INDIVIDUAL BEHAVIOURAL CHARACTERISTICS IN PIGS – INFLUENCES OF GROUP COMPOSITION BUT NO DIFFERENCES IN CORTISOL RESPONSES

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**ABSTRACT**

To determine the effect of group composition on backtest responses, and to determine the predictive value of the backtest for the physiological stress response to weaning and mixing, 814 pigs were backtested at 3, 10 and 17 days of age. 29% of all pigs were cross-fostered at 3 days according to backtest (Bt) responses and groups were formed of animals with high responses (HR) only, low responses (LR) only, or mixed groups of animals with high, intermediate and low responses (MISC). Original litters (OR, no cross-fostering) were used as controls. Cortisol responses were measured in saliva after weaning at 4 weeks of age and after moving and mixing at 9 weeks of age.

In HR groups, mean Bt responses decreased after cross-fostering while in LR groups, mean Bt scores increased. In both groups, Bt responses of individual animals before and after cross-fostering were not correlated. In MISC and OR groups, all Bt scores were correlated. Weaning and mixing caused a significant rise in cortisol in all animals, while moving or weighing did not. No relations were found between Bt scores and cortisol levels.

We conclude that backtest behaviour can change according to the social environment between 3 and 10 days. This could be intentional, to form a varied group, or it might be caused by a change in HPA function due to social stress. At an older age, this ability is lost and common farm practises such as regrouping, weaning and mixing of piglets at ages >10 days might have a negative effect on the piglets.

**INTRODUCTION**

Variations in behaviour and physiology are considered important biological individual characteristics to cope with relevant environmental changes that threaten homeostasis (Hessing et al., 1994).

It may be beneficial for the individual animal to behave differently depending on its age, sex or size or on its environment. Behavioural strategies may be depending on what other individuals are doing, or it may benefit animals to possess varying signals to indicate aspects of their identity. Variation may also occur because wide ranges of behaviour are tolerated by the selection process (i.e. evolutionary or an artificial selection), and the exact form of the behaviour is unimportant. Finally, in a varying and unpredictable environment, animals can only guess what behaviour may best be adopted, and a wide range of behaviour will remain in the population (Slater, 1981).

In pigs, individual variation in stress coping behaviour can be measured with the backtest (Hessing et al., 1993; van Erp-van der Kooij et al., 2001). In this test, a piglet is put on its back and restrained in this position for one minute, while the number of escape attempts is recorded (the ‘backtest score’). The extremes of the pig population can be defined as active (also referred to as ‘pro-active’) animals, with high backtest scores, and reactive (also referred to as ‘passive’) animals, with low backtest scores (for a discussion on terms, see Koolhaas et al., 1999)). We prefer the terms active and reactive over active and passive, because the latter might be interpreted negatively (‘passive’ as ‘not reacting, not doing anything’). Active animals are the ones that actively try to fight the stressor or try to get away from it, while reactive animals await the end of the stressor in certain test situations; in other situations the reactive animals show the most explorative behaviour. Reactive animals seem to adapt more easily to variable conditions and are more flexible, which is advantageous in changing or unpredictable conditions, while active animals develop routines and seem to anticipate situations, which is advantageous in stable and predictable conditions (Ruis et al., 2001b). Although the backtest has been criticised (Jensen et al., 1995), relations have been
reported between backtest results and other behavioural tests, and physiological and immunological parameters (Hessing et al., 1995; Hessing et al., 1994; Ruis et al., 2000; van Erp-van der Kooij et al., 2000).

Backtest scores range normally from 0 to 9 escape attempts in one minute. Mean backtest results at 10 days of age have risen in the population on the university farm in Utrecht, the Netherlands, from approximately 2 in 1995 to approximately 3 in 1999. Since a positive relation was found between lean meat percentage at slaughter and backtest score, this rise in backtest scores is probably due to the selection on lean meat percentage in commercial pigs (van Erp-van der Kooij et al., 2001; van Erp-van der Kooij et al., 2000).

Because experiences early in life such as cross-fostering and regrouping, which is common in commercial pig husbandry, might have an impact on the coping behaviour of the animals later in life, the first objective of this study was to investigate the backtest response of pigs that were cross-fostered and grouped according to backtest responses at a young age. We assume that backtest responses are relatively stable from 3 to 10 days of age if no major changes in the social environment take place, but if animals are regrouped between the tests, it is unknown what the effect on backtest responses is. Differences might be expected between cross-fostered animals and animals that stayed in the home pen and received new penmates, and between different groups when extreme social environments (with only active or only reactive animals) are created.

In the present study, pigs were weaned at 4 weeks and some were mixed at 9 weeks, to assess the animal’s response to those stressors, using salivary cortisol as a tool to assess the physiological stress response of the animals (Barnett and Hutson, 1987; Ekkel et al., 1996). The second objective of the study was to determine if animals that differed in coping responses to the backtest also differed in cortisol responses to weaning and mixing, because reactive animals might show a higher reactivity of the HPA system (Ruis et al., 2000).

