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Published in:
Applied Animal Behaviour Science

DOI:
10.1016/S0168-1591(01)00191-5

Publication date:
2002

ELSEVIER

Applied Animal Behaviour Science 75 (2002) 207–231

APPLIED ANIMAL BEHAVIOUR SCIENCE

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Implications of coping characteristics and social status for welfare and production of paired growing gilts

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Accepted 22 August 2001

Abstract

This paper considers the question whether knowledge on individual coping characteristics of growing pigs may be used to improve welfare and production after mixing. Gilts with either reactive or proactive coping characteristics were identified according to behavioural resistance in a backtest, respectively, being low (LR) and high resistant (HR) in this test. At 7 weeks of age, several pairs of unfamiliar gilts were formed, and pairs and dominance relationships were studied over a 3-week period. The following pairs (combinations) were established: two LR gilts (LR/LR; $n = 12$), two HR gilts (HR/HR; $n = 12$), one LR and one HR gilt (LR gilt dominant: LR(d)/HR; $n = 11$), and one LR and one HR gilt (HR gilt dominant: LR/HR(d); $n = 12$). Results showed that on the day of mixing, aggression subsided less quickly and increases in body temperature were higher in LR/HR(d) and HR/HR pairs. Also, during the first week post-mixing, feed efficiency was lower and skin damage was higher in LR/HR(d) and HR/HR pairs. Mixing of two HR gilts caused highest levels of stress, indicated by greater catecholamine concentrations in urine following the day of mixing, and higher baseline levels of plasma ACTH at 1 week post-mixing. The lower tendency of gilts within HR/HR pairs to contact a novel object may present higher fearfulness. In contrast to those of LR/HR(d) pairs, responses of LR(d)/HR pairs revealed much lower levels of stress, which emphasised the importance of dominance relationships, being independent of coping characteristics of individual gilts. We speculate that in LR/HR pairs, dominant LR gilts were able to suppress aggressiveness of HR subordinates. HR or proactive gilts, however, may become aggressive when

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0168-1591/02/$ – see front matter
PII: S0168-1591(01)00191-5

being dominant. General effects of social status, independent of combination, were also found. Compared to dominants, subordinates showed higher acute cortisol, body temperature and vocal responses to mixing. In the longer term, they showed a higher vocal and parasympathetic responsitivity towards the novel object, and their body growth was impaired. Measures not influenced by combination and social status included those of leucocyte subsets, prolactin, and average heart rates during novelty tests. To conclude, aggressive conditions in newly formed groups, and consequently welfare and production, may largely depend on coping characteristics of individual pigs, but also on dominance relationships. Accordingly, the practical value of the backtest is being discussed.

Keywords: Pig; Coping; Mixing; Stress; Aggression; Physiology; Social status

1. Introduction

Growing–finishing pigs may experience high levels of stress when mixed with unfamiliar conspecifics (Arey and Edwards, 1998), representing a major welfare concern in the pig industry. Mixing usually induces vigorous fighting and much aggression to settle hierarchy positions (McGlone and Curtis, 1985; Meese and Ewbank, 1973), and may be followed by less intensive aggression and social instability in the longer term (Ekkel et al., 1997; Stookey and Gonyou, 1994). Besides injuries, animals may suffer from health problems and growth retardation (Ekkel et al., 1995; Stookey and Gonyou, 1994). It is therefore of great significance to identify the factors that influence aggression and levels of stress after mixing.

Pigs vary individually in a consistent manner in aggressive behaviour, and aggressiveness represents therefore an important personality trait of pigs (Erhard and Mendl, 1997; Ruis et al., 2000). Moreover, aggressive features of individual pigs are linked to the way of coping with (adapting to) challenges in general. Low-aggressive pigs are more reactive to environmental stimuli, i.e. they are behaviourally more inhibited when (socially) challenged. High-aggressive pigs, in contrast, are less directed by these challenges (intrinsically driven), and show a more proactive type of behavioural response (Ruis et al., 2000). Physiologically, low-aggressive pigs predominantly show a hypothalamic activation (Ruis et al., 2000), whereas high-aggressive pigs are more sympathetically dominated (Hessing et al., 1994b). Although the extremes in the population do not represent distinct categories of pigs (no bimodality), the concept of coping 'styles' is supported. Irrespective of the distribution curve, different ways of coping are based on a differential and consistent use of various behavioural and physiological mechanisms to adapt to the environment, and may vary in the same direction consistently over species (Koolhaas et al., 1999).

The present study investigated whether knowledge on coping characteristics of individual growing pigs may be used to improve welfare and production after mixing. Behavioural studies in pigs related to this subject showed an important influence of group composition. Hessing et al. (1994a) suggested that stability of newly formed groups is stimulated by mixing pigs with different coping characteristics, based on large variation in individual aggressiveness. Erhard et al. (1997), on the other hand, proposed that the better

group integration is reached when pigs are mixed into groups of low-aggressive animals only. In the present experiment, gilts with specific coping characteristics were identified at a very young age by means of a backtest. It was previously shown that for pigs with extreme low or high resistance in the backtest, relationships exist between responses in this test and behavioural and physiological mechanisms to cope with environmental changes at a much later age (Ruis et al., 2000). Low resisting pigs in the backtest generally adopt a more reactive way of coping, whereas the high resistant pigs are the more proactive copers. At 7 weeks of age, unfamiliar gilts with similar or different coping characteristics were mixed into pairs (pairwise combinations). By forming pairs it was aimed to circumvent a complicate approach of testing pigs in larger groups, and basic and fundamental biological mechanisms may more easily be observed. Pairs were studied for 3 weeks, by examining a broad range of variables, including behavioural and neuroendocrine patterns, and production traits. Such a multifaceted approach was chosen for a better assessment of welfare status. Because it is known that mixing may differentially affect dominant and subordinate pigs (Tuchscherer et al., 1998), dominance relationships within the pairs were also studied.

2. Materials and methods

The care and use of animals in this experiment conformed with the requirements of the Animal Care and Use Committee of the Institute for Animal Science and Health in Lelystad (ID-Lelystad), The Netherlands. Fig. 1 shows the timing of management and experimental procedures.

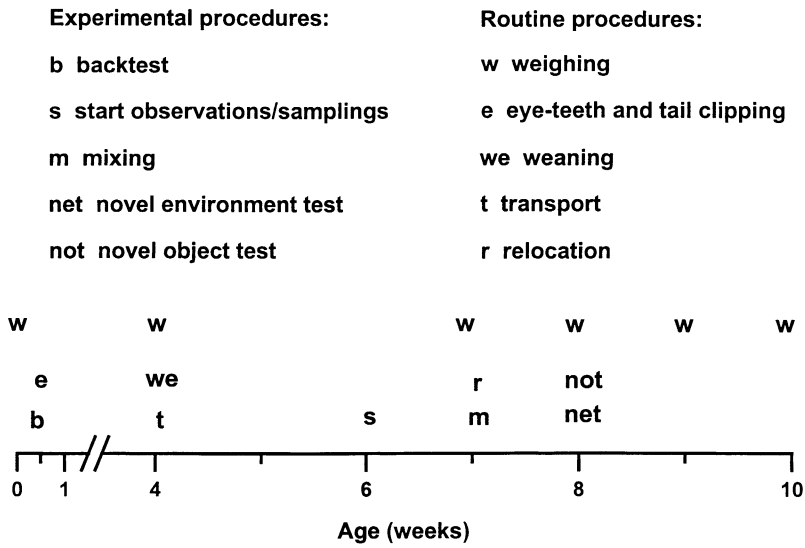

Fig. 1. Timing of management and experimental procedures.

2.1. Selection of gilts with (more) reactive and proactive ways of coping

This study consisted of three identical and consecutive trials, carried out over the period January to July (trials were about 2 months apart). Pigs (Great Yorkshire × (Great Yorkshire × Dutch Landrace)) were bred at the Experimental Farm for Pig Husbandry at Raalte, The Netherlands. They were kept in pens with half-slatted concrete floors of dimensions 3.60 m × 2.20 m until weaning at 4 weeks of age. Within 1 day after birth, piglets were weighed and received an ear tattoo. Between 2 and 4 days of age, identification and selection took place of gilts that were likely to adopt (more) reactive or proactive ways of coping. The identification was based on the level of behavioural resistance (escape behaviour) during manual restraint for 1 min in a backtest. On the basis of our earlier studies (Ruis et al., 2000), low resistant gilts (LR; two or less escape attempts) were considered to represent the more reactive animals, whereas high resistant gilts (HR; five or more escape attempts) were considered to be more proactive. There was an equal proportion of LR and HR gilts found in the tested population (see also Ruis et al., 2001a). Following the backtest, management procedures such as eye-teeth and tail clipping were carried out. Shortly after weaning, all selected gilts in a trial were brought at the same time to an experimental farm in Lelystad, The Netherlands, which is part of the Institute for Animal Science and Health (ID-Lelystad). During transport, and until the start of the actual experiment, litter-mates (3–5 LR and/or HR animals) were kept together and were not mixed with animals of other litters (38 l in total: 14 l in trial 1; 13 l in trial 2; 11 l in trial 3).

