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Grouping piglets by sex at weaning reduces aggressive behaviour

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Abstract

The aim of this work was to reduce aggressive behaviour when piglets are grouped at weaning. The experiment assessed whether it is better to group piglets by sex at weaning or to mix them in male–female groups. Large White piglets were weaned at 28 days and assigned to 4 conditions, with 6 groups of animals/condition: (L-MF) 4 males and 4 females reared together from birth, (A-M) 8 unfamiliar male piglets, (A-F) 8 unfamiliar female piglets, and (A-MF) 4 male and 4 female unfamiliar piglets. All groups were videotaped on days 28, 29, 31, 39 and 46 for 2 h. Aggressive interactions were quantified and scratches on each piglet were counted on days 27, 29 and 33. Feed consumption and piglets' weights were quantified from birth to day 63. On days 27, 29 and 33, urine was collected between 7:00 and 8:00 for measurements of catecholamines and glucocorticoids.

Fighting durations were longer in A-MF than in L-MF and both single-sex groups on day 29 ($p < 0.05$), and than in L-MF and A-F on day 28 ($p < 0.05$). Durations did not differ between L-MF and single-sex conditions. Fights were more severe and there were more scratches in the A-MF condition compared to the other three conditions. Conditions A-M and A-F did not differ. In the mixed-sex group, fights between two males tended to be more severe than fights between two females ($p < 0.1$) or than mixed-sex fights ($p < 0.1$); males initiated more aggression than females ($p < 0.05$). The presence of females seemed to increase males' aggressive behaviour in mixed-sex groups. Growth and food intake were slightly affected by treatments but were similar between

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conditions on day 63. A rise of noradrenaline levels was observed after weaning for the unfamiliar piglets ($p < 0.001$). However, the grouping criterion by sex did not lead to changes in neuroendocrine activity.

Grouping piglets by sex reduces aggressive behaviour at weaning and this should be taken into account in pig farms in order to improve animal welfare.

Keywords: Piglet; Weaning; Aggressive behaviour; Glucocorticoids; Catecholamines; Welfare

1. Introduction

In pig production, weaning induces abrupt modifications for young piglets which are not only separated from their mother at an early age (minimum 28 days, according to the revised European Directive no. 91/630/CE), but they also have to cope with new solid food, a novel environment and often with a new social group. The common management practice, which consists of sorting pigs at weaning by weight in order to give them equal opportunity in the weaning pen leads to severe fights between unacquainted piglets (Friend et al., 1983; Blackshaw et al., 1987). Fighting results in wounds (Olesen et al., 1996; Plyaschenko and Sidorov, 1987 in Ayo et al., 1998) and occasionally in death (Meese and Ewbank, 1972). In addition, aggression decreases the efficiency of food conversion and reduces weight gain (Tan et al., 1991), particularly when another stressor (limited food) is present (Sherrit et al., 1974; Graves et al., 1978).

The agonistic behaviour in pigs includes brief contacts between pairs of penmates (knocking head) and also more severe and longer fights involving two or more pigs and resulting in injuries. During fights, aggressive patterns such as threatening, pushing, head-knocking, biting, chasing and avoiding (turning away from the opponent) have been commonly observed in previous studies (Fraser, 1974; McGlone, 1985; Jensen and Yngvesson, 1998; Giersing and Andersson, 1998). The observed duration of the agonistic period following grouping differs across studies. It can vary from 3 h after mixing piglets (Friend et al., 1983) up to 3 weeks in growing pigs (Tan and Shackleton, 1990). Meese and Ewbank (1973) found that aggression stopped 24 h after grouping 8-week-old pigs, whereas Bazhov and Komlatsky (1989, in Ayo et al., 1998) observed a decrease in aggression 5–10 days after grouping.

Hormonal consequences of aggression have also been studied, with a rise in cortisol in plasma (Moore et al., 1994; Ruis et al., 2001; Otten et al., 2002) and saliva (Van Erp-van der Kooij et al., 2003; Merlot et al., 2004) after groups of piglets are formed. Rapid increases of plasma adrenaline and noradrenaline have also been observed (Otten et al., 2002). Another approach to the study of hormonal secretion has been developed by Hay et al. (2000) and consists of measuring catecholamines and glucocorticoids in urine. This method is less invasive for animals and secretion of such hormones in the urine sums up over several hours, and thus can be considered as more integrative than in plasma.

The revised European Directive 91/630/CE (November 2001) emphasizes the importance of preventing aggression at weaning or during the growing period. Various different ways of decreasing aggression between newly mixed pigs have been tested. The use of sedatives (Blackshaw, 1981; Gonyou et al., 1988; Tan and Shackleton, 1990) or the spread of pheromones (McGlone et al., 1987; McGlone and Morrow, 1988) have a positive, but temporary effect on social behaviour. Weary et al. (1999) found less aggressive behaviour at weaning when piglets from different litters were mixed in their farrowing pens, enabling them to become familiar at an early age when the level of aggressiveness is lower (Pitts et al., 2000). Mixing pigs of heterogeneous weights (Rushen, 1987; Andersen et al., 2000) or providing straw (Andersen et al., 2000) or toys (Blackshaw et al., 1997) reduces aggression in the group, whereas partitioning pens does not limit agonistic behaviour at weaning (Olesen et al., 1996).

Grouping piglets by sex at weaning is practiced by some breeders since males and females will be separated during the growing period. Other breeders consider this to be a supplementary grouping criterion in addition to weight, which complicates management at weaning. Behavioural consequences of grouping pigs by sex are presently unknown. According to Graves et al. (1978), no differences in behaviour were found between male castrates and females when grouped. However, some studies revealed that young intact males were more aggressive than females (Dobao et al., 1984–1985; Newberry and Wood-Gush, 1986). Furthermore, Giersing and Andersson (1998) found that males initiated more mixed-sex fights than females, and that frequencies of mixed-sex fights initiated by males were also higher than single-sex ones, but the authors did not discuss this last point. Nevertheless, this supports our previous unpublished observations which indicate that in a weaned mixed-sex group, fights involving two opposite sex penmates are more frequent than single-sex fights. We do not know whether the same effects would be found when comparing mixed- versus single-sex groups.

The aim of the present experiment was to elucidate behavioural, growth and neuroendocrine consequences of grouping unfamiliar piglets in same- or mixed-sex groups. The assumption was that same-sex groups would display less aggressive behaviour than groups including both sexes. In addition, a familiar mixed-sex group was included to compare the effects of both grouping variables (acquaintance and sex) on aggressive behaviour.