**MATERIALS AND METHODS**

An outline of the experiment is given in Fig.1.

**Figure 1: Experimental design**

| WEEK | DAY | W | BT | T | W | BT | T | F | C | BT | T | W | BT | T | F | C | BT | T | W | BT | T | F | C | BT | T | W | BT | T | F | C | BT | T | W | BT | T | F | C | BT | T | W | BT | T | F | C | BT | T | W | BT | T | F | C |
| 0    | 3   | 5 | 10 | 17 | 27 | 28 | 62 | 63 |

w=weighing
bt=backtest
f=formation of groups HR, MISC, LR and OR\(^1\) by cross-(Ruis et al., 2000) fostering
t=tail docking
c=castration of male piglet
we=weaning
cort=saliva sampling for cortisol
mo=only moving to fattening pens
mi=mixing and moving to fattening pens

\(^1\) HR=high resisting, Bt scores>3; MISC=miscellaneous Bt scores; LR=low resisting, Bt scores <3; OR=original litters, no cross-fostering
Housing and animals

The study was performed at the farm ‘De Tolakker’ of the University of Utrecht, The Netherlands. 882 piglets, born from 77 NL*GY sows, were used. Sows and piglets were housed in farrowing compartments (N=51 pens, 471 piglets) or multi-purpose compartments (N=40 pens, 411 piglets) and conventional farrowing crates were used. A heating lamp and floor heating were used when necessary. As of the second week, creep feeding was available for the piglets. An all in – all out system was used consistently. At birth, the ears of the piglets were tattooed for identification. 259 piglets were cross-fostered, according to the procedure described in the next paragraph. At approximately 5 days of age male pigs were castrated, tails of all pigs were docked and iron was given to the piglets by injection, according to Dutch standard procedures. At weaning, around 28 days of age, the sow was removed from the pen and the piglets remained in the same pen until 9 weeks of age. After weaning, animals received three plastic ear tags, one with the farm number (UBN number, obligatory in the Netherlands) and two others for experimental identification purposes.

At 9 weeks of age, the fattening period started. At that time, 379\(^2\) pigs from the farrowing compartments were moved to fattening units and 290 of these pigs were mixed (see next paragraph). 389\(^3\) piglets in the multi-purpose compartments were neither mixed nor moved but stayed in the same pens until slaughter. This is a specific-stress-free housing system (Ekkel et al., 1995; Scheepens et al., 1990)\(^4\). All fattening pens had a partly slatted, concrete floor with floor-heating. Feed and water were supplied ad libitum. Pigs were slaughtered at approximately 110 kilograms liveweight (around 6 months of age). This study only covers the period until 9 weeks of age.

Backtest and mixing procedure

A backtest was performed on all piglets (Hessing et al., 1993). In this backtest, each piglet was taken out of the home pen and placed on its back on a table, next to the pen. The animal was restrained manually in this supine position for one minute. One hand was placed loosely over the head of the pig, the other was placed loosely on the hind legs. The pressure was increased slightly when the animal started moving, in order to hold the piglet in the same place: the more an animal moved, the more pressure was needed. When the animal relaxed again, the pressure was decreased again. Each bout of wriggles that the piglet made without a pause was counted as one escape attempt. A pause was defined as a period of time in which the piglet did not move its hind legs, and that lasted at least 1 second. The total number of escape attempts is called the ‘backtest score’ and ranges from 0 to 11 in this study. A backtest was performed with every animal at 3, 10 and 17 days of age (plus or minus 1 day).

On day three, after performing the first backtest, different groups of approximately 10 piglets per sow were formed on the basis of the first backtest results by cross-fostering of some of the piglets, resulting in 20 HR groups (high resisting), 31 LR groups (low resisting), 18 MISC groups (miscellaneous) and 21 OR groups (original) as controls. In HR-pens only piglets with high backtest scores (>3) were placed, in LR-pens piglets with low scores (<3) were placed, MISC- pens consisted of animals with high, intermediate and low scores and OR-pens consisted of littermates only, independent of backtest score. The cut-off value of 3 was chosen because the mean backtest score on day 3 was 3.26. In the HR pens, 63 out of 192 piglets were cross-fostered (33%), in the MISC pens, 128 out of 304 (42%), and in the LR pens 67

\(^2\) The other 92 pigs either died or were not moved to the fattening pens due to animal density regulations for fattening pigs (min. 0.7 m\(^2\) per pig = max. 10 pigs per pen)

\(^3\) The other 22 pigs either died or were removed from the multi-purpose pens due to animal density regulations for fattening pigs (see footnote \(^2\))
out of 178 (38%). The number of cross-fostered piglets ranged from 1 to 11 in each litter, and cross-fostered piglets usually came from 1 other pen (in 42/64 pens) or 2 other pens (in 16/64 pens). In the remaining 6 pens, cross-fostered piglets came from 3, 4 or 5 other pens. All groups were balanced for gender. In MISC pens, 24% of piglets had high backtest scores (>3), 29% had low scores (<3) and 47% scored exactly 3 in the first backtest. Mean backtest scores per group after the cross-fostering procedure on day 3 are shown in Table 1.