2.2. Management and mixing procedures

Upon arrival at the experimental farm in Lelystad, groups of litter-mates were randomly assigned to one of three (adjacent) identical rooms, with constant climatological conditions. Ambient temperature was kept between 19 and 21 °C, and the light–dark cycle was 12 h light/12 h dark, with artificial lights on from 06:00 to 18:00 h (total lux varying from 50 to 100). Pens (2.35 m × 1.70 m) had partly slatted floors and contained a nipple drinker and a food trough. Throughout the experiment, animals were given ad libitum access to water and food (commercial pelleted dry diets). Gilts were allowed to acclimatise to their housing environment and were habituated to human contact and some procedures (e.g. saliva and urine samplings, measurements of body temperature) during 2 weeks.

At 7 weeks of age, mixing procedures were started by forming pairs (pairwise combinations) of unfamiliar gilts. Formation of different pairs was done on the basis of behavioural resistance in the backtest: two LR gilts (LR/LR; $n = 12$); two HR gilts (HR/HR; $n = 12$); and one LR and one HR gilt (LR/HR; $n = 24$), leading to a total of 96 gilts housed in 48 pens, i.e. 32 gilts in 16 pens during each trial. All litters which were brought to Lelystad were used in the experiment, with a maximum of 4 animals per litter. In trial 1, the 16 LR and 16 HR gilts were selected from 10 and 12 l, respectively. For trial 2, these numbers were, 10 and 11 l, respectively, and for trial 3, the numbers were 9 and 8 l, respectively. Gilts in each pair were weight matched and relocated in another room with pens sized 1.80 m × 0.85 m with partly slatted floors. For practical reasons, within each trial, mixings were started on four different days, with four pairs of unfamiliar gilts being formed on 1 day (one LR/LR pair, two LR/HR pairs and one HR/HR pair). To exclude room effects, pairs of each

treatment were as equally as possible divided over the three rooms. Mixings were always started between 08:00 and 10:30 h in the morning. The two pigs in each pen shared one food hopper and were not able to eat simultaneously. Pens were separated by 0.90 m high solid wooden partitions, preventing visual and physical contact between gilts of different pairs. One pair of the LR/HR combination was later excluded from data processing due to lameness of one animal. Gilts which were not allocated to mixing procedures became part of another experiment, not being described here. These animals were socially isolated and their ways to cope with this stressor were investigated (Ruis et al., 2001a).

2.3. Behavioural observations

The ethogram of behaviours which were recorded by scan-sampling is listed in Table 1. Behavioural data were collected by human observers. To minimise variability between observers, two well-trained persons did all the observations. Observations were always done during 30 min periods, in which the behaviour of each animal was scan sampled at 1 min intervals (a total of 31 observations for each 30 min period). On the day of mixing, observation periods started from time 0 of mixing and then at 30 min, 3 and 5 h post-mixing. Additionally, behavioural observations were made on 1, 2, 7, 14 and 21 days post-mixing. On each of these observation days, behaviour was scan sampled at 1 min intervals during a 30 min period (always between 08:00 and 10:00 h). For evaluation, behavioural data were presented as a percentage of all (total) behavioural observations (except for vocalising, which could coincide with other behaviours: vocalising is presented as a percentage of total scan samples).

To study dominance relationships, one animal was characterised as the dominant (winner) and one as the subordinate (loser) in each pair. During the above scan-sampling periods, special (continuous) attention was given to defence–offence behaviours. An animal was

Table 1
Ethogram of scan sampled behavioural measures

Behaviour	Definition
Exploring	Rooting, sniffing, touching the pen
Inactive	
Sleeping	Lying with eyes closed
Lying	Lying with eyes open
Sitting	Standing on fore-legs, hind quarter on the floor
Standing	Standing inactive, may be between activities
Ingestive	
Feeding	Time spent with head in the feeder and chewing feed
Drinking	Use of water nipple to obtain water
Interactive	
Manipulation	Sniffing, chewing, nosing any part of pen-mate
Display-aggression	Attacking (biting, headknocking, pushing) pen-mate
Vocalising	Total vocalisations: grunts and squeals
Walking	Walking or running through the pen

considered dominant when its opponent stopped fighting and started with defensive moves. This defensive behaviour was characterised by turning away from attacks (avoidance) and attempts to flee. At that time the dominant was offensive by biting its opponent in the head region, particularly the ears (McGlone, 1985; Rushen and Pajor, 1987). Accordingly, the subordinate was the pig which first stopped fighting and started with defensive moves.

2.4. Skin lesion score

At 2 and 7 days post-mixing, the body of each individual pig was examined for the frequency of all fresh cuts, scratches and wounds. The scoring included the actual number of fresh lesions, independent of size or length. Additionally, the actual length of each lesion was measured to obtain a cumulative measure of the total length of skin lesions.

2.5. Blood, saliva and urine samplings

Blood samples (about 10 ml of blood) were taken by puncturing the jugular vein while the pig was secured with a snare. The duration of handling and sampling took approximately 1 min per pig. Samples were taken at time-points 2 days before, and 1 and 3 weeks after mixing, always between 09:00 and 11:00 h. They were for the greater portion transferred to prolypropylene 10 ml centrifuge tubes containing EDTA (Vacuette®, Greiner B.V., The Netherlands), and put on ice. Within 30 min, blood was centrifuged and 1.5 ml aliquots of plasma were either frozen at −20 °C (for total cortisol measurements) or at −80 °C (for ACTH and prolactin determinations). Prolactin provides a valuable tool for stress assessments in pigs, and this hormone is released under conditions of fear (Ruis et al., 2001b). Smaller blood portions originating from samplings at the above time-points, were transferred to 5 ml centrifuge tubes containing heparin (Vacuette®, Greiner B.V., The Netherlands) and kept at room temperature. They were used within a few hours for leucocyte counts and differentiation.

Saliva samples, to determine free cortisol concentrations, were taken by simultaneous insertion of two veterinary cotton buds (on sticks) in the back of the mouth. The animals were allowed to chew for 1–2 min until the buds were thoroughly moistened. More detailed information on this widely used non-invasive procedure is given by Ruis et al. (1997). On the day of mixing, samples were taken at 15 min prior to, and 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min post-mixing. Some of these samplings (at 15, 30 and 45 min post-mixing) coincided with behavioural samplings. At these time-points, behavioural observations were interrupted, leading to one or two missing behavioural samples. Further saliva sampling took place at 2 days prior to and 1, 2, 7, 14 and 21 days after mixing, always between 08:00 and 10:00 h. These samplings did not interfere with behavioural samplings. Finally, saliva was gathered in the combined novel environment and novel object test (see further). Saliva was stored at −20 °C until analysis.

Spontaneously voided urine samples were collected in early morning periods (between 06:00 and 08:00 h) at 2 days prior to and 1, 3, 7, 14 and 21 days post-mixing. Urine was collected in buckets (500 ml) fastened on long sticks (2 m), which allowed to catch the urine from a distance, when an animal spontaneously started to urinate. By this method, disturbance of the animals, and faecal contamination in the urine, was kept to a minimum.

Samples were immediately placed at 4 °C and were adjusted to pH 3 within 2 h using 6 M HCl. One ml aliquots were frozen at −20 °C until analysis for noradrenaline, adrenaline and creatinine. In contrast to the other samplings, urine samplings were not 100% successful, but varied between 71 and 95% for each combination.

2.6. Hormone and immunological determinations

2.6.1. Hypothalamic–pituitary–adrenal (HPA) activity

Plasma ACTH was measured according to a radioimmunoassay procedure (RIA; Nichols Institute Diagnostics, San Juan Capistrano, USA), routinely performed in our lab (Ruis et al., 2001b). The intra- and interassay CV were 3.9 and 6.5%, respectively. The detection limit was 1.0 pg/ml. Plasma concentrations of total cortisol were quantified with a time-resolved fluoroimmunoassay (TR-FIA) assay (Erkens et al., 1998; Ruis et al., 2001b). Intra- and interassay CV were 6.5 and 8.1%, respectively. The detection limit was 1.6 ng/ml. Salivary cortisol was measured by using a solid-phase RIA kit (Coat-A-Count Cortisol® TKCO, Diagnostic Products Corporation, Apeldoorn, The Netherlands), modified and validated for pig salivary cortisol (Ruis et al., 1997). Intra- and interassay CV were 9.6 and 11.3%, respectively. The detection limit was 0.13 ng/ml.

2.6.2. Plasma prolactin

Plasma concentrations of prolactin were quantified in one assay, by means of a RIA, as previously described (Erkens et al., 1992). The intra-assay CV was 10.8% and the detection limit was 0.4 ng/ml.

2.6.3. Urinary catecholamines and creatinine

Urinary catecholamines (noradrenaline and adrenaline) were assayed using a high performance liquid chromatography (HPLC) procedure with electrochemical detection (Ruis et al., 2001b) following a two step extraction. One hundred μl urine was extracted using the sephadex extraction described by Westerink and Koolstra (1986). This first clean-up step resulted in a 2.5 ml extract of the urine sample. One ml of this extract was taken and subjected to the liquid extraction (twice) according to the procedure described by Smedes et al. (1982). One hundred μl of the extract obtained after this second clean-up step was injected into the HPLC system. Detection limits were 35 pg/ml for noradrenaline and 55 pg/ml for adrenaline. Creatinine levels were determined using a colorimetric quantitative reaction (Boehringer PAP-method). Color intensity was measured at 510 nm. Intra- and interassay CV were 2 and 5%, respectively. To correct for variable dilutions of urine related to water intake, catecholamine levels were expressed as ratios to creatinine concentrations: noradrenaline/creatinine (NC) and adrenaline/creatinine (AC) ratios.