2. Material and methods

2.1. Experimental procedure

The experiment was performed at the Pig Technical Institute experimental farm (ITP, Romillé, France). A total of 24 groups each containing 8 piglets from Large-White*Landrace-crossed sows were used ($n = 192$), corresponding to 6 different weaning batches. Groups were made from five litters equalized to 10 or 12 piglets per farrowing pen, fostered 24 h after farrowing. Previous studies have demonstrated that such brief contact is not sufficient for familiarity to be established between piglets (Stookey and Gonyou, 1998). Canine resection with a grinding wheel and tail docking were carried out on the day of birth. All males were castrated before they were 1 week old. Weaning occurred at 28 days (average body weight: 9.25 kg) and piglets were immediately assigned to a group in one of the four following experimental conditions: groups composed of littermates, four males and four females from one of the 5 litters (L-MF); four males and four females from four different litters (one male and one female from each) were assigned to alien mixed-sex groups (A-MF), eight females from 4 litters (two from each) were allocated to alien all-female groups (A-F) and eight males from 4 litters (two from each) were assigned to alien all-male groups (A-M). In this manner, six groups of eight piglets were assigned to each condition. Excess piglets were assigned to the classic breeding conditions out of the experiment.

Weaning pens measured 3.9 m^2 (1.70 m × 2.30 m) and consisted of partially slatted concrete floor in one of the two weaning rooms used for this experiment (three replicates per room) and of complete slatted floor in the second room. Control temperature was maintained at 27–28 °C. Rooms were automatically illuminated from 7:30 to 19:30. The starter diet used in farrowing pens was maintained after weaning up to 41 days and given ad libitum; then piglets received another pelleted diet. Animals had permanent access to water.

All piglets were weighed at birth and on postnatal days 21, 28, 32, 36, 40 and 63 (end of post-weaning). Feed consumption was measured per group between each weighing day.

2.2. Behavioural measures

Agonistic behaviours were recorded on postnatal days 28 (weaning day), 29, 31, 35, 39 and 46. A video camera was positioned directly overhead of each group and the piglets were filmed in real-time for 8 h. We initially filmed piglets at night under red light but the test revealed that they were asleep from 19:00 to 7:00; then, we decided to limit our observations to the period when the rooms were illuminated. On day 28, piglets were weaned between 9:30 and 10:00, and observed for the first 2 h after grouping. On day 29, observations started in the afternoon, 2.5 h after the end of our interventions (urine sampling and scratch counting) when piglets became active again. On days 31, 35 and 39, scan samplings were performed 8 h/day, every 15 min, to assess the number of piglets standing, in order to determine a 2 h-period per day of high activity. Two periods of high activity were thus identified, 0.5 h in the morning (between 7:30 and 8:30) and 1.5 h in the afternoon (between 15:30 and 18:00). On day 46, all piglets were resting in the morning, thus we observed them for 2 h in the afternoon only (between 15:30 and 19:00).

Each piglet had a number marked with a black pen on its back. Observers viewed the videotapes and recorded occurrence and duration of fights by continuous sampling during the 2 h-period of high activity/day. Four trained observers were assigned to this task.

Agonistic behaviour was defined as dyadic encounters during which one piglet attacked another one. We classified fights according to the degree of severity, determined by reciprocity and/or speed of the fights. Four aggression scores were thus defined: (1) a piglet thrusts (head knock) another with mouth closed; (2) a piglet thrusts another smoothly (no chase, no running), biting or with intent to (mouth opened); (3) two options: reciprocal jerks of the head with bites (mouth opened) without chase, or unilateral fight (one piglet chases and bites another that does not retaliate); (4) reciprocal fight with jerk, chase and bite.

Aggression was considered to begin when one of these defined behaviours was observed. The aggression ended when piglets lost contact with each other for at least 15 s.

In mixed-sex groups, we recorded the sex of the piglets involved in dyadic aggressive interactions and the one which began the interaction.

On days 27, 29, 33 and 61, wounds were counted on the four following regions of the pigs' bodies: (1) ears; (2) head; (3) neck-shoulders; (4) flanks-abdomen. Wounds included scratches and bites. Each discrete scratch was counted as one scratch. Until day 33, the 8 piglets of the four experimental groups were individually caught by one handler and another counted scratches. On day 61, the handler separated one piglet from the others behind a PVC wall while the counter noted scratches with a torch sweeping over that pig. The four people successively assigned to this task were previously trained and used the exact same counting method. On each region of the pig's body, the maximum number of scratches was set at 50, after which we noted >50. On the entire body, the maximum scratch score was set at 100.

2.3. Endocrine measures

Prior to counting scratches, urine was collected between 7:00 and 9:00 on days 27, 29 and 33. Urine from spontaneous urination was obtained from at least four piglets per group (two males and two females in the mixed-sex groups). Urine was collected in a beaker attached to a 50 cm stick to avoid contact between the human and the piglet, which could be disturbing. If four piglets (minimum) were not sampled by 8:00, 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. Piglets sampled on day 27 were also sampled on days 29 and 33. The samples were acidified using 6 M HCl (1% of urine volume) and immediately frozen at −20 °C.

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

Urinary cortisol and cortisone (the oxidized metabolite of cortisol) were assayed using a solid phase extraction procedure followed by HPLC with UV absorbance detection (254 nm), as previously described by Hay and Mormède (1997b). Briefly, 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 color derived from the reaction between creatinine and alkaline picrate

(Jaffe's reaction) when the mixture is acidified. Thus, the difference in color intensity measured at 500 nm before and after acidification of the mixture is proportional to creatinine concentration. Catecholamines and glucocorticoids levels are expressed as ng/mg of creatinine to take into account urine dilution.

2.4. Statistical analyses

2.4.1. Behavioural analyses

The Kolmogorov–Smirnov test was used to assess the normality of our behavioural data. Because these data lacked normality, non-parametric tests were used.

Median values for each group of eight individual piglets were the statistical units ($n = 6$ groups in each treatment condition). Within each pen, fighting duration was summed across the 2 h/day. Data from the four independent experimental conditions were compared using the Kruskal–Wallis test (H), followed by post-hoc Mann–Whitney U-tests when $p < 0.1$. Within each experimental condition, median number of fights were compared across the four levels of aggression (1–4) using the Friedman test (χ^2, d.f. = 3), followed by the Wilcoxon matched-pairs signed-ranks test (if $p < 0.1$). Intensities of single-sex fights were compared between experimental conditions using the Kruskal–Wallis test, followed by Mann–Whitney U-tests (if $p < 0.1$). Samples used for that comparison were percentages of level-4 fights among all the agonistic interactions.

