred on d0.

comparisons revealed that W21 had a greater level of cohesion than C21 on d + 4 and d + 6 ($p < 0.05$). The same weaning effect occurred earlier in W28, with a higher percentage of cohesion than in C28 on d0 ($p < 0.01$) and d + 1 ($p < 0.05$). In addition, W21 had a higher level of cohesion on d + 6 than W28 (73% versus 55%; $p = 0.05$) (Fig. 6).

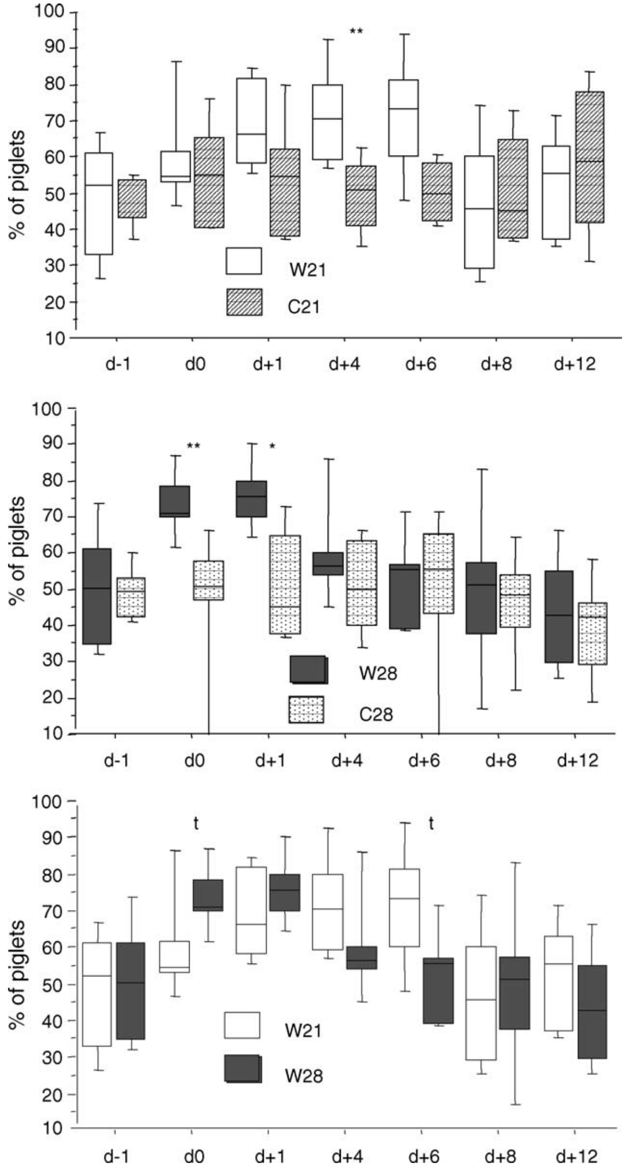

Fig. 6. Litter cohesion: median (quartiles: 25 and 75%) percentages of W21, W28, C21 and C28 piglets observed lying in litter cohesion; $^{*}p < 0.05$; $^{**}p < 0.01$, t: $0.05 < p < 0.1$ (Mann–Whitney U-test). Weaning occurred on d0.

3.3. Endocrine data (Fig. 7)

In the W21 group (compared to C21), although cortisol levels did not change after weaning, the metabolite cortisone remained lower on d22 and d26 ($p < 0.01$ and <0.05, respectively). Adrenaline levels were lower in the W21 group on the day of weaning