In the first 10 farrowing pens, some behavioural observations were done after cross-fostering. Approximately 30 minutes after cross-fostering, cross-fostered pens were observed for 10 minutes per pen, and aggressive interactions were scored. The following day, during one suckling bout it was checked if all cross-fostered piglets were suckling.

At 9 weeks, all HR, MISC and LR groups from the farrowing compartments were mixed with other HR, MISC or LR groups from the same compartments to create 17 HR, 27 MISC, 17 LR and 18 OR groups in the fattening compartments. Mixing was done only within groups (HR+HR, MISC+MISC and LR+LR). Group sizes sometimes differed before and after mixing, because a) the available space in the fattening pens was limited (a maximum of 10 pigs per pen was allowed) and b) we tried to use the fattening pens as efficiently as possible (e.g. by mixing 20 piglets from 3 farrowing pens into 2 fattening pens, provided that they came from the same group, LR, HR or MISC). Animals from the OR groups and animals in the multi-purpose pens were not mixed.

Table 1: Mean backtest results (LSMeans) and differences between means at day 3, 10 and 17 in pens with high backtest results on day 3 (>3, HR), with low backtest results on day 3 (<3, LR), with mixed results on day 3 (range 0-8, MISC) or original litters (OR). Groups are formed on day 3, on the basis of the first backtest results.

<table>
<thead>
<tr>
<th>MEAN BACKTEST SCORE</th>
<th>DAY 3</th>
<th>DAY 10</th>
<th>DAY 17</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>4.74±0.24</td>
<td>3.23±0.31</td>
<td>3.40±0.35</td>
</tr>
<tr>
<td>MISC</td>
<td>3.07±0.18</td>
<td>2.68±0.23</td>
<td>3.23±0.26</td>
</tr>
<tr>
<td>LR</td>
<td>1.52±0.25</td>
<td>1.66±0.33</td>
<td>2.29±0.37</td>
</tr>
<tr>
<td>OR</td>
<td>3.53±0.24</td>
<td>2.66±0.33</td>
<td>3.42±0.36</td>
</tr>
</tbody>
</table>

Means with a different superscript in the same column differ significantly.

Saliva sampling procedure and radioimmunoassay for cortisol

The saliva sampling and the radioimmunoassay were performed as described previously (Ekkel et al., 1996). Saliva samples were taken from approximately 20% of the animals (2 animals per pen, randomly chosen) on the pre-weaning (cort1) and on the weaning day (cort2), and at 9 weeks of age on the day before weighing (cort3) and after weighing and/or mixing and/or moving (cort4) (Fig. 1). The same animals were used for all the samples. Weaning and weighing started at 9 a.m., saliva sampling started at 11 a.m. (and was finished at the latest at 1 p.m.). Because of practical reasons, only 4 samples per animal were taken. The saliva was collected by allowing the pigs to chew on cotton buds (9679396, Hartman, Nijmegen, the Netherlands) until they were thoroughly moistened. Pigs were handled and trained before saliva sampling, therefore fixing was never necessary. The cotton buds were centrifuged for 5 minutes at 400 x g to remove the saliva, which was then stored at –20 °C until analysis.

Concentrations of cortisol in saliva were estimated by a previously validated (Ekkel et al., 1996) direct solid-phase ^125^I RIA method (Coat-A-Count TKCO; Diagnostic Products Corporation, Los Angeles, CA, U.S.A.) in general in duplicate samples of 200 µl. For 17% of the samples smaller aliquots were used due to small amounts of collected saliva.
The main cross-reactivities were 11.4, 0.98, 0.94 and 0.26% for 11-deoxycortisol, cortisone, cortisosterone and 11-deoxycortisosterone, respectively, according to the manufacturer. Comparison of added and estimated values when cortisol was added to a pooled saliva sample over the range of the standard curve (0.5-50 ng/ml) produced an average intra-assay coefficient of variation of 7.9% and a recovery of 96.8%. The limit of quantitation was 0.25 ng/ml and the interassay coefficient of variation was 9.5% for the range of 0.5 to 15 ng/ml (n=8).