Within mixed-sex groups, the percentage of fights (considering only the level-4 ones), according to opponents' sex was calculated across the six observation days. The observed percentage of fights between a male and a female (F-MF) relative to all the fights involving at least one female was compared to an expected percentage: 57% (a female can fight with four different males among the seven potential partners). The same method was applied to males' fights: M-MF percentage was compared to the theoretical percentage. Observed and expected percentages were compared using the Mann–Whitney U-test.

In addition, a Wilcoxon matched-pairs signed-ranks test was performed in order to compare the correlated proportions of fights according to whether a male (M) or a female (F) was involved as initiator. The percentage of level-4 fights computed among all the agonistic interactions (levels 1–4) was compared between each sex combination (two males: MM, two females: FF, one male and one female: MF) in littermate mixed-sex groups (L-MF) and alien mixed-sex groups (A-MF) using the Kruskal–Wallis test, followed by Mann–Whitney U-tests (if $p < 0.1$).

Proportions of piglets in each group showing more than 50 scratches in the region 'flank-abdomen' and more than 100 scratches in the entire body were compared between groups using the Kruskal–Wallis test (H), followed by Mann–Whitney U-tests. Skin lesions in alien all-female groups (A-F) and alien all-male group (A-M) did not differ significantly. We combined data into a new group called alien-unisex (A-UNI, $n = 12$).

2.4.2. Endocrine analyses

Data were transformed into their logarithmic scores for normalization. Two tests were performed on data corrected for the litter effect. In the first analysis, unfamiliar piglets and littermates within the mixed-sex groups were compared (A-MF versus L-MF). The effects of familiarity, sex, day and their interactions were estimated using a mixed linear model using the day as a repeated factor. In the second test, we compared mixed-sex and single-sex piglets within unfamiliar piglet groups. The effects of sex mixing, sex of the piglets, day and their interactions were assessed with a mixed linear model using the day as a repeated factor. All calculations were performed with SAS Version 8 (SAS Institute, 1999).

2.4.3. Growth and food intake analyses

Feed intake was analysed during the following periods: 28–32, 32–36, 36–40 and 40–63 days. Average daily gain (ADG) was calculated from weaning (28 days) to days 32, 36, 40 and 63. Analysis of variance was performed using the General Linear Model of SAS, including treatment and weaning batch in the model. Weight at weaning was used as a covariate for these analyses. Post-hoc tests were done when an overall treatment effect was found at $p < 0.1$.

3. Results

3.1. Behavioural data

3.1.1. Inter-group comparisons

On day 28, the total duration of fights during the 2 h observation period differed significantly between conditions ($H = 10.19$, $p < 0.05$, Fig. 1). It was significantly lower in the L-MF group than in the A-MF ($U = 1$, $p < 0.01$) and only a slight difference appeared between L-MF and A-F ($U = 7$, $p = 0.07$). Fights were significantly longer in the A-MF than in the A-F group ($U = 3$, $p < 0.05$). Significant differences were also found between conditions on day 29 ($H = 10.37$, $p < 0.05$). Median fight duration was higher in condition A-MF (62.8 min/2 h) than in the L-MF (1.3 min, $U = 2$, $p < 0.05$), A-M (26.4 min, $U = 4$,

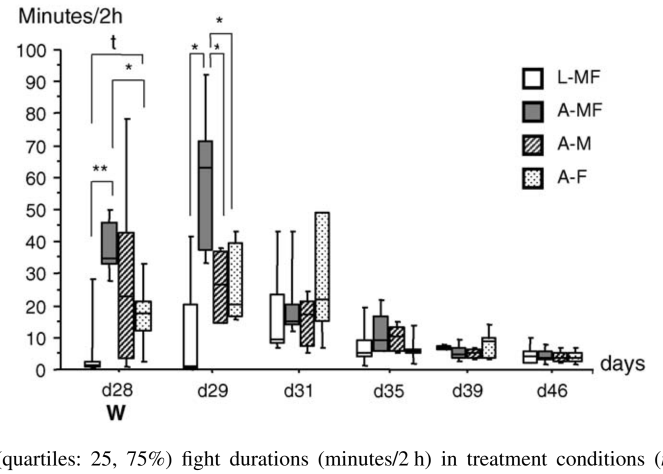

Fig. 1. Median (quartiles: 25, 75%) fight durations (minutes/2 h) in treatment conditions ($n = 6$ each) after weaning at 28 days. L-MF (littermates, four males–four females), A-MF (aliens, four males–four females), A-M (aliens, eight males), A-F (aliens, eight females). Medians were compared using the Mann–Whitney U-test ($^{t}0.05 < p < 0.1$; $^{*}p < 0.05$; $^{**}p < 0.01$).

$p < 0.05$) and A-F (20.2 min, $U = 4$, $p < 0.05$) conditions but L-MF, A-M and A-F did not differ significantly on this day.

From days 31 to 46, fight durations did not differ between conditions.

Table 1 shows the distribution of fights according to severity (scores 1–4). On day 28, number of fights differed significantly between severity classes in condition L-MF only ($\chi^2 = 9.25, p < 0.05$). We observed more level-1 fights than aggression with scores 2, 3 or 4

Table 1
Median (quartiles: 25, 75%) number of fights according to level of aggression (1–4) on each observation day (2 h/day)