2.6.4. Leucocyte differentials

As a measure of immune function, leucocyte enumeration and differential leucocyte counts were performed in peripheral blood. Total leucocyte numbers were determined by means of an automated cell counter (Sysmex®, F-800, TOA Medical Electronics, Kobe, Japan). After staining of blood smears with a Hema-Tek slidestainer, a total of 100 cells was counted using a light microscope, and leucocytes were identified as lymphocytes,

monocytes, neutrophils, eosinophils, or basophils. Because only 1–5% of leucocytes were eosinophils, basophils and monocytes, analysis for treatment differences was restricted to % lymphocytes and % neutrophils.

2.7. Body temperature

Body temperature was measured by means of a thermometer inserted in the ear (ThermoScan®, IRT 3020, Braun, Germany). This thermometer, which is developed for human use, measures the infrared heat generated by the eardrum and surrounding tissue. To validate this way of thermometry in pigs, a pilot was done prior to the experiment in which rectal temperatures were compared with ear temperatures. Although rectal temperatures were on average 0.6 °C higher, the correlation with ear temperature was rather high ($R_s = 0.60$; $P = 0.001$; $n = 30$). At a certain time-point, two temperature samples were taken during a period of approximately 10 s (average value used for analysis). For this purpose, a pig was held (mild restraint) by one person, while another person inserted the thermometer in the ear. Samples were taken before mixing: at −7 days and −15 min, and following mixing, at 1, 3 and 5 h, and on days 1, 2, 7, 14 and 21. Samples were always taken between 09:00 and 11:00 h (except for those on the day of mixing), and did not overlap with the collection of behavioural data.

2.8. Behavioural, cortisol and cardiac responses to novelty

At 1 week after mixing, two novelty tests were performed according to procedures described by Ruis et al. (2001b). In a novel environment test (NET), gilts were allowed to enter a novel arena following opening of a startbox. The gilt was then allowed to explore the arena for 10 min. Behaviour was recorded on videotape and analysed afterwards with the software programme EthoVision® (Noldus Information Technology, Wageningen, The Netherlands). Behavioural parameters that were determined were the latency time to leave the startbox (all four legs outside the box), and locomotion (distance travelled in the arena). Numbers of vocalisations in the arena were directly scored during the test. Immediately following the NET, a novel object test (NOT; 5 min period) was performed in which gilts in the arena were confronted with a novel object consisting of a yellow and a grey bucket, tied together. The following behavioural parameters were studied: contact latency, number of contacts, total time of contact, and number of vocalisations. The cortisol response to the overall test (NET and NOT) was determined by sampling saliva 5 min prior to, and 5 and 15 min after testing.

During the NET and NOT, heart rate and time domain heart rate variability (HRV) measures were obtained via the heart rate monitor Vantage® NV (Polar Electro Oy, Kempele, Finland), which allows to determine beat-to-beat (R–R) intervals. The heart rate monitor was fastened to a pig, immediately after being driven into the startbox. The following parameters were quantified, according to Sgoifo et al. (1999): (1) mean heart rate (beats per minute: bpm), as measured from R–R interval durations (RR, ms); (2) overall HRV (sympathetic–parasympathetic autonomic balance), as estimated by (a) the standard deviation of the mean RR (S.D., ms) and (b) the ratio between the standard deviation of the mean RR and the mean RR (S.D./RR, coefficient of variance); and (3) parasympathetic

influence on HRV, as expressed by the root mean square of successive RR differences (r-MSSD, ms). Values were quantified for the first min and total duration of the NET and NOT.

2.9. Growth, feed intake and gain/feed ratio

Pigs were weighed shortly before the start of mixing and weekly thereafter. Feed intake was calculated by keeping a daily record per pen of all feed added to, and the weight of, the feed hoppers. From these data, feed intake, live-weight gain, and gain/feed ratio were calculated per week.

2.10. Statistical analysis

Means of pairs were analysed by analysis of variance with main effects for trial and combination. For analysis of percentages a logistic regression model was employed with a multiplicative overdispersion factor. The analysis was based on maximum quasi-likelihood (McCullagh and Nelder, 1989). Effects for expected fractions p (= percentage/100) were introduced on the logit scale:

$$\text{logit}(p) = \log\left(\frac{p}{1-p}\right) = \text{sum of main effects for trial and combination.}$$

A variance function of the form $c^* p^*(1-p)$ was assumed, where the unknown dispersion factor c was estimated from Pearson's Chi-square statistic. Similarly, counts were analysed as overdispersed Poisson data on a logarithmic scale, i.e. for expected counts m:

$$\log(m) = \text{sum of main effects for trial andcombination,}$$

and a variance function $c^* m$ was assumed. Latency times were analysed on a logarithmic scale as well, again with variances assumed to be proportional to the means. For the number and length of skin lesions following mixing, linear and quadratic covariables for levels prior to mixing were included in the model. Pairwise comparisons were made employing t-tests with a pooled dispersion estimator.

For analysis of the effect of social status for continuous variables, such as hormone concentrations, differences $y_d - y_s$ between the dominant (d) and subordinate (s) animals within pairs were calculated and analysed. For count data, conditional upon the total count within a pair, say $n = n_d + n_s$, the fraction n_d/n for the dominant animal was analysed. Covariables for skin damage prior to mixing, in the comparison between dominant and subordinate gilts, did not significantly add to the model, and were therefore deleted from the model. Model and analysis parallelled the analysis of percentages. For fractions (= percentages/100) a new fraction $z = (p_d - p_s + 1)/2$ was evaluated and analysed. Effects were introduced on the logit scale and equal variances were assumed. Again a maximum quasi-likelihood analysis was performed. For continuous variables no effect of social status corresponds to an expected value of $y_d - y_s$ of 0, for counts and fractions the expected value of n_d/n or z is 0.5, which corresponds to value 0 on the logit scale. Effects of trial and combination represent interactions with social status. All calculations were performed with the statistical programming package Genstat 5 (1993). Differences were considered significant if $P < 0.05$ and data are presented as mean ± S.E.M.

3. Results

Overall, most aggressive interactions were observed in the first hour after mixing, being at much lower levels thereafter. Therefore, we give a separate description of the more acute (day of mixing) and the more long-term (from the day of mixing onwards) effects of mixing. Scores of skin lesions at 2 days after mixing were considered to result predominantly from aggressive interactions shortly after mixing, and were described in the acute effects section. Accordingly, this was also done for concentrations of urinary catecholamines on the day following the day of mixing.

3.1. Acute effects of mixing

3.1.1. Dominance relationships, aggression and skin lesions

During the first day of mixing, dominant and subordinate gilts became identified in all pairs. Because in LR/HR pairs there was an almost equal number of LR and HR dominants, we differentiated between LR/HR pairs according to the social status of individuals: LR(d)/HR (LR gilt dominant; $n = 11$) and LR/HR(d) (HR gilt dominant; $n = 12$). Characteristics of aggressive behaviour (attacks directed to pen-mate) of pairs of gilts on the first day of mixing are shown in Fig. 2. During the first 30 min, aggression was relatively high, with no significant effect of combination. Within-pair differences in aggressive behaviour were

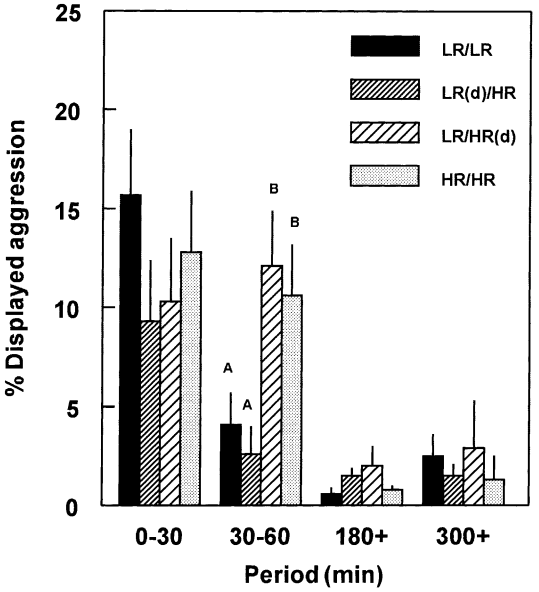

Fig. 2. Mean (±S.E.M.) percentage of displayed aggression of pairs of gilts during 30 min periods on the day of mixing. Low (LR) and high (HR) resistant gilts in the backtest were mixed in the following combinations: LR/LR ($n = 12$), LR(d)/HR ($n = 11$), LR/HR(d) ($n = 12$) and HR/HR ($n = 12$). Combination significantly affected aggressive behaviour in the second 30 min period ($P < 0.05$). Means with different letters differ significantly ($P < 0.05$).

also not observed. In the second 30 min period, however, combination was significantly ($P < 0.05$) related to differences in levels of aggression, and most aggressive acts occurred in LR/HR(d) and HR/HR pairs. In this period, aggressive behaviour became less bidirectional, and most dominance relationships were established (in 10 of 12 LR/LR pairs; in 10 of 11 LR(d)/HR pairs; in 10 of 12 LR/HR(d) pairs; and in 10 of 12 HR/HR pairs). Overall, dominants displayed significantly more aggression ($4.2 \pm 1.78\%$; $P < 0.05$), with a relatively small difference in HR/HR pairs (difference in %: 1.2 ± 0.68; combination × social status interaction: $P < 0.05$). After the first hour of mixing, levels of aggressive behaviour had much declined and between-pair differences were not detected anymore. Similarly, differences in aggressive acts between dominants and subordinates were small and negligible.