Statistical analysis
All analyses were performed using the statistical package SAS (Ekkel et al., 1996; SAS_Institute_Inc., 1989). Pearson correlation coefficients were calculated for individual backtest results on different test days. Confidence intervals around the correlation coefficients were calculated using a Fisher’s Z transformation. To compare correlations between day 3 and day 10 for the different groups (HR, LR, MISC and OR), the following model was used (PROC GLM, SAS):

\[ Bt3 = \mu + Bt10 + pen(group) + group + Bt10*group \]

in which \( Btx \) is the backtest score at \( x \) days of age, pen is the pen number (within group) and group is HR, LR, MISC or OR. If the interaction \( Bt10*group \) is significant, this means that the correlation between \( Bt3 \) and \( Bt10 \) is different in the different groups. The estimates for the betas in the different groups are calculated relative to the OR group (option /solution in SAS); this way we can determine if the correlations in the cross-fostered groups HR, LR and MISC differ from the OR group (original litters).

Log-transformations were used in the analyses for the cortisol rise at weaning (\( \log(cort2/cort1) \)) and at 9 weeks (\( \log(cort4/cort3) \)) since cortisol levels were not normally distributed, and for group comparisons the following models were used at weaning (1) and at 9 weeks (2):

\[ (1) \log(cort2/cort1)=group \]
\[ (2) \log(cort4/cort3)=log(cort2/cort1) + group + ssf \]

where group=HR, MISC, LR or OR and ssf=SSF-housing system (no mixing, no moving) or conventional system (moving and/or mixing of pigs at 9 weeks).

To determine the relation between individual backtest result and cortisol rise, the following models were used at weaning (1) and at 9 weeks (2):

\[ (1) \log(cort2/cort1)=backtest score \]
\[ (2) \log(cort4/cort3)=\log(cort2/cort1) + backtest score + ssf \]

where backtest score is the mathematical mean of the backtest result on day 10 and day 17 for each individual.

A general linear mixed model (PROC MIXED in SAS) was used, and models were corrected for batch*compartment*pen in the random statement.

Results were considered statistically significant when P-values were below 0.05; P-values between 0.05 and 0.10 are called trends.

RESULTS

Backtest
Mean backtest results were 3.26 ± 0.06 on day 3 (range 0-9, n=813), 2.76 ± 0.06 on day 10 (range 0-11, n=787) and 2.97 ± 0.07 on day 17 (range 0-11, n=785). Gilts had higher backtest results than barrows on day 3, 10 and 17, but this was significant only on day 10.
By cross-fostering on day 3, we created pens with mean backtest scores on that day of 4.74 in the HR pens, 3.07 in the MISC pens, 1.52 in the LR pens and 3.53 in the OR pens (Table 1). At day 10 and day 17, the differences between the pens were much smaller (Fig.2) and only the mean backtest result of the LR pens was significantly lower than in the other pens. In both the LR pens and in the HR pens, on day 10 almost the full range of backtest results was seen again. This resulted in correlations between backtests on different test days that were dependent on group composition. Correlations between day 3 and 10 and between day 3 and 17 were not significant for the animals in the HR and LR pens. The correlations between day 3 and day 10 for all animals in the LR and HR groups differed from the correlation between day 3 and day 10 in the OR group (P<0.05). The correlation between day 3 and day 10 for all animals in the MISC group did not differ from that in the OR group (P=0.18).

Figure 2: Mean backtest score for HR, MISC, LR and OR pens. n=186, 294, 170 and 197 pigs for HR, MISC, LR and OR pens, respectively. Cross-fostering to create HR, MISC and LR pens took place just after the first backtest, on day 3.

Not only the mean backtest scores changed, but also the individual ranking (in backtest results) of the animals changed. Backtest results in typical HR and LR pens are in Figures 3a and 3b.
Figure 3a: Backtest scores of individual piglets in a representative HR pen, before and after regrouping. Lines represent one or more piglets, numbers are between brackets.

Figure 3b: Backtest scores of individual piglets in a representative LR pen, before and after regrouping. Lines represent one or more piglets, numbers are between brackets.

Mean backtest results for all animals in the HR, MISC and OR pens were on an equal level on 10 and 17 days. Backtest results on day 10 and 17 were correlated for all groups (Table 2a) but backtest results on day 3 and day 10 were only correlated for the high resisting animals (Bt scores >3) in the MISC and OR groups. (Table 2b).
Table 2a: Correlations (with confidence intervals) between backtests in pens with high backtest results on day 3 (>3, HR), with low backtest results on day 3 (<3, LR), with mixed results on day 3 (range 0-8, MISC) or original litters (OR).

<table>
<thead>
<tr>
<th></th>
<th>DAY3-DAY10</th>
<th>P-VALUE</th>
<th>DAY3-DAY17</th>
<th>P-VALUE</th>
<th>DAY10-DAY17</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>0.08 (-0.06-0.22)</td>
<td>0.26</td>
<td>0.08 (-0.06-0.22)</td>
<td>0.27</td>
<td>0.38 (0.25-0.49)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MISC</td>
<td>0.23 (0.12-0.34)</td>
<td>&lt;0.0001</td>
<td>0.22 (0.11-0.33)</td>
<td>&lt;0.001</td>
<td>0.47 (0.37-0.55)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LR</td>
<td>0.06 (-0.09-0.21)</td>
<td>0.40</td>
<td>-0.07 (-0.22-0.08)</td>
<td>0.36</td>
<td>0.25 (0.11-0.39)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OR</td>
<td>0.25 (0.12-0.38)</td>
<td>&lt;0.001</td>
<td>0.36 (0.23-0.47)</td>
<td>&lt;0.0001</td>
<td>0.44 (0.32-0.54)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 2b: Correlations between backtest results for high (HR) or low (LR) resisting animals in groups with high backtest results (>3, HR), with low backtest results (<3, LR), with mixed results (range 0-8, MISC) or original litters (OR).