Days[a]	Conditions[b]	Aggression scores[c]				$\chi^{2\ d}$	Statistical effect
		1	2	3	4		
Day 28 (W)	L-MF	26.5 (10–31) a	3 (0–10) b	1 (0–3) b	0 (0–0) b	9.25	*
	A-MF	6.5 (5–8)	10.5 (9–17)	11 (10–12)	22 (9–27)	5.55	NS
	A-M	11 (8–14)	12.5 (11–18)	10 (3–11)	10 (1–16)	2.25	NS
	A-F	8.5 (6–15)	5.5 (3–10)	4 (2–7)	13 (7–13)	3.85	NS
Day 29	L-MF	26.5 (9–30)	7 (0–14)	1 (0–12)	0 (0–10)	6.15	NS
	A-MF	10.5 (10–14) a	11 (9–16) a	8.5 (4–12) b	17.5 (13–22) c	7.15	t
	A-M	17 (8–18)	18.5 (10–29)	7 (6–13)	11.5 (7–15)	3.65	NS
	A-F	6 (5–9)	10.5 (7–14)	7 (7–15)	10.5 (6–16)	2.6	NS
Day 31	L-MF	18 (8–20)	38.5 (23–51)	13 (1–29)	3.5 (2–12)	5.15	NS
	A-MF	17 (9–29) ab	32 (23–37) a	16.5 (5–24) cb	8.5 (5–14) c	13.8	**
	A-M	20.5 (15–33) a	36 (27–42) b	11.5 (6–17) ac	9 (1–11) c	10.6	*
	A-F	15.5 (11–20)	36.5 (25–42)	14.5 (9–18)	5 (4–31)	3.65	NS
Day 35	L-MF	15 (8–63) a	41 (24–58) a	8 (7–17) b	0 (0–1) c	12.12	**
	A-MF	46 (28–51) a	29.5 (24–64) a	13.5 (11–16) b	3 (1–5) c	14.6	**
	A-M	28.5 (23–58) a	32.5 (24–77) a	9 (7–26) a	1 (1–3) b	13.4	**
	A-F	41.5 (23–48) a	20 (19–24) a	9 (6–14) b	2 (1–3) b	11.6	**
Day 39	L-MF	32 (16.5–66) a	29 (20–40) a	15 (13.5–17) a	0 (0–5) b	10.68	*
	A-MF	41 (18–67.5) a	24 (23–33) a	9 (6–11.5) b	2 (0–3.5) c	13.56	**
	A-M	35 (24–38) a	31.5 (26–36) a	9 (6–13) b	0 (0–1) c	16.2	**
	A-F	32 (22.5–96) a	27 (24–39) a	9 (7–10) b	2 (2–4) c	14.04	**
Day 46	L-MF	40 (32–71) a	29 (21–48) a	8 (6–13) b	0 (0–3) c	16.4	***
	A-MF	39 (18–57) a	25.5 (23–28) a	8 (6–18) b	0 (0–1) c	14.6	**
	A-M	38.5 (26–65) a	28 (18–36) a	8.5 (7–13) b	0 (0–0) c	15	**
	A-F	27.5 (26–49) a	24 (18–39) a	7 (3–12) b	0 (0–1) c	14.6	**

Within rows medians with different letters are significantly different (Wilcoxon matched-pairs signed-ranks test; $p < 0.05$).

[a] Weaning occurred on day 28.

[b] L-MF (littermates, four males–four females), A-MF (aliens, four males–four females), A-M (aliens, eight males), A-F (aliens, eight females) ($n = 6$).

[c] Medians are given with interquartile range in parentheses.

[d] The four correlated samples within each condition were compared with the Friedman test (d.f. = 3).

[*] $p < 0.05$.

[**] $p < 0.01$.

[***] $p < 0.001$.

[t] $0.05 < p < 0.1$.

NS: non-significant.

($p < 0.05$, respectively). No further differences appeared in the three other conditions. On day 29, the distribution of the four scores tended to be non-random in the A-MF condition only ($\chi^2 = 7.15$, $p = 0.067$): the frequency of level-4 aggression was higher than that of levels 1, 2 or 3 (17.5 versus 10.5, 11 and 8.5, respectively, $p < 0.05$ in each case). However on this day, neither all-male nor all-female groups showed any difference when comparing the number of fights according to degree of aggression.

On day 31, in the A-MF condition, score 2 was observed significantly more often than scores 3 and 4 ($p < 0.05$). Score 1 was also more observed than score 4 ($p < 0.05$). In the A-M condition, score 2 was observed significantly more often than the other scores ($p < 0.05$). No differences appeared in L-MF or A-F conditions.

On days 35, 39 and 46, scores 1 and 2 were observed significantly more often than score 4 in each group ($p < 0.05$, in each case).

The percentages of level-4 fights among male to male aggression (MM) differed significantly between the three conditions concerned ($H = 8$, $p < 0.05$, Fig. 2). This percentage was significantly higher in A-MF (19.7%) than in L-MF (2.1%, $U = 1$, $p < 0.01$) and A-M (7.6%, $U = 7$, $p < 0.05$).

Those percentages related to dyadic fights involving two females differed significantly between the three conditions concerned ($H = 8.4$, $p < 0.05$, Fig. 2). We observed less level-4 aggression between two females in L-MF than in A-MF and A-F (0.5% versus 11.3 and 9.2%, respectively, $p < 0.05$), but A-MF and A-F did not differ significantly.

The only treatment effects found for scratches according to the body region were on day 29, for flank-abdomen ($H = 7$, $p = 0.07$) and entire body ($H = 13.7$, $p < 0.01$, Fig. 3). A-M and A-F did not differ significantly. The following comparisons were thus carried out between conditions L-MF, A-MF and A-UNI (A-M + A-F). 18.7% of piglets from A-MF showed more than 50 scratches on the flank-abdominal zone, versus 0% in L-MF ($U = 2.5$, $p < 0.05$). For the entire body zone, A-MF differed from the two other conditions: 75% of piglets in A-MF had more than 100 wounds on their body, versus 0% in L-MF ($U = 0$, $p < 0.01$) and 50% in A-UNI ($U = 15$, $p < 0.05$). A-UNI also differed from the L-MF condition ($U = 0$, $p < 0.01$).

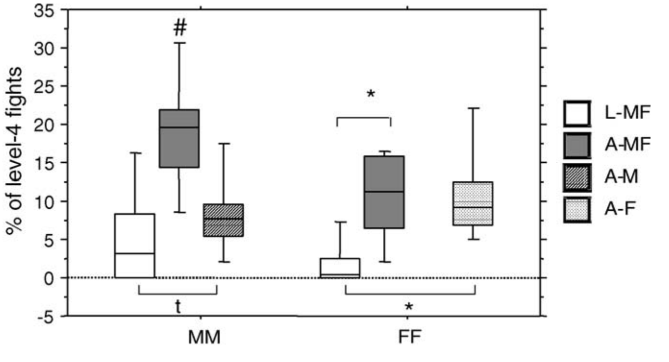

Fig. 2. Median (quartiles: 25, 75%) percentages of level-4 fights within male–male fights (MM) and female–female fights (FF). L-MF ($n = 6$): littermates (four males–four females); A-MF ($n = 6$): aliens (four males–four females); A-M ($n = 6$): aliens (eight males); A-F ($n = 6$): aliens (eight females). [t]$0.05 < p < 0.1$; [*]$p < 0.05$; [#]A-MF differs from the two other conditions ($p < 0.05$) (Mann–Whitney U-test).