At 2 days after mixing, total numbers of fresh skin lesions differed significantly ($P < 0.05$) between the different pairs (Fig. 3). Scores were highest in LR/HR(d) and HR/HR pairs, differing significantly ($P < 0.05$) from LR(d)/HR pairs. Subordinate gilts had on average more skin lesions than dominant gilts ($P < 0.01$), but the magnitude of differences between gilts depended on the type of combination (combination × social status interaction: $P < 0.05$; Fig. 3). Similar results (patterns and differences), according to combination and social status, were obtained for total length of skin lesions (data not shown).

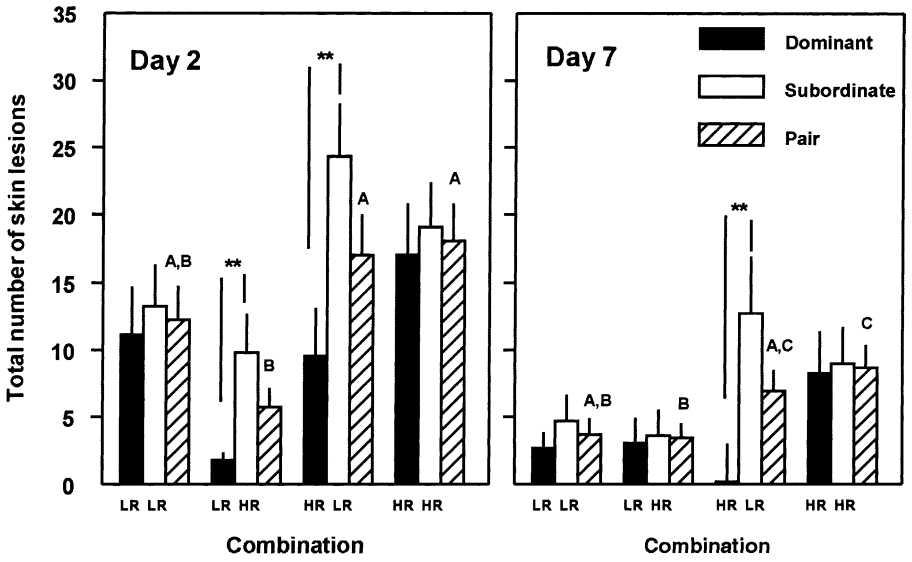

Fig. 3. Total number (mean ± S.E.M.) of fresh skin lesions for pairs of gilts, and for dominant and subordinate gilts. Low (LR) and high (HR) resistant gilts in the backtest were mixed in the following combinations: LR/LR ($n = 12$), LR(d)/HR ($n = 11$), LR/HR(d) ($n = 12$) and HR/HR ($n = 12$). Skin damage was scored at 2 (left panel) and 7 (right panel) days after mixing. At pair-level, combination significantly affected scores of skin lesions at both time-points ($P < 0.05$), and means with different letters differed significantly ($P < 0.05$). Effects of social status on skin damage were significantly related to combination at both time-points (interaction: $P < 0.05$). The asterisks indicate a significant difference between dominant and subordinate gilts ($^{**}P < 0.01$).

3.1.2. Other behaviours

On the day of mixing, profiles of behaviours not involving aggression did not differ between combinations. Overall, percentages of exploratory, inactive and ingestive behaviours, and vocalising, were 18.3 ± 1.15, 60.0 ± 1.44, 9.8 ± 0.85 and 7.4 ± 0.78, respectively. Walking and manipulation behaviours were less prevalent (below 2%). Only vocalising seemed to be affected by dominance status, as demonstrated in the second 30 min period after mixing: subordinates vocalised more often than dominants (overall difference in %: 3.1 ± 1.21; $P < 0.01$), but the contrast was largest in HR/HR pairs (difference in %: 6.11 ± 1.90; combination × social status interaction: $P < 0.05$).

3.1.3. Salivary cortisol and urinary catecholamines

During the first 6 h post-mixing, salivary cortisol responses (maximum change from baseline (baseline: average value of −2 days and −15 min) in ng/ml: LR/LR: 3.36 ± 0.37; LR(d)/HR: 2.78 ± 0.39; LR/HR(d): 3.45 ± 0.37 and HR/HR: 3.54 ± 0.37) and peak (maximum) values of salivary cortisol (in ng/ml: LR/LR: 4.83 ± 0.36; LR(d)/HR: 4.12 ± 0.39; LR/HR(d): 5.08 ± 0.37 and HR/HR: 4.97 ± 0.37) did not differ between pairs. Quantitative total responses (areas under the curves: AUCs) during this period, however, were significantly affected by combination ($P < 0.05$; Fig. 4), with the lowest

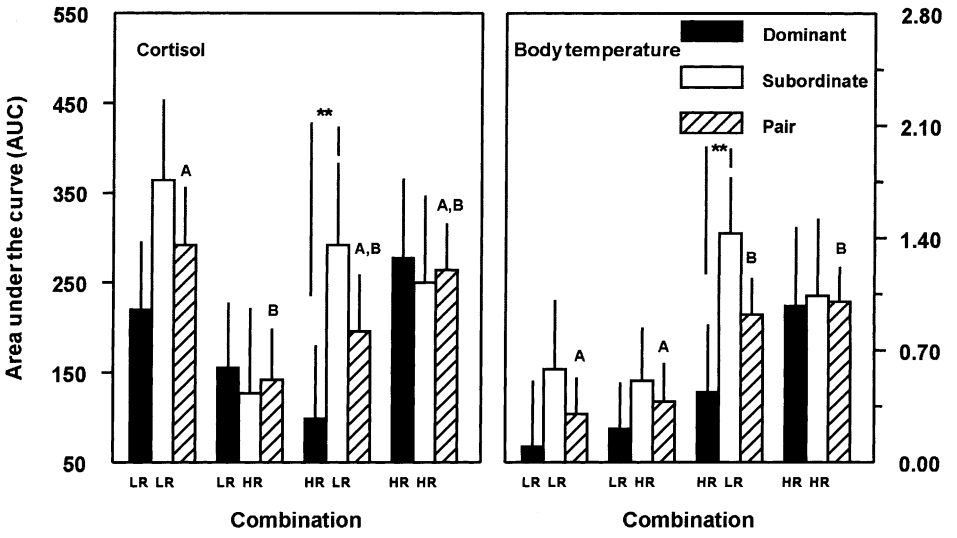

Fig. 4. Total salivary cortisol (left panel) and total body temperature (right panel) responses (mean ± S.E.M.) on the day of mixing, of pairs of gilts, and of dominant and subordinate gilts. Low (LR) and high (HR) resistant gilts in the backtest were mixed in the following combinations: LR/LR ($n = 12$), LR(d)/HR ($n = 11$), LR/HR(d) ($n = 12$) and HR/HR ($n = 12$). Total salivary and body temperature responses of pairs of gilts, expressed in area under the curves (AUC), were significantly influenced by combination ($P < 0.05$). Pair means with different letters differ significantly ($P < 0.05$). Effects of social status on total salivary cortisol (tendency for an interaction: $P = 0.09$) and total body temperature (interaction: $P < 0.05$) responses were depending on combination. The asterisks indicate a significant difference between dominant and subordinate gilts ($^{**}P < 0.01$).