<table>
<thead>
<tr>
<th>BACKTEST RESULTS DAY 3</th>
<th>GROUP</th>
<th>CORRELATION DAY 3-DAY 10</th>
<th>CORRELATION DAY 3-DAY 17</th>
<th>CORRELATION DAY 10-DAY 17</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR (0,1 OR 2) HR</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MISC</td>
<td></td>
<td>0.15 (NS)</td>
<td>0.17 (NS)</td>
<td>0.59 (P&lt;0.0001)</td>
<td>80</td>
</tr>
<tr>
<td>LR</td>
<td></td>
<td>0.05 (NS)</td>
<td>-0.05 (NS)</td>
<td>0.29 (P&lt;0.0005)</td>
<td>147</td>
</tr>
<tr>
<td>OR</td>
<td></td>
<td>0.17 (NS)</td>
<td>0.26 (NS)</td>
<td>0.31 (P&lt;0.05)</td>
<td>46</td>
</tr>
<tr>
<td>HR (&gt;3) HR</td>
<td></td>
<td>0.09 (NS)</td>
<td>0.09 (NS)</td>
<td>0.38 (P&lt;0.0001)</td>
<td>166</td>
</tr>
<tr>
<td>MISC</td>
<td></td>
<td>0.40 (P&lt;0.001)</td>
<td>0.22 (P&lt;0.1)</td>
<td>0.45 (P&lt;0.0001)</td>
<td>71</td>
</tr>
<tr>
<td>LR</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>OR</td>
<td></td>
<td>0.28 (P&lt;0.01)</td>
<td>0.31 (P&lt;0.005)</td>
<td>0.53 (P&lt;0.0001)</td>
<td>91</td>
</tr>
</tbody>
</table>

When, within the groups, cross-fostered piglets were compared with animals that received new penmates, no differences in backtest results were found (Table 2c). No aggressive interactions were seen after cross-fostering, and the following day all cross-fostered piglets were suckling.

Table 2c: Backtest results on day 3 and 10 and difference (diff) between day 3 and day 10 (Bt day 3-Bt day10) for cross-fostered animals and animals that stayed in the same pen but received new penmates, in pens with high backtest results on day 3 (>3, HR), with low backtest results on day 3 (<3, LR) or with mixed results on day 3 (range 0-8, MISC). In original litters (OR), no cross-fostering was performed.

<table>
<thead>
<tr>
<th></th>
<th>NOT CROSS-FOSTERED</th>
<th>CROSS-FOSTERED</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>BT DAY3 4.91±1.08</td>
<td>4.86±0.95</td>
</tr>
<tr>
<td></td>
<td>BT DAY10 3.24±1.66</td>
<td>3.33±1.87</td>
</tr>
<tr>
<td></td>
<td>DIFF 1.69±1.93</td>
<td>1.46±1.95</td>
</tr>
<tr>
<td>MISC</td>
<td>BT DAY3 2.94±1.34</td>
<td>3.18±1.21</td>
</tr>
<tr>
<td></td>
<td>BT DAY10 2.59±1.65</td>
<td>2.76±1.68</td>
</tr>
<tr>
<td></td>
<td>DIFF 0.35±1.78</td>
<td>0.41±1.98</td>
</tr>
<tr>
<td>LR</td>
<td>BT DAY3 1.47±0.70</td>
<td>1.48±0.68</td>
</tr>
<tr>
<td></td>
<td>BT DAY10 1.92±1.33</td>
<td>2.06±1.50</td>
</tr>
<tr>
<td></td>
<td>DIFF -0.44±1.41</td>
<td>-0.58±1.67</td>
</tr>
</tbody>
</table>
Cortisol levels in saliva (mean ± SE) were 2.90 ± 0.30 and 4.62 ± 0.26 before and after weaning, and 1.83 ± 0.13 and 2.37 ± 0.20 before and after the start of the fattening period, respectively. The mean difference in cortisol levels before and after weaning was 2.05 ± 0.41 and the mean difference at 9 weeks (before and after the start of the fattening period) was 0.73 ± 0.24. This rise in cortisol levels was significant at weaning, and the animals that were mixed and moved at 9 weeks also showed a significant rise in salivary cortisol at that time. No animals were mixed without moving. Only moving the pigs (no mixing) had no effect on cortisol levels (Table 3).