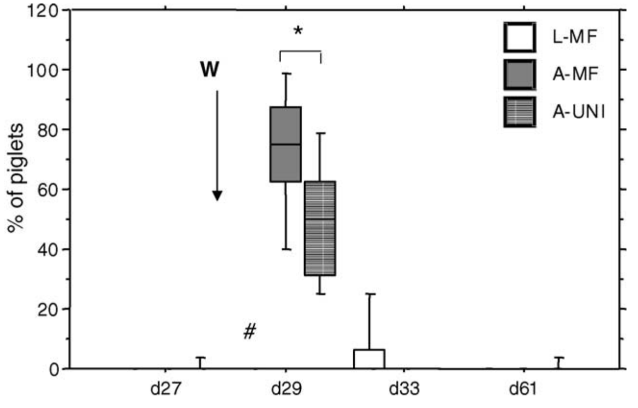

Fig. 3. Median (quartiles: 25, 75%) percentages of piglets showing more than 100 skin lesions on the entire body (measured on 4 days from days 27 to 61). L-MF ($n = 6$): littermates (four males–four females); A-MF ($n = 6$): aliens (four males–four females); A-UNI ($n = 12$): aliens unisex (eight males or eight females). $^{*}p < 0.05$ (Mann–Whitney U-test); #L-MF differs from the two other conditions ($p < 0.01$). Weaning occurred on day 28.

3.1.2. Intra-mixed-sex groups comparisons

The percentage of level-4 fights involving a male and a female relative to all-females fights (F-MF) was higher than the expected percentage (72.6% versus 57%, $U = 0$, $p < 0.01$, Fig. 4). As a result, the percentage of aggression between two females among all the fights involving at least one female was significantly lower than expected (i.e. 43%) (27.4% versus 43%, $U = 0$, $p < 0.01$, not shown in the figure). In contrast, the percentages of male–female fights (M-MF) among all the fights involving at least one male did not

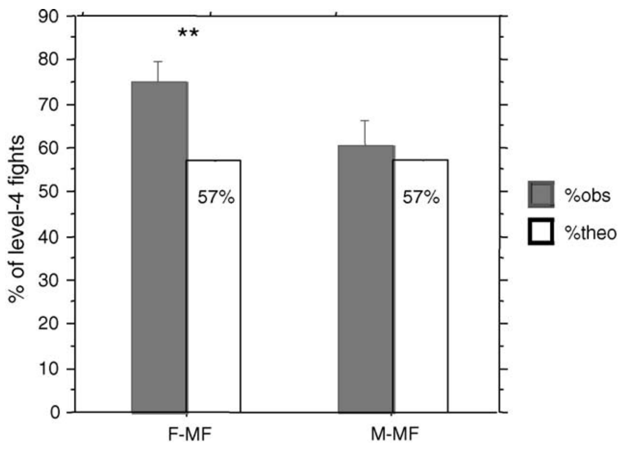

Fig. 4. Percentages of fights (% obs) by different combination of pairs in the alien mixed-sex condition (A-MF) relative to theoretical expectations (% theo): mixed-sex fights (F-MF) among all the level-4 fights involving at least one female and mixed-sex fights (M-MF) among all the level-4 fights involving at least one male. $^{**}p < 0.01$ (Mann–Whitney U-test).

differ from the theoretical percentage. A male fought against either a male or female opponent without displaying any preference.

No difference between sex composition in dyadic fights was found within the group composed of littermates (L-MF).

In A-MF, the median proportion of level-4 fights (computed among all levels) differed between the 3 sex combinations ($H = 4.64$, $p < 0.01$, Table 2). The percentage of level-4 fights involving two males was higher (but not significantly so) than that involving two females ($U = 6$, $p = 0.054$) or both sexes ($U = 7$, $p = 0.078$). In L-MF, the proportions of level-4 fights did not differ significantly between sex combinations.

In A-MF, males were involved as initiators of 62.2% of level-4 mixed-sex fights versus 37.8% initiated by females (Wilcoxon test, $p < 0.05$, Table 2). In the L-MF condition, the proportions of aggression did not differ significantly according to sex of the initiator.

3.2. Animal production data

We did not observe any difference in feed intake between the four conditions (Table 3), except for days 36–40 period, when A-MF ate more than A-F and L-MF (22.2 kg/pen versus 20.5 and 19.1 kg/pen, respectively, $p < 0.05$).

Table 3 also shows that growth rate tended to differ between conditions during the first period (days 28–32), with better growth in L-MF than in A-F (270 g/day versus 192 g/day, $p < 0.05$). Twelve days after weaning (days 28–40 period), conditions L-MF and A-F showed the poorest growth, with the best results obtained by A-MF (397 g/day and 404 versus 443 g/day, respectively, $p < 0.05$). At the end of the post-weaning period, no further differences were observed between conditions.

Table 2
Median (quartiles: 25, 75%) percentages of level-4 fights relative to all the levels (1–4) across all observation days in each sex combination: two males (MM), two females (FF), one male and one female (MF), and in relation to sex of the initiator (M or F) within mixed-sex groups ($n = 6$)

	% of level-4 fights among all the levels (1–4) in each sex combination			Statistical effect[a]	% of level-4 fights among all the levels (1–4) according to sex of the initiator	
	MF	MM	FF		M	F
A-MF						
Median	11.3	19.7[t]	11.3	<0.01	62.2[*]	37.8
Quartiles	9.4–14.5	14.3–21.8	6.4–15.8		59.1–68.2	31.8–40.9
L-MF						
Median	3.2	2.1	0.4	NS	56.4	43.6
Quartiles	0–8.3	0–5.3	0–2.4		48.7–65.8	34.2–51.3

MF: male–female fights, MM: male–male fights, FF: female–female fights. L-MF ($n = 6$): littermates (four males–four females); A-MF ($n = 6$): aliens (four males–four females).

[a] Kruskal–Wallis test.

[*] M differs significantly from F, in A-MF (Wilcoxon test, $p < 0.05$).

[t] MM tends to differ from MF and FF, in A-MF (Mann–Whitney U-test, $0.05 < p < 0.1$).

NS: non-significant.