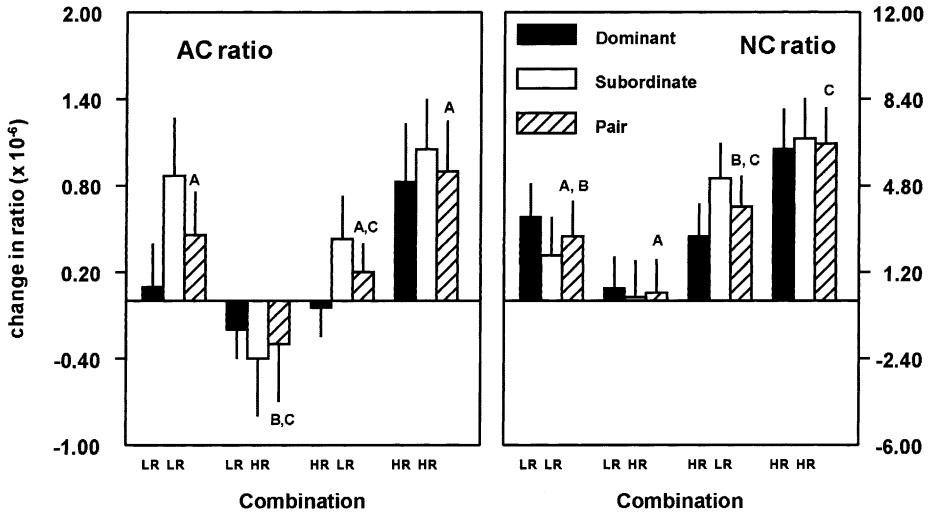

Fig. 5. Mean (±S.E.M.) catecholamine levels in urine after the day of mixing, in pairs of gilts, and in dominant and subordinate gilts within these pairs. Noradrenaline and adrenaline are expressed relative to creatinine concentrations: NC and AC ratios, respectively. Low (LR) and high (HR) resistant gilts in the backtest were mixed in the following combinations: LR/LR (sample size: $n = 11$), LR(d)/HR (sample size: $n = 10$), LR/HR(d) (sample size: $n = 10$) and HR/HR (sample size: $n = 9$). At pair-level, NC and AC ratios were significantly influenced by combination ($P < 0.05$). Pair means with different letters differ significantly ($P < 0.05$). NC and AC ratios did not differ between dominant and subordinate gilts.

responses in LR(d)/HR pairs. In general, total cortisol responses tended to be higher in subordinates than in dominants ($P = 0.1$), and the difference was largest in LR/HR(d) pairs (combination × social status interaction: $P = 0.09$; Fig. 4).

Early morning urine samplings for determinations of catecholamines showed that after the first day of mixing, noradrenaline/creatinine (NC) and adrenaline/creatinine (AC) ratios were most strongly increased (compared to pre-mixing values) in HR/HR pairs and to a much lower extent in LR(d)/HR pairs (significant effect of combination: $P < 0.05$; Fig. 5). The magnitude of increases in NC and AC ratios did not differ between dominant and subordinate gilts.

3.1.4. Body temperature

During the first 5 h post-mixing, body temperature responses (maximum changes from baseline (baseline: average value of −7 days and −15 min)) were not influenced by combination (change in °C: LR/LR: 0.48 ± 0.13; LR(d)/HR: 0.45 ± 0.14; LR/HR(d): 0.68 ± 0.13 and HR/HR: 0.62 ± 0.14). Peak (maximum) body temperatures did also not differ between pairs (in °C: LR/LR: 39.48 ± 0.14; LR(d)/HR: 39.49 ± 0.15; LR/HR(d): 39.55 ± 0.14 and HR/HR: 39.67 ± 0.14). Notwithstanding, there was a significant ($P < 0.05$) effect of combination on the total body temperature response (AUCs) during the above period (Fig. 4), being greatest in LR/HR(d) and HR/HR pairs. Overall, body temperature responses were higher in subordinates (difference in °C: 0.20 ± 0.06; $P < 0.01$) than in dominants, being most pronounced within LR/HR(d) pairs (difference

in °C: 0.30 ± 0.10; combination × social status interaction: $P < 0.05$). A similar pattern was observed for total body temperature responses (Fig. 4).

3.2. Long-term effects of mixing

3.2.1. Behaviour and skin lesions

Beyond the first day of mixing, no relationships were found between combination and levels of aggressive behaviour. Observed incidences of aggressive acts were rather low (overall percentage: $1.48 \pm 0.21\%$), and differences between pigs of different social status were not significant. Indirect assessments of levels of aggression, however, by scores of fresh skin lesions, indicated that gilts in HR/HR pairs were more involved in aggressive interactions in the first week after mixing (significant effect of combination: $P < 0.05$). At the end of this week, these pairs displayed more injuries than LR/LR and LR(d)/HR pairs ($P < 0.05$; Fig. 3). Subordinates showed on average twice as many injuries than dominants (overall effect of social status: $P < 0.05$), but this effect was mainly caused by a large contrast within LR/HR(d) pairs (combination × social status interaction; $P < 0.05$; Fig. 3). Profiles of total length of skin lesions followed those of numbers (data not shown).

Neither combination, nor social rank, did have any effect on other behavioural parameters, at any of the 30 min sampling periods. During the 3-week period, average percentages for pairs of exploration, inactivity, ingestion, manipulation, walking and vocalising were 8.8 ± 0.79, 71.8 ± 2.35, 9.6 ± 0.86, 2.9 ± 0.58, 0.7 ± 0.10 and 5.7 ± 0.61, respectively.

3.2.2. HPA-axis activity, prolactin and immunological characteristics

Beyond the day of mixing, salivary cortisol concentrations were similar between the different pairs, and were not different from pre-mixing values: they ranged between 0.85 ± 0.15 and 1.33 ± 0.25 ng/ml. Differences between dominants and subordinates in salivary cortisol were also not observed. An influence of combination on changes in blood variables was only found for ACTH (Table 2). Plasma ACTH concentrations were relatively high in HR/HR pairs, compared to the other pairs. For all plasma variables, dominant and subordinate gilts did not differentially contribute to the mean pair values (Table 2).

3.2.3. Urinary catecholamines

Analyses of NC and AC ratios showed that from the day of mixing onwards, changes in catecholamine concentrations did not differ according to combination. Relative to pre-mixing values, average NC ratios were 2.7 ± 0.69, 0.29 ± 0.41, -0.92 ± 0.38 and -1.06 ± 0.29, respectively, on day 3, 7, 14 and 21 day post-mixing. These values were -0.08 ± 0.11, -0.01 ± 0.08, 0.08 ± 0.15 and -0.33 ± 0.15, respectively, for changes in AC ratios. At all sampling points, differences in (changes in) NC ratios between dominants and subordinates depended on the type of combination (combination × social status interaction: $P < 0.05$). Differences (dominants minus subordinates) in NC ratios were rather small in LR/LR, LR(d)/HR and LR/HR(d) pairs (between -3.05 ± 2.08 and 1.58 ± 1.66; no differences between these pairs). However, they were between 6.54 ± 2.68 and 9.69 ± 2.67 in HR/HR pairs, differing significantly from the other pairs ($P < 0.05$). Changes in AC ratios were not influenced at all by social status (data not shown).

Table 2
Changes in plasma hormone concentrations and percentages of circulating leucocyte subsets (mean ± S.E.M.), in relation to combination and social status[a,b]

Variable		Combination							
		Day 7				Day 21			
		LR/LR ($n = 12$)	LR(d)/HR ($n = 11$)	LR/HR(d) ($n = 12$)	HR/HR ($n = 12$)	LR/LR ($n = 12$)	LR(d)/HR ($n = 11$)	LR/HR(d) ($n = 12$)	HR/HR ($n = 12$)
ACTH (pg/ml)	Pair[*]	−36.6 ± 15.0 A	−3.3 ± 15.7 A, B	−32.1 ± 15.0 A	13.8 ± 14.9 B	−31.5 ± 13.1	−21.7 ± 13.7	−36.8 ± 13.1	−13.5 ± 13.1
	Dom-sub	19.6 ± 39.8	−18.5 ± 39.9	42.6 ± 39.9	−40.3 ± 38.2	24.6 ± 32.5	9.50 ± 32.4	47.6 ± 32.4	−32.1 ± 31.0
Cortisol (ng/ml)	Pair	0.53 ± 3.2	5.04 ± 3.4	4.07 ± 3.2	1.93 ± 3.3	−1.22 ± 3.3	5.75 ± 3.2	3.52 ± 3.0	3.22 ± 2.9
	Dom-sub	7.50 ± 5.8	−4.29 ± 5.6	−1.0 ± 5.8	6.96 ± 5.6	7.76 ± 5.3	−2.37 ± 5.4	6.22 ± 5.4	3.96 ± 5.1
Prolactin (ng/ml)	Pair	0.04 ± 0.2	−0.19 ± 0.2	0.02 ± 0.2	0.24 ± 0.2	0.16 ± 0.1	−0.14 ± 0.2	0.46 ± 0.2	0.23 ± 0.2
	Dom-sub	−0.08 ± 0.4	−0.31 ± 0.4	0.45 ± 0.4	0.05 ± 0.3	0.33 ± 0.4	−0.33 ± 0.4	0.47 ± 0.4	−0.09 ± 0.3
Lymphocytes (%)	Pair	−4.15 ± 2.7	−0.98 ± 2.9	−0.98 ± 2.7	−4.48 ± 2.7	0.08 ± 3.0	−3.88 ± 3.1	−2.08 ± 3.0	1.63 ± 3.0
	Dom-sub	−3.11 ± 4.8	−3.88 ± 5.0	−2.11 ± 4.8	−1.15 ± 5.0	−1.08 ± 5.7	−1.17 ± 5.7	−4.87 ± 5.5	−5.26 ± 5.6
Neutrophils (%)	Pair	4.60 ± 2.7	1.59 ± 2.9	0.18 ± 2.7	4.89 ± 2.8	−1.21 ± 3.0	3.41 ± 3.1	3.46 ± 3.0	−1.12 ± 2.9
	Dom-sub	2.06 ± 4.9	3.39 ± 5.1	2.06 ± 4.9	1.39 ± 5.1	−0.20 ± 5.7	0.80 ± 5.7	1.30 ± 5.5	3.98 ± 5.7

[a] Values at 7 and 21 days were compared with those at 2 days prior to mixing. Pair: pairs (combinations) of gilts. Dom-sub: dominants minus subordinates (difference).
[b] Means with different letters (A, B) within the same row differ significantly ($P < 0.05$).
[*] Significant effect of combination at 7 days after mixing ($P < 0.05$).