**Table 3:** Cortisol rise in saliva at the start of the fattening period, for animals that are mixed compared to animals that are not mixed and animals that were moved but not mixed compared to animals that were not moved nor mixed.

<table>
<thead>
<tr>
<th></th>
<th>CORTISOL RISE</th>
<th>LOG (CORTISOL)</th>
<th>P-VALUE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO MIXING</td>
<td>0.10 ± 0.14</td>
<td>0.007</td>
<td>&lt;0.0001</td>
<td>73</td>
</tr>
<tr>
<td>MIXING</td>
<td>1.48 ± 0.47</td>
<td>0.526</td>
<td></td>
<td>61</td>
</tr>
<tr>
<td>NO MOVING</td>
<td>0.14 ± 0.12</td>
<td>0.006</td>
<td>NS</td>
<td>56</td>
</tr>
<tr>
<td>MOVING</td>
<td>-0.03 ± 0.50</td>
<td>0.010</td>
<td></td>
<td>17</td>
</tr>
</tbody>
</table>

In the statistical analysis, log transformations were used.

**Group effects on cortisol and relations with individual backtest scores**

Differences in cortisol levels for the different groups (HR, MISC, LR and OR) before and after weaning, and at the start of the fattening period are presented in Table 4. Relations were found only with housing system (SSF or CONV). No relations were found between individual backtest results at 3, 10 or 17 days of age and cortisol rise at weaning or at 9 weeks.

**Table 4:** Cortisol rise (mean ± SE) after weaning and at 9 weeks in in pens with high backtest results on day 3 (>3, HR), with low backtest results on day 3 (<3, LR), with mixed results on day 3 (range 0-8, MISC) or original litters (OR), in different housing systems; SSF=specific stress free (no mixing or moving); CONV=conventional, moving and/or mixing at 9 weeks. Until 9 weeks, housing is identical.

<table>
<thead>
<tr>
<th>CORTISOL RISE</th>
<th>HR</th>
<th>MISC</th>
<th>LR</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFTER WEANING</td>
<td>2.31 ± 0.59</td>
<td>2.42 ± 0.58</td>
<td>1.29 ± 1.65</td>
<td>1.84 ± 0.40</td>
</tr>
<tr>
<td>AT 9 WEEKS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSF</td>
<td>0.21 ± 0.25</td>
<td>0.23 ± 0.22</td>
<td>0.18 ± 0.20</td>
<td>-0.26 ± 0.26</td>
</tr>
<tr>
<td>CONV (MOVING, NO MIXING)</td>
<td>1.38 ± 0.87</td>
<td>1.20 ± 0.68</td>
<td>2.20 ± 0.96</td>
<td></td>
</tr>
<tr>
<td>CONV (MOVING AND MIXING)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Asterisks indicate if the cortisol rise differs from 0 (analyses on log-transformed data). P<0.10

**DISCUSSION AND CONCLUSIONS**

**Backtest results**

The backtest is a behavioural test, which measures the reaction of an individual in a certain stressful situation. Mean and range of the backtest results in this study were comparable with earlier studies, and also gilts had higher backtest results than barrows at day 3, 10 and 17, but this was significant only at day 10 (van Erp-van der Kooij et al., 2001; van Erp-van der Kooij et al., 2000).
So far, it is unknown which specific aspects of the coping style of the animals are measured with this test. According to a recent study (Gosling and John, 1999), five dimensions of the personality can be distinguished in animals. These are organised in the categories Neuroticism (e.g. anxiety), Antagonism (e.g. aggression), Extraversion (e.g. activity), Openness (e.g. curiosity-exploration) and Dominance. It is possible that the backtest measures, for example, a combination of fear of humans, aggression and activity. In order to reveal some of the underlying dimensions of the personality, several extra parameters should be recorded during the backtests such as vocalisations, latency to first escape attempt and defecating behaviour. Because of practical and logistical reasons, this was not done in the present study.

Some authors claim that the backtest is a non-social test (Hessing et al., 1993), but others suggest that being restrained in a supine position during a backtest may represent forced submission, by its resemblance with certain aspects of social fighting (Ruis, 2001). Reactive animals (with low backtest scores) might more readily accept their forced subordinate status and react less fiercely, whereas active animals (with high backtest scores) become frustrated by bodily forced submission and are more eager to resist. In that view, the backtest represents (elements of) a social test. Our results support the idea that the backtest has a social component, since the group composition (=social environment) influenced test results.

**Backtest results and group composition**

Mean backtest results for all piglets in the HR pens decreased after cross-fostering and correlations before and after cross-fostering were absent, while HR animals in the MISC and OR pens behaved consistently in the successive backtests.