Table 3
Mean (±S.E.M.) food intake and average daily weight gain by period

	Treatments				S.E.M.	Statistical effects		
	L-MF	A-MF	A-M	A-F		Treatment	Batch	Weaning weight
Feed intake (kg/pen)								
Days 28–32	8.2	7.6	8.4	7.7	1.2	NS	*	NS
Days 32–36	12.3	13.5	13.1	13.3	1.2	NS	**	*
Days 36–40	19.1 a	22.2 b	20.7 ab	20.5 a	1.4	*	***	*
Days 40–63	178.3	176.5	175.7	175.5	11.2	NS	**	*
Growth (g/day/piglet)								
Days 28–32	270 b	219 ab	243 ab	192 a	148	0.1	***	NS
Days 28–36	315	354	342	322	110	NS	**	*
Days 28–40	397 a	443 b	431 ab	404 a	102	0.1	***	***
Days 28–63	563	562	550	550	75	NS	**	***

Means with common letters do not differ ($p < 0.05$). L-MF ($n = 6$): littermates (four males–four females); A-MF ($n = 6$): aliens (four males–four females); A-M ($n = 6$): aliens (eight males); A-F ($n = 6$): aliens (eight females).
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
NS: non-significant.

3.3. Endocrine data

We compared unfamiliar pigs (A-MF) and littermates (L-MF) (Fig. 5). Urinary cortisol levels increased on day 29 ($p < 0.05$); familiarity and sex had no effect, and the interaction between familiarity and sex was significant, the levels being higher in females than in males in littermates only ($p < 0.05$). Urinary cortisone concentrations were lower on day 33 ($p < 0.05$), higher for littermates ($p < 0.05$) and for females ($p < 0.01$). There was no interaction between familiarity and sex. On day 33, urinary noradrenaline levels decreased ($p < 0.05$). Familiarity and sex had no effect, but an interaction between familiarity and day was observed: on day 29, alien piglets showed much higher noradrenaline levels than littermates ($p < 0.001$). Urinary levels of adrenaline decreased on day 33 ($p < 0.05$). The transient increase on day 29 was not significant. Levels were higher in males ($p < 0.05$).

In a second analysis, mixed-sex alien piglets were compared to single-sex alien piglets (Fig. 6). On day 29, urinary cortisol levels increased ($p < 0.01$); sex of the piglets had no effect by itself and there were no significant differences between mixed-sex versus single-sex groupings. Urinary cortisone concentrations were lower on day 33 ($p < 0.01$) and higher for females ($p < 0.001$). The urinary catecholamines levels increased on day 29 ($p < 0.0001$); sex per se and sex-mixing had no effect.

4. Discussion

In unfamiliar groups, no difference appeared between A-M and A-F, either in fight length, severity of fights, or injured piglets. When they were penned together, males did not

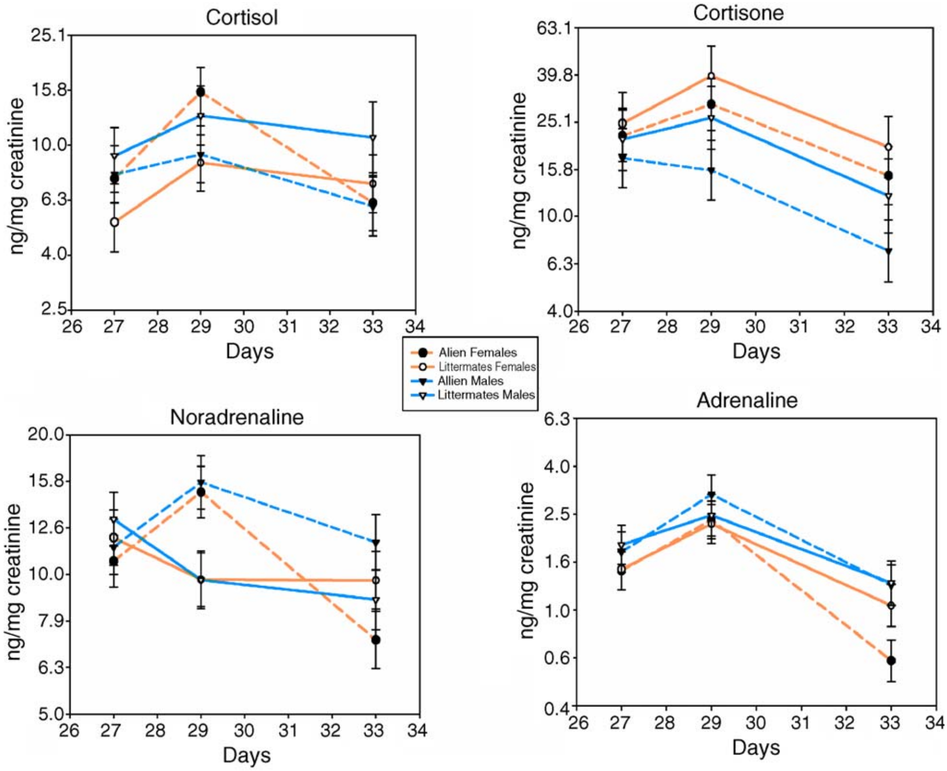

Fig. 5. Mean (±S.E.M.) urinary cortisol, cortisone, noradrenaline and adrenaline concentrations (ng/mg creatinine) in alien piglets and littermates. Weaning occurred on day 28.

display more aggressive behaviour than did females penned together. However, comparisons between A-MF and the single-sex conditions showed longer durations of fights in A-MF on days 28 and 29, and more 'score 4' than other scores in A-MF only. As a result, we observed more injured piglets in A-MF on the day after weaning than in both single-sex groups. According to Olesen et al. (1996), long fights seem to be correlated with the number of scratches. Moreover, length of fights at weaning did not differ between the single-sex groups and the familiar mixed-sex group where this length was very low, confirming that grouping piglets by sex considerably decreases aggression at that time.

To summarize, A-M and A-F showed the same level of aggression when the piglets were grouped at weaning, lower than that of A-MF and close to that of L-MF, while mixing both sexes in a group increased total aggressive behaviour. In contrast, in laying hens, mixing sexes in groups leads to decreased agonistic behaviour (Bhagwat and Craig, 1979). However, in this study, conditions such as group size and sex ratio (1 male for 25 females on average) were very different from those of the present experiment and the males, which were larger than females, clearly exerted social dominance over the hens.