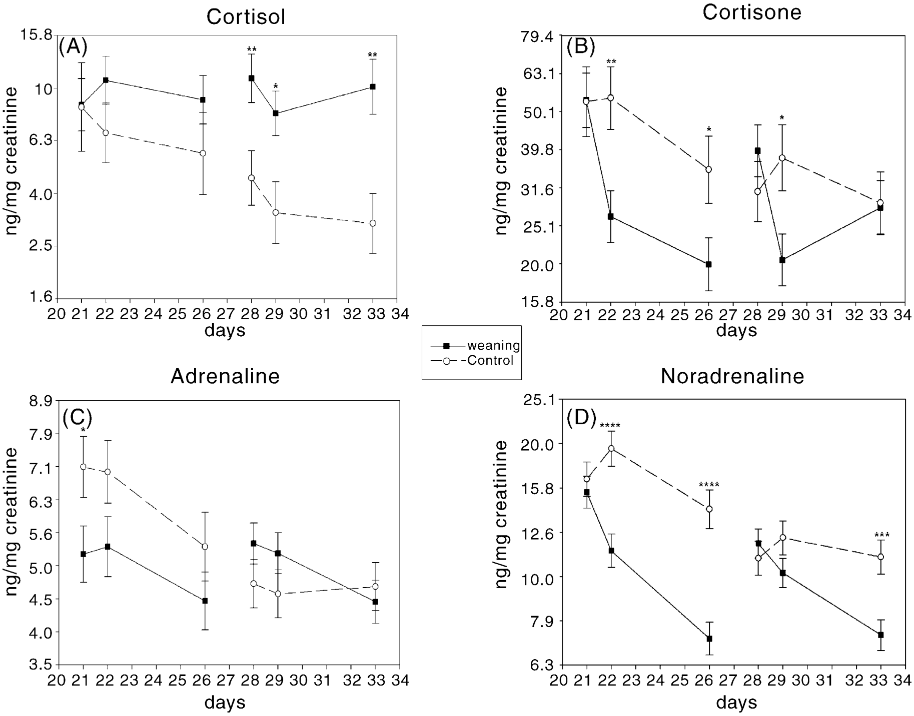

Fig. 7. Mean ± S.E.M.: urinary cortisol (A), cortisone (B), adrenaline (C) and noradrenaline (D) concentrations (ng/mg of creatinine) in weaned and control piglets ($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$). Weaning occurred on days 21 and 28.

($p < 0.05$) but reached C21 levels on d22. In the W21 group, noradrenaline levels were lower than in the C21 group on d22 ($p < 0.0001$) and remained significantly lower on d26 ($p < 0.0001$) (Fig. 7).

In the W28 group, cortisol levels were higher than C28 at each time point (d28, $p < 0.01$; d29, $p < 0.05$; d33, $p < 0.01$), but there was no day-to-day difference in either group. Cortisone levels were only lower on d29 ($p < 0.05$). Adrenaline did not differ between groups and noradrenaline levels were lower in W28 on d33 ($p < 0.001$).

Results for each variable are summarised in Table 1.

4. Discussion

Abrupt modifications of the environment and feeding at weaning reduce weight gain and induce immediate or subsequent adaptive changes in the behaviour and physiology of piglets compared to those suckling their mother. The intensity of these changes can be used to compare different procedures, such as weaning age (21 days versus 28 days).

Daily weight gain decreased significantly in both weaned groups compared to the control group: between d21 and d28 in group W21 and between d28 and d30 in group W28.

Table 1
Table summarising all results

Animal production, behavioural and physiological variables	Days	W21 vs. W28	W21 vs. C21	W28 vs. C28
Weight (Fig. 1A)				
	d28	W28 > W21		
	d30	W28 > W21 t		
ADG (Fig. 1B)				
	d21/d23	W28 > W21**	W21 < C**	
	d23/d28	W28 > W21**	W21 < C**	
	d28/d30	W21 > W28		W28 < C
Feeding behaviour (Fig. 2)				
	d + 1		W21 > C**	
	d + 4		W21 > C	
	d + 6		W21 > C**	W28 > C**
	d + 8		W21 > C	
	d + 12		W21 > C**	W28 > C**
Aggressive behaviour (Fig. 3)				
	d + 4			W28 > C**
	d + 6	W28 > W21		W28 > C**
Nosing behaviour (Fig. 4)				
	d + 6			W28 > C
	d + 8		W21 > C**	W28 > C**
	d + 12		W21 > C**	W28 > C t
Vocalisations				
	d0		W21 > C**	W28 > C**
	d + 1		W21 > C t	W28 > C t
	d + 4	W21 > W28 t		
Lying (Fig. 5)				
	d0			W28 > C
	d + 1			W28 > C
	d + 6	W21 > W28	W21 > C	
Litter cohesion (Fig. 6)				
	d0	W28 > W21 t		W28 > C**
	d + 1			W28 > C
	d + 4		W21 > C	
	d + 6	W21 > W28 t	W21 > C	
Cortisone (Fig. 7B)				
	d22		W21 < C**	
	d26		W21 < C	
	d29			W28 < C
Noradrenaline (Fig. 7D)				
	d22		W21 < C****	
	d26		W21 < C****	
	d33			W28 < C***