Mean backtest results for all piglets in the LR pens increased after cross-fostering and correlations before and after cross-fostering were absent, but LR animals did not seem to behave consistently in the MISC and OR pens either. This suggests that in extreme circumstances, the animals can change their behaviour. Furthermore, the behaviour of LR or reactive animals might be more directed by environmental stimuli (extrinsic behavioural control), whereas the behaviour of the active animals might be more routine-like and intrinsically determined, as was suggested in a study with reactive and active rodents (Benus et al., 1991).

Why did the animals’ behaviour change? We believe there are two explanations possible. The first explanation is, that the animals changed their behaviour intentionally, because behavioural variation is beneficial for a group. In a study on individual behavioural characteristics in pigs (Hessing, 1994) it is stated that the complementary individual behavioural characteristics of the active and the reactive pigs under stress will result in a better socially integrated group. A socially well integrated group will be more successful in solving their problems, which is beneficial for each individual and for the group as a whole. This is shown in 2 studies, where active (HR) and reactive (LR) pigs were mixed. It was found that the most stable social relationship existed between HR/LR pairs where the LR animal was dominant (Ruis et al., 2002) and that HR/LR groups performed better (Hessing, 1994).

In a study where pigs were categorised as high aggressive (H) or low aggressive (L), it was found that the number of pairs fighting was lowest when H pigs were mixed with L pigs, compared to when only H or only L pigs were mixed. However, the L/L groups integrated faster and the L pigs fought less vigorously than H pigs and therefore it was concluded that it is preferable to mix pigs into groups with low-aggressive pigs only, which has been stated in another paper as well (Erhard et al., 1997; Erhard and Schouten, 2001). If LR pigs are low-
aggressive and HR pigs are high-aggressive, then we might deduce that homogeneous LR or mixed HR/LR groups would be preferred over homogeneous HR groups. In the above mentioned studies (Hessing, 1994; Ruis et al., 2002) pigs were mixed at 7 and 9 weeks of age. The fact that the results in the different pairs or groups could be related to backtest behaviour before mixing, suggests that mixing did not change backtest behaviour. In the present study, animals were mixed at a much younger age, and we assume that that is why the behaviour of these animals could still change.

A second explanation of the change in backtest results after regrouping, is that in some animals HPA axis functioning and behavioural reactivity might have been affected by the regrouping procedure. This is suggested in a study with calves, where regrouped calves showed an increased sensitivity of the adrenal cortex to ACTH (Veissier et al., 2001) and regrouped calves were more agitated during restraint (Boissy et al., 2001). In another study (Weaver et al., 2000) it was shown that neonatal handling permanently affected HPA function in boars. It is concluded from that study that there is a sensitive period during early postnatal development in the pig, during which environmental manipulations can result in permanent changes in HPA function, behaviour and body weight. Differences in body weight were not seen in the present study, but the changes in backtest behaviour might have been a result from changes in HPA function, due to environmental manipulations such as regrouping and handling, that took place in the sensitive period (between 0 and 14 days of age).

**Dominance**

If the animals changed their behaviour in order to create a more varied and possibly more stable social environment, than dominance hierarchy in the group should be taken into account. In a study with paired gilts (Ruis et al., 2002), HR gilts that were dominant over LR gilts showed much more aggression than LR gilts that were dominant over HR gilts. LR and HR gilts had almost equal chances of becoming dominant. The authors suggest that LR dominant gilts were able to suppress aggression in the HR subordinates, while HR gilts became aggressive when dominant. Mixing of two HR gilts caused the highest levels of stress. Unfortunately, in the present study we have no information on the dominance hierarchy.

**Cross-fostering**

No differences were found between cross-fostered animals and animals that received new littermates, within the same group. Differences we found between the groups, were dependent on group composition only. The attachment between sow and piglets is established in the first 3-4 days postpartum (Petersen et al., 1989) so it is possible that cross-fostering at 3 days of age will affect the piglets: they might miss their mother.

No aggression was observed after cross-fostering. We did not expect to see much aggression after cross-fostering, since it was done within a few days after birth (≤ day 4). In a study where piglets were cross-fostered frequently, more fighting occurred in cross-fostered litters compared to control litters when cross-fostering was performed on day 4, 7, 10, 13 or 16 postpartum, but not when cross-fostering was performed on day 1 (Robert and Martineau, 2001). Probably, in our study we cross-fostered early enough not to induce fighting. Under free-range conditions, piglets will join the social group at about 2 weeks postpartum, and in the following period (2-7 weeks pp), mostly friendly interactions are seen. Aggression between residents and intruders is described in older pigs (Ekkel et al., 1995; Erhard and Mendl, 1997; Ruis et al., 2001a).
Cortisol was measured in saliva. This non-invasive technique has proven to be reliable and easy to perform. Salivary cortisol is mainly unbound and reflects the biologically active, plasma free fraction (Cook et al., 1996; Ruis et al., 1997). Because a limited number of samples was taken, only major changes in cortisol levels could be found and temporal dynamics of cortisol were not taken into account.