In order to understand why piglets were more aggressive when both sexes were mixed, it is necessary to assess opponents' sex in A-MF. We observed that males initiated more

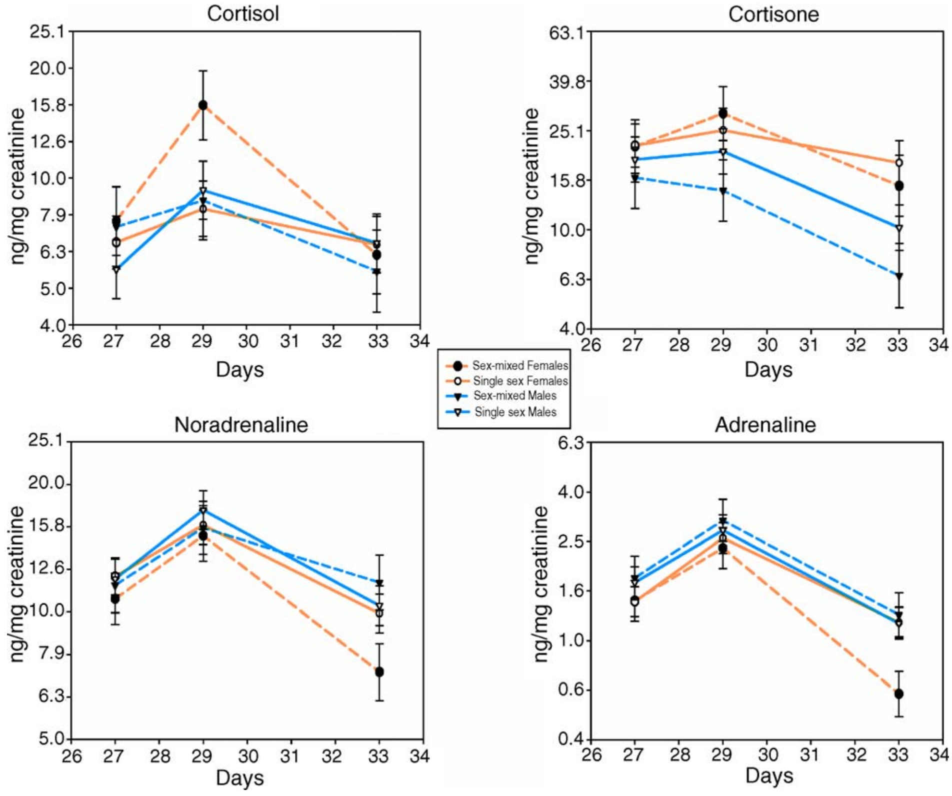

Fig. 6. Mean (±S.E.M.) urinary cortisol, cortisone, noradrenaline and adrenaline concentrations (ng/mg creatinine) in sex-mixed and single-sex piglets. Weaning occurred on day 28.

severe aggressive interactions than females did, which is in accordance with results obtained by Giersing and Andersson (1998) with 8–24-week-old pigs. In addition, dyadic fights mainly involved two piglets of the opposite sex. In fact, the observed percentage of mixed-sex fights among the most severe fights involving at least one female was higher than 57% (i.e. expected percentage). However, the observed percentage of mixed-sex fights computed among fights involving males did not differ from the expected percentage. This suggests that in the mixed-sex group, males fought indiscriminately against another male or a female, and in most cases when females had males as opponents, the latter initiated fights more frequently than did females. These results seem to contrast with those of Dobao et al. (1984–1985) who included pushing, butting and biting in play behaviour and reported more ‘‘play’’ between partners of the same sex. However, these authors also included sexual behaviour in their ethogram (mounting), while for these intra-group results, we only considered the level-4 fights (pushing, biting, chasing). In addition, in A-MF, the level-4 fights seemed to be more frequent than other levels of fights when two males were involved than when either two females or both sexes were involved, which suggests that dyadic fights were the most severe when the opponents were two males. As a result, male-to-male fights were more severe in A-MF than in A-M (more level-4 fights), whereas female-to-female fights had the same severity in A-MF as in A-F. Dobao et al. (1984–1985) also described ‘‘behavioural dimorphism’’ between young males and females, with males showing higher ‘‘play activity’’, including agonistic behaviour. Furthermore, Newberry and Wood-Gush (1988) observed that pushing and butting were performed more frequently by males than females, under semi-natural conditions. However, in the present experiment, aggression in male groups is as high as that of females. It seems thus to be the presence of females in a mixed-sex group which provoked the aggressive behaviour of the young males. The presence of females in the group might induce competitive behaviour in the young males. The increased level of aggression in the mixed-sex group compared to the same-sex groups confirms our previous unpublished observations, which indicated that in a mixed-sex group, fights involving two opposite sex penmates were more frequent than single-sex fights. This is in accordance with Giersing and Andersson (1998) who observed that mixed-sex fights initiated by a male were more frequent than those initiated by females and also than single-sex ones, but the authors did not discuss this last point.

The present study reveals that this higher level observed in the mixed-sex group is due to male aggressiveness towards penmates of both sexes, and suggests the existence of sexual discrimination at this age. In adult pigs, intact males are more aggressive than gilts and castrates (Lundström et al., 1987; Signoret et al., 1989; Giersing, 1998; Cronin et al., 2003). As a result, Warriss and Brown (1985) have observed more carcass damage in boars than in castrates. The aggressiveness of intact males is directly influenced by testosterone secreted by the testicles. However, our observations showed that after unacquainted piglets were grouped, prepubertal castrated males were the most aggressive when penned with females, even though their testicles had been removed 1 week after birth. If we assume that hormonal mechanisms are involved in this behavioural dimorphism between the two sexes, these may be related to the elevated blood level of testosterone recorded in neonatal males (Meusy-Dessolle, 1975; Ponzilius et al., 1986). These levels are low prenatally but increase early after birth, before castration. They may yet induce maturation of structures involved in aggressive behaviour of males in their second month of life.

Twelve days after weaning, feed consumption and growth rate were better in the mixed-sex group than in the all-females group. At this stage of development, female growth potential may start to differ from that of males, which could explain this difference. Gaudré and Granier (2004) also observed a sex effect on growth during the first 19 days after weaning.

In alien groups, fights following weaning were longer and more severe in the mixed-sex than in the same-sex conditions, but these did not influence performance. The energetic loss related to fights was low and access to the feeder did not seem compromised in that case.

In the two mixed-sex conditions, grouping piglets from the same litter at weaning induced shorter and less severe fights than grouping unfamiliar piglets at weaning. This supports findings from various studies of newly-mixed pigs (Tan et al., 1991) or newly-weaned piglets (Friend et al., 1983; Ekkel et al., 1995; Puppe et al., 1997), where aggression is higher when unacquainted animals are penned together, than in groups formed by familiar pigs. However, durations of agonistic interactions – which also include low intensity levels – in the familiar mixed-sex condition and in unfamiliar single-sex conditions did not differ after weaning. As seen earlier in the discussion, aggression durations seem similar when groups are composed of familiar males and females, or when piglets are in single-sex groups even with unfamiliar individuals.