3.2.4. Body temperature

After the day of mixing, body temperatures of the four combinations did not differ significantly, and fluctuated between 38.73 and 39.06 °C. Also, social rank did not have any significant effect on this variable (largest overall difference in °C: 0.06 ± 0.05).

3.2.5. Behavioural, cortisol and cardiac responses to novelty

Of the behavioural variables studied in both novelty tests, only total time of contact in the NOT was significantly affected by combination (Table 3). Gilts of HR/HR pairs spent relatively short time in contacting the novel object. Compared to dominants, subordinate gilts generally vocalised more: 126 ± 6.5 versus 99 ± 5.9 times (overall effect of social status: $P < 0.05$; Table 3). Neither combination nor social status did influence salivary cortisol responses to the combined test (NET + NOT; Table 3).

Heart rate and time domain parameters of heart rate variability in the NET were influenced neither by combination, nor by social status (Table 4). In the NOT, however, the S.D. of RR intervals was significantly ($P < 0.05$) elevated in HR/HR pairs compared to LR/LR and LR/HR(d) pairs (significant effect of combination: $P < 0.05$). The other

Table 3
Effects of combination and social status on behavioural and cortisol responses (mean ± S.E.M.) to novelty tests at 1 week post-mixing[a,b]

Variable		Combination			
		LR/LR ($n = 12$)	LR(d)/HR ($n = 11$)	LR/HR(d) ($n = 12$)	HR/HR ($n = 12$)
NET					
Latency time (s)	Pair	52.0 ± 13.7	42.0 ± 13.1	18.1 ± 8.1	60.5 ± 14.8
	Dom-sub	−13.5 ± 18.7	−2.5 ± 18.6	−2.1 ± 18.6	−8.3 ± 18.7
Locomotion (m)	Pair	102 ± 7.7	97 ± 8.0	115 ± 8.2	97 ± 7.5
	Dom-sub	8 ± 13	−1 ± 14	14 ± 12	12 ± 13
Vocalisations (number)	Pair	138 ± 22	120 ± 22	158 ± 24	139 ± 22
	Dom-sub	−6 ± 39	28 ± 38	−45 ± 39	−48 ± 39
NOT					
Contact latency (s)	Pair	42.2 ± 12.1	41.6 ± 12.5	29.9 ± 10.2	39.5 ± 11.7
	Dom-sub	−16.4 ± 19.5	−3.8 ± 19.3	−3.4 ± 19.8	−7.0 ± 19.5
Number of contacts	Pair	10.0 ± 1.0	9.8 ± 1.1	10.9 ± 1.0	8.9 ± 0.9
	Dom-sub	1.0 ± 2.0	−1.6 ± 2.0	−1.6 ± 2.0	1.2 ± 2.0
Contact time (s)	Pair*	43.1 ± 6.3 A	47.1 ± 6.9 A	39.9 ± 6.1 AB	28.1 ± 5.1 B
	Dom-sub	6.6 ± 11.9	−4.0 ± 12.0	−14.2 ± 12.0	4.2 ± 11.8
Vocalisations (number)	Pair	109 ± 11	97 ± 10	123 ± 12	118 ± 11
	Dom-sub**	−16 ± 18.1	−12 ± 18.2	−50 ± 18.0	−28 ± 18.1
NET + NOT					
Cortisol response (ng/ml)	Pair	2.74 ± 0.3	2.33 ± 0.3	2.53 ± 0.3	2.39 ± 0.2
	Dom-sub	0.19 ± 0.6	−0.44 ± 0.6	0.12 ± 0.5	0.38 ± 0.5

[a] NET: novel environment test. NOT: novel object test. Pair: pairs (combinations) of gilts. Dom-sub: dominants minus subordinates (difference).

[b] Means with different letters (A, B) within the same row differ significantly ($P < 0.05$).

* Significant effect of combination ($P < 0.05$).

** Significant overall difference between dominant and subordinate gilts ($P < 0.05$).

Table 4
Effects of combination and social status on heart rate and heart rate variability (mean ± S.E.M.) during novelty tests at 1 week post-mixing[a,b]

Variable	Test		Combination			
			LR/LR ($n = 12$)	LR(d)/HR ($n = 11$)	LR/HR(d) ($n = 12$)	HR/HR ($n = 12$)
HR (bpm)	NET	Pair	165 ± 3.1 (173 ± 4.8)	161 ± 3.2 (164 ± 5.0)	163 ± 3.1 (172 ± 4.8)	163 ± 3.2 (173 ± 5.0)
		Dom-sub	6.2 ± 9.7 (8.9 ± 10.7)	−0.5 ± 9.5 (3.6 ± 10.5)	4.8 ± 11.1 (−4.1 ± 12.1)	2.5 ± 9.6 (−2.6 ± 10.6)
	NOT	Pair	160 ± 3.0 (170 ± 3.7)	157 ± 3.2 (170 ± 3.9)	156 ± 3.0 (167 ± 3.7)	157 ± 3.2 (164 ± 3.9)
		Dom-sub	4.2 ± 9.1 (7.5 ± 9.0)	−5.9 ± 9.5 (−5.6 ± 8.8)	−2.2 ± 10.3 (−0.6 ± 9.3)	3.1 ± 8.9 (−1.1 ± 9.5)
S.D. (ms)	NET	Pair	43.1 ± 3.0 (27.6 ± 2.3)	40.1 ± 3.2 (28.7 ± 2.4)	43.8 ± 3.0 (27.7 ± 2.3)	41.4 ± 3.2 (27.6 ± 2.4)
		Dom-sub	4.9 ± 7.5 (−3.3 ± 7.1)	−2.6 ± 7.5 (−3.4 ± 7.0)	−3.1 ± 8.7 (4.9 ± 7.9)	−7.4 ± 7.6 (2.4 ± 7.0)
	NOT	Pair*	42.8 ± 1.9 A (41.4 ± 3.0) a	45.9 ± 2.0 AB (45.7 ± 3.0) ab	43.2 ± 2.0 A (42.3 ± 2.0) a	48.8 ± 1.9 B (49.1 ± 2.9) b
		Dom-sub	6.2 ± 7.5 (−5.3 ± 9.1)	5.3 ± 7.8 (−1.9 ± 8.9)	−3.0 ± 8.5 (−5.4 ± 9.5)	−2.7 ± 7.4 (1.9 ± 9.6)
S.D./RR	NET	Pair	0.11 ± 0.01 (0.08 ± 0.01)	0.10 ± 0.01 (0.08 ± 0.01)	0.12 ± 0.01 (0.07 ± 0.01)	0.11 ± 0.01 (0.08 ± 0.01)
		Dom-sub	0.03 ± 0.02 (−0.01 ± 0.01)	0.00 ± 0.01 (−0.01 ± 0.01)	−0.02 ± 0.01 (0.02 ± 0.01)	−0.02 ± 0.02 (0.01 ± 0.01)
	NOT	Pair	0.11 ± 0.02 (0.11 ± 0.01)	0.12 ± 0.02 (0.12 ± 0.01)	0.11 ± 0.02 (0.11 ± 0.01)	0.13 ± 0.02 (0.13 ± 0.08)
		Dom-sub	0.02 ± 0.08 (−0.01 ± 0.02)	0.02 ± 0.08 (0.01 ± 0.02)	−0.01 ± 0.09 (−0.01 ± 0.03)	−0.01 ± 0.07 (0.00 ± 0.02)
r-MSSD (ms)	NET	Pair	0.6 ± 0.05 (1.7 ± 0.17)	0.5 ± 0.06 (1.4 ± 0.18)	0.5 ± 0.06 (1.2 ± 0.17)	0.5 ± 0.06 (1.3 ± 0.18)
		Dom-sub	−0.3 ± 0.19 (−0.8 ± 0.56)	0.1 ± 0.17 (−0.5 ± 0.55)	−0.3 ± 0.20 (0.4 ± 0.54)	−0.2 ± 0.17 (−0.6 ± 0.56)
	NOT	Pair	0.9 ± 0.11 (2.2 ± 0.36)	0.8 ± 0.12 (2.0 ± 0.38)	0.8 ± 0.10 (1.9 ± 0.35)	0.9 ± 0.12 (2.6 ± 0.38)
		Dom-sub**	−0.3 ± 0.30 (−1.2 ± 0.86)	0.5 ± 0.33 (0.4 ± 0.84)	−0.3 ± 0.36 (−0.8 ± 0.99)	−0.1 ± 0.30 (−0.8 ± 0.90)

[a] Average heart rate (HR), standard deviation (S.D.), coefficient of variance (S.D./RR) and root mean square of successive RR differences (r-MSSD) during the NET (novel environment test; total 10 min period (first minute between parentheses)) and NOT (novel object test; total 5 min period (first minute between parentheses)). Pair: pairs (combinations) of gilts. Dom-sub: dominants minus subordinates (difference).