$p < 0.05$; "t" means that groups tend to differ ($p < 0.1$); $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

However, the intensity and duration of this effect was greater and lasted longer in animals weaned at 21 days than those weaned at 28 days. After this immediate effect of weaning, animals caught up with their suckling controls and there was no long-term reduction of growth. Our results confirm those of Landrain et al. (1997) who obtained a similar short-term effect on growth of piglets weaned at 21 and 28 days of age. In contrast to piglets weaned at 6 days of age, which show a period of starvation after separation from the mother (Orgeur et al., 2001), piglets weaned at 21 or 28 days of age did not avoid food after weaning. Petersen et al. (1989) also reported that 2-week-old piglets only began to eat substantially on day 2 post-weaning, whereas feed intake started at weaning in a 4-week-old group. However, the transient decrease of daily weight gain suggests that the quality and/or quantity of the dry feed replacing the mother's milk did not correspond perfectly to the piglets' needs.

Despite the familiarity between piglets at weaning, aggressive behaviour was observed in both weaned groups, which is in line with previous results (Van Putten and Dammers, 1976; Mason et al., 2003). However, the level of occurrence was quite low, with a maximum observed in W28 on d + 6 (20 aggressive interactions within 2 h). Aggressive behaviour generally increased in both groups between d0 and subsequent days. In contrast, Mason et al., (2003) found that aggression was higher on the day of weaning than the 2 following days, which is similar to results obtained by Friend et al. (1983). However, in these earlier experiments, piglets were unfamiliar with each other and displayed the usual aggressive behaviour associated with establishing a social order. A similar, but less intense period seems to occur later among familiar piglets after a traumatic event, such as weaning. Only piglets from the W28 group had a significantly higher level of aggression than that of their control group (on d + 4 and d + 6). On d + 6, W28 piglets were also more aggressive than those in the W21 group. This is in accordance with Mason et al.'s study (2003) showing that aggression was higher in piglets weaned at 35 days than those weaned at 21 days, but differs from the results obtained by Worobec et al. (1999) who found no differences in aggressive behaviour related to the age at weaning (7, 14 and 28 days). However, in pair encounters, young unweaned piglets spend less time fighting and have fewer lesions than older pigs (Pitts et al., 2000). Furthermore, Jensen (1994) showed that aggression is higher at 5 and 9 weeks of age than at 1 week. Therefore, comparison of agonistic behaviour between different ages at weaning seems difficult because the level of aggression displayed by unweaned piglets varies according to age. For this reason, it seems important to compare weaned piglets with a control group of the same age. In our study, this comparison (W28 versus C28) clearly showed a greater level of aggression in the group weaned at 28 days, whereas no difference appeared between groups W21 and C21. Therefore, it seems that weaning induces aggressive reactions even if piglets are acquainted, and this aggression increases with weaning age.

The levels of belly-nosing and chewing pen-mates increased in both weaned groups compared to their nursed controls. Nosing behaviour increased progressively after weaning, reaching a maximum on d + 8 in W21 and on d + 12 in the W28 group. These results are consistent with the bulk of literature showing that weaning induces 'chewing–nosing littermates' responses (Van Putten and Dammers, 1976; Fraser, 1978; Blackshaw, 1981). We did not find any significant difference between the two weaned groups, and this finding differs from that of previous studies, which reported more intense nosing behaviour, appearing earlier the younger the age at weaning (Metz and Gonyou, 1990; Bøe, 1993; Weary et al., 1999; Worobec et al., 1999; Orgeur et al., 2001). However, the youngest weaning ages studied by these authors were 1 or 2 weeks, which is younger than our 21-day-old piglets. This could explain the absence of difference in nosing between piglets weaned at this older age (21 days) and piglets weaned only 1 week later (28 days).