In the unfamiliar groups, fights occurred immediately after mixing, but decreased thereafter as animals became familiar with one another. In 8-week-old (Meese and Ewbank, 1973) and 4-week-old piglets (Olesen et al., 1996), the establishment of the social order is determined 48 h after mixing. In the present experiment, the maximum duration of fights was reached 24 h after weaning in the A-MF condition, with a total of 1 h of fighting within the 2 h-period of observation. Three days after weaning (day 31), the duration of fights declined to a low level and did not differ from the unfamiliar condition, which probably indicates that the social relationships were clarified at that time.

On the day of weaning, we observed a higher number of level-1 interactions in L-MF than in the three unfamiliar conditions. These unilateral interactions were brief head knocks, mainly displayed at feeding or by a piglet disturbed by the trampling of another one. The low level of this type of behaviour in the alien compared to the familiar condition can be explained by the vigorous fights (level 4) occurring among the aliens, preventing therefore any other types of social interactions. When severe fights occurred between two or more piglets, other penmates interrupted their activity and seemed to become wary. In the L-MF condition, brief head-knocks were the only agonistic interactions recorded on the day of weaning. Fraser (1974) has shown that conflicts between previously acquainted pigs were restricted mainly to butting, while fighting between unacquainted animals primarily involved biting. The different reasons for these butting (or level-1 interactions) in L-MF were easily observable (place at the feeder or at the trough, trampling, belly-nosing, etc.), whereas the origin of level-4 fights recorded in newly mixed groups were not so obvious. Generally, an individual threatened another one, approaching and biting or only staring without physical contact. Then, as Ewbank and Bryant (1972) described, the threatened pig retaliated strongly, causing the threatening pig to reinforce its threat and escalation to occur. According to Ewbank (1976), the type of aggression necessary for the maintenance of social order is different from that used in its establishment. In condition L-MF, the maintenance of the social order immediately after weaning involved only brief thrusts but the appearance of fights with scores 2 and 3 on days 35 and 39, respectively, indicates a frail social stability despite the familiarity between penmates. We even observed a slight, but non-significant, difference in total duration of fights on day 39, higher in the L-MF than in the A-M condition, although the length of fights in each group on that day remained very short (6 min in average, per 2 h). The maintenance of social order, as observed between littermates reared together since nursing, seems to require recurring contact between the individuals. According to Fraser (1974) and Ewbank and Meese (1971), threshold levels of interactions are necessary to maintain familiarity and dominance relationships.

The level-1 interactions observed in condition L-MF immediately after weaning did not cause injuries, compared to the unfamiliar groups. In these groups however, the severe fights resulted in 50–75% of piglets showing more than 100 skin lesions on their body on day 29. This is in accordance with various studies indicating that mixing of unfamiliar pigs, hierarchy formation and competition lead to skin lesions (Ellis et al., 1983; Gonyou et al., 1988; Olesen et al., 1996; Fredriksen et al., 2003). Furthermore, piglets in the A-MF condition had more injuries on the flank-abdominal region than did L-MF on day 29.

Olesen et al. (1996) have shown also that this region was the most severely damaged during long fights.

The wounds counted on day 29 in the unfamiliar groups were closing on day 33, which reflects that despite the impressive and serious fights observed between unacquainted piglets, injuries were in most cases superficial. The relative lack of severe injuries may be attributable to the youth and thus the low weight of the animals used in this study, while traumatic injuries are more frequent when pigs are heavier (Olesen et al., 1996; Tan et al., 1990).

The long lasting decrease in the performance related to social mixing has been demonstrated particularly when animals had limited access to food (Sherrit et al., 1974; Graves et al., 1978), which is not the case in the present study where piglets were fed ad libitum. Immediately after weaning, we did not observe a clear difference in growth between familiar and unfamiliar groups, and L-MF even showed worse performance than A-MF between weaning and day 40. The modification of social order observed in L-MF at that time may be the explanation (more difficult access to the feeder). However, these differences were only transient and the conditions did not differ at the end of post-weaning period, which confirms findings of Friend et al. (1983) and Blackshaw et al. (1987) relative to social mixing.

The general pattern for glucocorticoids and catecholamines levels in urine was a transient increase after weaning (day 29) followed by a large decrease on day 33. However, neither familiarity nor sex mixing influenced cortisol production. Five days after weaning, cortisone production decreased. Indeed, there is a general decrease of plasma and urinary levels after birth until adult basal level are reached (Kattesh et al., 1990; Hay et al., 2001). On the other hand, weaning and social confrontation have been shown to increase stress hormone levels in plasma or urine (Hay et al., 2001; Dantzer and Mormède, 1981; Kanitz et al., 1998; Carroll et al., 1998; Fernandez et al., 1994). Experimental treatments had a limited influence on this general pattern, the most noticeable being the effect of familiarity on noradrenaline levels on day 29. The rise of noradrenaline levels after weaning for the unfamiliar piglets may be related to the intensity of the fights observed in these groups. Indeed, Fernandez et al. (1994) showed that plasma catecholamines levels and aggressive behaviour were strongly correlated, part of this relationship may be related to increased physical activity. Thus, grouping unfamiliar piglets may stimulate the sympathetic nervous system as a result of an increased physical activity.

5. Conclusions

Grouping piglets according to familiarity had a great influence on the reduction of aggression. However, the duration of agonistic interactions in the familiar mixed-sex condition and in unfamiliar single-sex conditions did not differ after weaning. Effects in term of fight durations seem similar in groups composed of familiar piglets of both sexes, or when piglets are grouped with unfamiliar individuals of the same sex. However, these behavioural conclusions are not consistent with the familiarity found for endocrine measures—i.e. a rise of noradrenaline levels after weaning for the unfamiliar piglets only.

Within unfamiliar conditions, behavioural data revealed more fights in mixed-sex than in single-sex groups after weaning. Grouping unfamiliar piglets by sex seemed to decrease the immediate aggressive behaviour even if it did not lead to noticeable changes either in neuroendocrine systems or in long-term animal production results. Aggressive behaviour observed in the mixed-sex group seemed to result from an acute aggressiveness displayed by young males, despite their castration. The reduction of aggressive behaviour found in both single-sex groups compared to the mixed-sex group suggests that piglets' sex should be taken into account when groups are formed at weaning, in order to increase animal welfare.

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