[b] Means with different letters (A, B and a, b) within the same row differ significantly ($P < 0.05$).

* Significant effect of combination ($P < 0.05$).

** Significant overall difference between dominant and subordinate gilts (only in the first minute; $P < 0.05$).

Table 5
Effects of combination on production characteristics of pairs of gilts (mean ± S.E.M.) during 3 weeks post-mixing[a]

Item	Combination			
	LR/LR ($n = 12$)	LR(d)/HR ($n = 11$)	LR/HR(d) ($n = 12$)	HR/HR ($n = 12$)
Feed intake (kg)				
Week 1	16.67 ± 0.98	16.90 ± 1.03	16.49 ± 0.98	16.97 ± 0.98
Week 2	19.16 ± 0.99	19.49 ± 1.03	19.27 ± 0.99	19.15 ± 0.99
Week 3	21.78 ± 1.15	22.51 ± 1.20	22.19 ± 1.15	21.94 ± 1.15
Total period	57.61 ± 2.92	59.10 ± 3.11	57.95 ± 3.06	58.06 ± 2.92
Weight gain (kg)				
Week 1	14.48 ± 0.85	14.59 ± 0.89	13.70 ± 0.85	14.05 ± 0.85
Week 2	12.60 ± 0.79	12.30 ± 0.82	12.54 ± 0.79	12.22 ± 0.79
Week 3	13.77 ± 0.85	15.08 ± 0.89	13.54 ± 0.85	13.35 ± 0.85
Total period	40.85 ± 1.82	41.97 ± 1.91	39.78 ± 1.82	39.62 ± 1.82
Gain/feed (kg/kg)				
Week 1*	0.87 ± 0.02 A	0.87 ± 0.02 A	0.82 ± 0.02 B	0.83 ± 0.02 B
Week 2	0.65 ± 0.08	0.63 ± 0.08	0.65 ± 0.07	0.64 ± 0.08
Week 3	0.62 ± 0.09	0.66 ± 0.10	0.60 ± 0.09	0.60 ± 0.09
Total period	0.71 ± 0.02	0.70 ± 0.03	0.69 ± 0.03	0.68 ± 0.02

[a] Means with different letters (A, B) within the same row differ significantly ($P < 0.05$).
* Significant effect for combination ($P < 0.05$).

measure of RR variability, i.e. the coefficient of variance (S.D./RR), was not significantly influenced by combination, and this also applied for average heart rate and parasympathetic activity (r-MSSD). Significant variation in the latter parameter, however, was observed when dominant and subordinate gilts were compared in the NOT. Overall, the r-MSSD was 0.64 ± 0.37 ms higher ($P < 0.05$) in lower ranking gilts, but only during the first minute of the NOT. Social status did not significantly affect average heart rate and measures of total variance in heart rate.

3.2.6. Production parameters

Analyses of production parameters of pairs of gilts showed a significant ($P < 0.05$) effect of combination on gain/feed ratio in the first week post-mixing, but not in the following weeks, and averaged over the whole 3-week period (Table 5). Body weight gain and feed intake of pairs were not influenced by combination. Regarding characteristics of individual gilts, a highly significant ($P < 0.01$) overall effect of social status on body growth was found: during the 3 weeks following mixing, body weight gain was higher in the dominant gilts (difference in kg: 1.47 ± 0.47).

4. Discussion

4.1. Aggression

Immediately following mixing, the likelihood of fighting was not influenced by individual coping characteristics of gilts, as shown by similar levels of aggressive acts in pairs of different

composition. However, levels of aggressive behaviour subsided more quickly in LR/LR and LR(d)/HR pairs, compared to LR/HR(d) and HR/HR pairs. The latter two pairs also showed higher acute (total) rises in body temperature, which may be indicative for higher stress levels (De Jong et al., 1998) and/or higher physical activities, due to the more severe aggressive conditions in these pairs. After the first hour of mixing, however, aggression was strongly diminished in all pairs, which concurs with several other observations of mixed pigs (Friend et al., 1983; Rushen, 1987). Although not reflected in body temperatures, other measures indicated that pigs in LR/HR(d) and HR/HR pairs remain in a higher stressful state beyond the day of mixing. In the first week post-mixing (but not thereafter), feed efficiency as reflected by gain/feed ratio, was lowest in LR/HR(d) and HR/HR pairs, compared to the other pairs. It can be argued that the LR/HR(d) and HR/HR pairs met higher energy demands, which were, however, not significantly interfering with body growth. Because behavioural activities did not differ between pairs, it is more likely that stress was involved in this effect on feed efficiencies (Stookey and Gonyou, 1994). Animals in LR/HR(d) and HR/HR pairs, especially in the latter ones, also suffered from more skin damage. Because this was also observed at 7 days post-mixing, incidences of aggressive acts, previously shown to correlate with lesion scores (Barnett et al., 1992), seemed to persist for a prolonged time, indicative for unstable social relationships (social instability).

4.2. Stress and fear

Most obvious negative effects of mixing were observed when two HR gilts were mixed. Besides the above mentioned indications for relatively poor welfare, other variables additionally emphasise highest levels of stress and fearfulness in these pairs. Both urinary noradrenaline/creatinine and adrenaline/creatinine ratios are validated measures of sympathetic nervous system (SNS) activity (Hay et al., 2000) and were typically higher in HR/HR pairs 1 day after mixing, indicative of the greatest stress responses to the mixing procedure (Otten et al., 1999; Ruis et al., 2001b). Specifically, adrenaline production is believed to be associated with increased mental stress, whereas elevations in noradrenaline also may represent a higher physical activity (Otten et al., 1999). Beyond the day of mixing, no further differences in levels of urinary catecholamines between pairs were detected, suggesting that more prolonged (chronic) stress differences did not exist. However, relatively high baseline plasma ACTH concentrations in HR/HR pairs were found up to 1 week post-mixing. Increments in HPA activity are often associated with increased stress levels, also known to occur when pigs are socially challenged (Arnone and Dantzer, 1980; Otten et al., 1999; Ruis et al., 2001b). The other feature of HPA activity, i.e. adrenocortical activity (total and free cortisol), did not vary. This suggests a physiological adaptation at the level of the adrenal glands, possibly due to a desensitisation of the adrenals to ACTH (Ladewig and Smidt, 1989). Percentages of leucocyte subsets and prolactin levels, known to be affected under conditions of social stress (Ruis et al., 2001b), did not differ between the pairs. In contrast, differences existed in stress-reactivities to the specific novelty stress of the NOT, 1 week post-mixing. Gilts of HR/HR pairs typically vocalised more during the test and spent less time in contacting the novel object than the other gilts. Although vocalising cannot unequivocally be interpreted as measures of fear (Ruis et al., 2001b), lower inclinations to contact a novel object may indicate a higher level

of fearfulness (cf. Hopster et al., 1999). Behavioural variables in the novel environment test (NET) did not at all discriminate between pairs, which is in accordance with our previous thoughts that fear is not the only factor to determine responses to this test (Ruis et al., 2001b). For instance, locomotory behaviour may be a reflection of both fear and exploratory motivations. In line with this, in a novelty test comparable to our NET, Andersen et al. (2000b) were not able to demonstrate anxiolytic-like effects of benzodiazepines on pig behaviour. Finally, some differences between pairs in the autonomic regulation of heart rate emerged in our study. Overall heart rate variability, quantified by the standard deviation of the mean RR, was significantly elevated in HR/HR pairs in the NOT (not in the NET), as compared to the other pairs. However, we cannot unambiguously interpret these findings in terms of balance between sympathetic and parasympathetic activities (sympathovagal balance). The r-MSSDs, specifically quantifying parasympathetic activities (Sgoifo et al., 1999), and average heart rates did not differ between pairs. Accordingly, one might argue that the sympathetic tone is reduced, but there is no evidence to conclude this, particularly because direct assessments of sympathetic activity, i.e. of plasma catecholamine concentrations, were not obtained during the test. We therefore conclude that our measures of cardiac activities did not provide additional information on differences in emotional distress between pairs. This also applies for the cortisol responses to the combined NET and NOT, which were not found to differ between the pairs.

Although some of the differences may not only be related to state variables such as stress and fear, but may also reflect aspects of personality or coping (Koolhaas et al., 1999), welfare seemed to be most seriously compromised in HR/HR pairs. When extrapolated to larger groups, one may argue that it should be avoided to form groups consisting of many HR pigs. Hessing et al. (1994a) suggested that group integration is slowed down when pigs with similar coping characteristics, and hence similar aggressiveness are mixed. We were only able to demonstrate this for paired HR pigs. Erhard et al. (1997) suggested that it is preferable to mix only low-aggressive animals. We could only partially substantiate this for LR/LR pairs, which were generally ''intermediate''. In contrast to bringing together many low-aggressive pigs, mixing pigs with different aggressive features is of much more practical relevance for pig husbandry. In the next paragraph we describe that it may very well be possible to create socially stable pairs of LR and HR gilts, also indicated by Hessing et al. (1994a) for larger groups, but this depends explicitly on the outcome of dominance relationships.