Nosing littermates has been commonly referred to as a behavioural indicator of 'stress' in early-weaned piglets. Factors involved in its origin are currently under study, but it has not yet been determined whether belly-nosing is a general indicator of social stress (Dybkjaer, 1992; Li and Gonyou, 2002), feeding stress (Gardner et al., 2001), or more simply a motor pattern similar to the massaging movements that piglets direct towards the sow's udder (Gill and Thomson, 1956). The causes of belly-nosing may in fact differ according to the age when weaning occurs, and the earlier appearance of the pattern in a very-early-weaned group (Orgeur et al., 2001) may be associated with starvation observed at the same time. In this case, belly-nosing to some extent substitutes for eating behaviour. In the groups weaned at 21 and 28 days, chewing and nosing behaviour may be associated with a lack of environmental stimuli. After a period of exploration and feeding adaptation, piglets may develop these patterns towards stimuli only offered by littermates. It can have a calming effect and it may be important for piglets to perform this behaviour at either weaning age.

The calls given by piglets when separated from the sow are another possible indicator of stress. Indeed, vocalisations increase in length and intensity when weaning age decreases (Weary and Fraser, 1997; Weary et al., 1999). In the present experiment, grunts and squeals were frequent on the day of weaning in both weaned groups (W21 and W28), then rapidly decreased. Control piglets that were nursed by their mother never emitted that kind of vocalisation. Orgeur et al. (2001) explained these vocalisations by diet frustration because the calls stopped when piglets began to eat new food, on average 21 h after weaning. In our study, starvation was not observed, therefore, grunts and high calls may both be attributed to the active searching of the sow and exploration of the new environment. On d + 4, vocalisations stopped in W28 (when their growth rate reached control levels), while they were still observed in W21 (which had a lower growth rate than controls).

Weaning at either age was followed by increased resting. The new environment may be involved in the display of this behaviour. Bøe (1993) found that piglets weaned at 28 days in flat-deck pens spent more time lying than piglets weaned in their farrowing pen. In group W28, the response was immediate; 80% of piglets from group W28 were recorded lying down on d0 and d + 1. We did not find such a difference between W21 and C21 on these days. However, the difference appeared later with a higher level in W21 than in C21 and W28 on d + 6. This lack of activity, which is a typical symptom of a situation of chronic stress (Broom, 1996), was only displayed by the oldest piglets (W28) immediately after weaning then decreased, whereas younger piglets (W21) remained lying down for a longer time.

With regard to lying behaviour, differences in litter cohesion were observed between weaned and control groups, but more importantly there were different kinetics between the two weaned groups. After weaning, an increase of litter cohesion was observed in both weaned groups, appearing earlier in the W28 group and lasting longer in the W21 group. This behaviour suggests an impairment of thermoregulation, which could be due to the absence of the dam, and to low food intake in the post-weaning period. The observation of this behaviour immediately after weaning in group W28 suggests that these piglets also had problems coping with the new situation, even though they were older and able to regulate their temperature earlier (from d + 4) than piglets weaned at 21 days (from d + 8).

The main weaning-related endocrine changes were decreased levels of cortisone and noradrenaline (and to a lesser extent adrenaline) in the W21 compared to the C21 group, these changes being considerably less in the W28 versus C28 group. A large reduction of urinary catecholamine levels has already been described after very early weaning (Hay et al., 2001) and interpreted in terms of food intake deficit. Changes in growth rate indicated that weaning at 21 days induced a much greater deficit than weaning at 28 days. No change in cortisol levels were observed to be related to weaning itself. However, a difference, which started before weaning (d28), was seen between W28 and C28 groups on each sampling day. In fact, cortisol levels were higher in W28 than in C28, but the reasons for these differences are currently unknown. On the other hand, the profile of cortisone levels in urine was very similar to noradrenaline levels. Taken together with the stability of cortisol levels across days, this result suggests that the metabolism of cortisol into cortisone by 11-β hydroxysteroid dehydrogenase (Palermo et al., 1996) is influenced by early weaning and that noradrenaline may be involved in this process. However, further experiments are required to substantiate this hypothesis.

In conclusion, our results show that weaning piglets at 21 days has more negative consequences on growth rate and stress endocrine responses than weaning at 28 days, but behavioural disturbances exist in both weaned groups.

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