Weaning caused a significant rise in cortisol levels in the piglets of 58%, which we considered important and possibly harmful for the animal (Barnett and Hemsworth, 1990; Mendl, 1991). Since weaning is a known stressor for mammals, this is not surprising. A large variation was found between the animals as was also shown by other authors (Moberg, 1987) and some animals even showed a decrease in cortisol level after weaning. This could be due to some unknown event happening just before or during the first saliva sampling procedure, such as an aggressive encounter with a penmate or a sow vocalising, causing a very high cortisol level at that time. Cortisol levels in pigs may rise quickly in response to an acute stressor (Ruis et al., 2001b).

The procedure at the start of the fattening period (at 9 weeks of age) included weighing of animals and for a number of animals also mixing and moving to the fattening compartments. Animals that were mixed and moved showed a considerable increase in cortisol of 69%, which is also possibly harmful for the animals. It is known that aggression during mixing can increase cortisol in pigs (Parrott et al., 1990) and that mixing of pigs can have a long lasting effect on the physiology and behaviour of pigs (Ekkel et al., 1997; Ekkel et al., 1995). However, cortisol can decrease quickly and be back to basal levels one hour after the stressor (Becker et al., 1985; Geverink et al., 1998; Stricklin et al., 2001). (Weighing and moving without mixing had no effect on cortisol. In the present study, the weighing and moving procedures were new to the animals. Moving meant that a group of pigs was driven through the corridors of the farm to another compartment. Weighing at birth and at weaning was performed with a small balance on which animals were lifted by the researcher, while at 9 weeks pigs were driven on a large weighing device. We therefore expect these treatments to have been stressful for the animals. However, these procedures might have caused a short-lived increase of cortisol, and at the time of measuring (appr. 2 hours later) the effect may already have disappeared.

Cortisol changes and backtest results

Several studies indicate that some differences in cortisol response to stressors in pigs, depend on coping style (Haemisch, 1990; Hessing et al., 1994; Ruis et al., 2001b; Ruis et al., 2000) while others do not (Geers, 1995).

In the present study, no differences were found in mean cortisol rise after weaning or at 9 weeks between the groups. This is not surprising, since at 10 days of age mean backtest results in all groups were approximately at the same level, and the whole range of backtest results was found again in the originally different groups. However, relations between individual backtest scores and cortisol rise were not found either. This means that coping style, as measured with the backtest, could not predict the individual stress responses to weaning or to mixing at 9 weeks of age for these pigs. If a relation with backtest response at 3 days would have been found, we would have concluded that animals had kept their original coping strategy (before cross-fostering) but a phenotypical change in test results after regrouping had occurred, while a relation with backtest responses at day 10 or 17 would have suggested that animals had changed their coping behaviour permanently.

It is possible that the time between the start of the stressor and the cortisol sampling was too long in our study. The animals that differed in coping style, may also have had a different
cortisol curve, meaning that the time and the level of the cortisol peak has differed, in accordance with the results of a study with gilts differing in coping response to the backtest (6). It was found that LR gilts (<3 escape attempts in the backtest on day 3) showed higher cortisol responses than HR gilts (>4 escape attempts) at 15 and 30 minutes after the start of an isolation period, but subsequent levels at 45, 90, 180 and 300 minutes did not differ between HR and LR animals. With the single saliva sample approximately 2 hours after the start of the stressor in the present study, this could not be determined. In another study by the same author (21), it was found that not only backtest behaviour at 3 days, but also dominance and the interaction between dominance and backtest behaviour influenced the stress response to mixing at 7 weeks. In future studies on mixing of pigs, dominance should therefore be taken into account as an important factor which might explain differences in cortisol responses after mixing, especially when group stability is discussed.

**CONCLUSION**

The backtest measures some aspects of the coping style, but which ones exactly is still unknown. The coping response in the backtest is a relatively consistent characteristic of the animal in a stable social environment. When piglets with similar backtest responses (HR or LR) are put together at a young age, backtest responses of the animals change and the group becomes heterogeneous again, containing active (HR) and reactive (LR) individuals. This change could be intentional, because a varied group might be more beneficial for the animals, or might be caused by a change in HPA function due to stress. Salivary cortisol was increased after weaning and also after mixing of unfamiliar pigs at 9 weeks, but we found no relations with backtest scores in this study.

It is important that people who work with pigs realise that common farm practices such as regrouping of piglets and mixing of older pigs have such an influence on the behaviour and the physiology of the animals. Although cross-fostering has less (visible) effects than regrouping at a later age, the procedure can change the animals' behaviour. We therefore advise strongly not to mix pigs unless it is absolutely necessary, and if mixing is unavoidable, to do it at a very young age. Hopefully, if farmers (and consumers) see our production animals as individuals again, each with their own personality, they will treat them better (or demand that they be treated better), and spare them some of the (unnecessary?) stressors of everyday life on the commercial pig farm.

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Chapter 4