4.3. Causal explanations for aggression: relationships with proactivity and social status

In LR/HR pairs, levels of aggressive interactions and consequently social stability strongly depend on the social status of LR and HR gilts. The most stable social relationship existed between a dominant LR and a subordinate HR gilt. In this situation, aggression decreased rather quickly and was predominantly observed in the first 30 min after mixing. Moreover, acute HPA-axis and SNS responses to mixing were lowest compared to the other pairs. In contrast, when a HR gilt became dominant, a less favourable social situation existed (see Section 4.2). These findings essentially address the question how coping characteristics and social status may influence aggression and stability of social relationships when unacquainted pigs are mixed. One important difference between proactive and

reactive animals is the difference in the individual's propensity to start offensive encounters. As shown in rodents, aggressive or proactive animals generally take the initiative to attack others, whereas low-aggressive or reactive animals only respond with aggression when absolutely necessary (Koolhaas et al., 1999). Individual variation in proneness to behave aggressively is also observed in pigs (Erhard et al., 1997). We therefore hypothesise that in LR(d)/HR pairs, characterised by a fast decline in levels of aggression, this behaviour was limited to initial fighting to settle disputes for social hierarchy positions. This implies that although LR gilts were reluctant to start aggression they nevertheless were able to fight back properly when being attacked. Indeed, in the present study, both types were equally able to acquire the dominant position, which indicated that coping characteristics of individual pigs were not predictive of social dominance status. When a LR gilt became dominant, it is basically low intention to use physical aggression may have suppressed aggressive intentions in the subordinate HR gilts, especially when the latter animals assessed their chance of winning as being low (Rushen and Pajor, 1987). In LR/HR(d) pairs, on the other hand, inhibitions in aggressive acts that should rise from dominance relationships, were not manifested. Dominant HR gilts expressed much aggression, and subordinate LR gilts served as targets, even beyond settlements of social hierarchies. This was unequivocally demonstrated by the large contrast in fresh skin lesions at 2 days, and especially at 7 days post-mixing. Thus, aggressive behaviour by a HR gilt seemed to be related to its dominant position. The persistence of aggressive behaviour, even in the absence of any further direct provocation to behave aggressively, may imply that in this situation HR gilts are more driven by impulsive and intrinsic aggression. This is in accordance with another feature of proactivity, i.e. a behaviour which is rather independent from actual environmental stimuli. Also, proactive animals have generally a high demand to control the environment, being manifested by an aggressive mode of behaviour of top ranking HR gilts.

Our findings may thus indicate that from a welfare perspective, the situation is beneficial when reactive animals prevail among the top positions in social rank. Although it may be difficult to control the outcome of social fighting, variations in body weight may have some predictive potential. Although it is still common practice to match pigs for weight upon mixing, less aggression is observed and dominance relationships are more quickly established between pigs of different weight, with the larger animals having an advantage to become dominant (Andersen et al., 2000a; Rushen, 1987). When combined with our findings, this advantage for pig welfare by providing variation in weight may especially arise when the larger pigs have more reactive features and the smaller ones are more proactive, thereby increasing the chance that (a) reactive pig(s) become(s) highest in social rank. On the other hand, when resources are limited, the accessibility of smaller and subordinate pigs to food might be limited. This might further increase the differences in weights between smaller and larger animals, through a differentiation in growth rates. This will not be appreciated by farmers.

Finally, the survey of the onset and persistence of aggression may be extended by the observations on gilts within HR/HR pairs. Contrary to LR/HR(d) pairs, the relatively high level of aggressive acts was bidirectional and was equally displayed by the two animals. We argue that the hostility and aggression of dominant HR gilts triggers aggressive inclinations in HR subordinates. In this case, the subordinate pigs were likely to retaliate

against the dominant pigs. Evidence of aversively stimulated aggression was given in humans as well as in animals (Berkowitz, 1989), including the pig (Arnone and Dantzer, 1980), and may be provoked in situations such as ''irritation'', ''annoyance'', ''frustration'' and physical pain. Although this phenomenon is widespread (Berkowitz, 1989), we believe that in accordance with the above description of proactive individuals, particularly HR gilts are prone to house these negative emotions, leading to aggressive acts. As a consequence of retaliation of HR subordinates, also dominant HR pigs may be negatively affected, which may explain their relatively high SNS activity, reflected in noradrenaline (but not adrenaline) levels beyond the first day of mixing.

4.4. Dominance relationships

Despite the differences in overt aggression and social stability between the different pairs, general effects of social status on several measures were observed, with the impact of mixing being most pronounced in subordinate gilts. Although SNS activities did not differ between dominants and subordinates on the day of mixing, which was also shown by Fernandez et al. (1994) following social encounters, other measures elucidated higher stress responses in subordinates. This was presently shown by relatively high cortisol, body temperature and vocal responses in the subordinate animals. This may be related to social defeat, known to result in high levels of social stress (Ruis et al., 2001b). It seems likely that the adversity of social defeat is primarily related to the unpredictability and uncontrollability of the stress (Tuchscherer et al., 1998). Social rank also caused differences in the longer term. When challenged in the NOT, indications were obtained for a higher level of distress in subordinates compared to dominants. The frequency of vocalising was higher in the subordinates and their cardiac activity was characterised by an initially higher r-MSSD, and thus higher parasympathetic activity. Again, we do not know the contribution of the sympathetic branch of the autonomic nervous system. However, because social rank did not affect average heart rates, we argue for an increased sympathetic activation in subordinates, being parasympathetically antagonised. Such a maintenance in sympathovagal balance under increasing stress levels was recently also found in situations of social stress in pigs by De Jong et al. (2000). Despite variations in aggressive interactions between pairs, subordinates also had on average twice as much skin damage than dominants in the first week after mixing. However, this may particularly result from the high skin damage in LR subordinates within LR/HR(d) pairs. Finally, social rank had a pronounced effect on body growth during the 3-week period post-mixing, as characterised by a depressed body growth in subordinate gilts compared to dominants. Although dominance status might have had a direct influence on individual feed intake and accordingly body growth (Morgan et al., 1999), we hypothesise that stress played a predominant role. We recently showed that body growth of defeated gilts was not impaired when gilts lost a social encounter and were subsequently removed from the dominant (Ruis et al., 2001b). We therefore argue that for subordinates, the continuing presence of dominants (social cohabitation) following social defeat, leads to a persisting higher state of (psychosocial) stress. It may not be the bodily exposures as such that determine levels of stress, but also threats (Stookey and Gonyou, 1994) and visual contact (Stefanski, 1998). However, concentrations of hormones measured in the current experiment, including cortisol, did not depend on dominance status

beyond the day of mixing, and may consequently not have accounted for the differences in body growth. To conclude, differences in social status are not always found to lead to differences in levels of stress (Sachser et al., 1998), which may be related to the degree of competition for resources and possibilities to hide (McGlone and Curtis, 1985; Rushen, 1987). Increasing competition for space and food, together with a lack of hiding places, triggers more interactions between animals, and is likely to increase the vulnerability of subordinate gilts to the adverse effects of being defeated. With increasing group-size, it may be expected that avoidance between certain group-members is better possible. However, one may wonder how big this advantage is under current intensive housing conditions. In the present study, the possibility to avoid pair-members was low and competition was high. Only one feeder space was available in each pen, and the space per pig was limited (0.75 m^2 per pig). It is therefore expected that the social conditions in the present experiment with pair-housing were far from optimal, leading to the observed higher impact of mixing on subordinate gilts. However, in larger groups of mixed pigs, it was also seen that defeated animals show a depressed growth (Albinsson and Andersson, 1980), and have a reduced immune function (Tuchscherer et al., 1998). This suggests that current intensive housing and management routines represent a threat to welfare and production of whole groups, not in the last place through the adverse effects on subordinates.

5. Conclusions

Knowledge on specific individual coping characteristics, obtained from behavioural resistance in a backtest, may be used to improve welfare and production of pigs after mixing. Combining pigs with different coping characteristics could lead to low levels of aggression and stress, but this strongly depended on the outcome of social fighting. Although the outcome of social fighting may be guided by provision of variation in weight upon mixing, this does not seem to be realistic at farm level. This means that the optimal social situation hardly can be established. However, the opposite, i.e. the worst situation, may easily be prevented. Bringing two high resistant or proactive gilts together was most detrimental for welfare, and proactive animals are particularly aggressive when being dominant or frustrated. This implies that, at farm level, mixing of many potentially aggressive pigs in one group should be avoided, especially under intensive housing conditions. For this purpose, the backtest is a valuable tool to be implemented by pig farmers. The backtest has a practical value for the formation of groups of growing–finishing pigs and sows, in which the numbers of animals allow the establishment of strong social hierarchies. However, more research is needed to determine whether pigs adopt different agonistic strategies when the number of animals in a group become too large to form social hierarchies. When social relationships become weak in very large groups, the backtest may have no practical value.

Acknowledgements

The authors express their gratitude to the personnel of the Experimental Farm for Pig Husbandry at Raalte, and of our experimental farm in Lelystad, especially to Henk

Altena and Gerrit Vink. We further thank Goffe de Boer, Jelle Duyf and Onno Nieuwenhuis for their practical assistance, and Arie Hoogendoorn, Joop Testerink, Michel Breuer, Jo Erkens and Gerdien Korte-Bouws for performing the hormone and immunological analyses.

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