Platelet serotonin uptake, N/L ratio and (abnormal) behaviour of pigs with a low or high social breeding value in barren or enriched housing

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Skara 2012

MSc Animal Sciences

Studentarbete
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Preface

This major thesis study was performed as partial fulfilment of the Master's degree in Animal Sciences at Wageningen University and the Swedish University of Agricultural Sciences. The study was conducted in Wageningen and took place from November 2010 until July 2011. The thesis was part of a PhD project called 'Seeking sociable swine', which focuses on (amongst other subjects) maladaptive behaviour and social breeding value in pigs.

Hereby I would like to thank Nanda Ursinus and Liesbeth Bolhuis, both from Wageningen University, for supervising my master's project in Wageningen. From the Swedish University of Agricultural I was supervised by Linda Keeling, thank you for your comments on my thesis report. I would like to thank Irene Camerlink for getting me familiarised with the statistical analysis in SAS. Finally, I would like to thank Ger de Vries Reilingh on her help explaining the white blood cell count, Merel Verhoeven for helping me in the laboratory and Fleur Bartels and Monique Ooms for their help in the stables and for processing the data.

Summary

Pigs that are kept intensively and in groups may show excessive aggressive and/or manipulative behaviour. These behaviours may cause both welfare problems for the pigs and economic problems for the farmer. These problems may be solved by adjusting the environment the animals are kept in, and by (genetic) selection of animals. A social breeding value (SBV) has been designed that is used to estimate the heritable effect of an animal on the traits of its pen mates. Selecting for high social breeding values for the growth of pen mates might be an indirect way to reduce behavioural problems related to social interactions, but so far the effects of selecting for SBV for growth on behaviour and physiology of pigs are unknown.

The main aim of the current study was to examine the relation between SBV, serotonin, Neutrophil to Lymphocyte (N/L) ratio, (abnormal) behaviour and aggression in animals with a high or low SBV, housed in an enriched or barren environment. The experiment was set up as a 2x2 arrangement, with SBV (high vs. low) and housing (barren or enriched) as factors. In total, 32 groups of pigs (192 animals) divided over two batches were used, the pigs were housed barren (partially slatted) or enriched (with sawdust and straw) in groups of six (1:1 sex ratio). Animals diverged in SBV, and half of the pigs were high SBV animals, with an average estimated genetic effect of +2.72 on average daily gain (ADG) of pen mates, and the other half were low SBV animals, with on average a negative effect of -1.5 g ADG on pen mates. To study both SBV and housing, behavioural observations in the home pen were conducted, a regrouping test (i.e. mixing of individuals from the same SBV) was performed, lesion and tail bite scores were recorded, and at three times some blood was taken from the pigs to determine N/L ratios and serotonin uptake velocity by platelets.

Housing was found to have a greater impact on explorative, active and manipulative behaviour during this experiment than SBV, and no interactions were found between SBV and housing. Pigs in enriched pens were more active compared to barren housed animals, showed more explorative behaviour and less oral manipulation of pen mates. Furthermore, tail damage scores were lower in enriched housing. Body lesion scores after regrouping were not affected by housing, but under stable social conditions, pigs in enriched pens showed more lesions than barren housed pigs. SBV or its interaction with housing did not affect behaviour, body lesion scores or tail damage of pigs. SBV did affect platelet serotonin uptake velocity, with a higher uptake for the low SBV than for the high SBV pigs. Also N/L ratio was affected by SBV, with a higher N/L ratio in low SBV animals than in high SBV animals. Housing did not significantly influence serotonin (5-HT) uptake or N/L ratio.

In conclusion, SBV did not affect behaviour of pigs, but effects on physiological variables were found. It is possible that selection for a high SBV may genetically make the animals less prone to show damaging behaviours, but in the experimental environment or method of this study, this was not observed. Alternatively, as this study was part of a larger experiment, the sample size may have been too small to reveal potential effects of diverging SBVs on behaviour of pigs. Further research is needed to unravel which traits of pigs are changing when selecting for SBV for growth of pen mates.

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1. **Introduction**

When groups of pigs are kept together intensively, aggressive behaviour and maladaptive oral behaviours directed at pen mates like tail biting, ear biting or belly nosing may occur. Excessive fighting may occur because the animals are not able to retreat from the aggressors (Andersen et al., 2004). Maladaptive oral behaviours may occur due to the barren environments pigs are often kept in. The lack of suitable substrates in these environments restricts highly motivated and species-specific behaviour of pigs, like foraging (Wemelsfelder et al., 2009). Aggressive and maladaptive behaviour may cause both welfare problems for the pigs (e.g. tail biting) and economic problems for the farmer, for example by veterinary bills and reduced weight gain (Heinrichs and Richard, 1999). The provision of straw could function as an outlet for highly motivated foraging and explorative behaviours that are restricted by a barren environment, and in this way decrease manipulative behaviour (Young et al., 1994, Day et al., 2002). Another solution to prevent undesired behaviour can be through breeding management. In current animal production systems, selection is aimed at the individual and its production traits while the animal's effect on group mates is not taken into account (Muir, 2005). This may potentially lead to an increase in negative social behaviour, as the traits selected may not be optimal for functioning in a group of conspecifics (Rodenburg et al., 2010). To describe the positive or negative effects of pigs on their pen mates, a social breeding value (SBV) formula has been designed. This formula describes the genetic, heritable effect of one pig on the growth of its pen mates. It has been shown that social genetic effects on growth of pen mates contribute profoundly to the genetic variation in growth (Bergsma et al., 2008). Yet, it is unknown why or how pigs with a high SBV enhance the growth of pen mates. It can be hypothesized that less detrimental or more positive social interactions between animals underlie these social genetic effects. Therefore in the current study, some behavioural and physiological characteristics that are expected to be related to SBV and to be affected by housing will be examined. The physiological factors are related to stress (Neutrophil to Lymphocyte (N/L) ratio) and behaviour (serotonin uptake). The behavioural and physiological characteristics were examined during socially stable periods, but also during a socially unstable period (regrouping of the animals). This to test the characteristics mentioned during an 'extreme' event, and because regrouping pigs is a common practise in pig production, and therefore the functioning of high and low SBV animals is important to examine under these circumstances as well. Behaviour observed during this experiment consists of explorative, aggressive, social, non-active and manipulative behaviour because both the environment and SBV are expected to affect these behaviours.

The main aim of this study was to examine the relation between social breeding value, serotonin, N/L ratio, (abnormal) behaviour and aggression in pigs housed barren or enriched. The following research question was formed to reach the aim of the research: *What is the relation between serotonin, N/L ratio, abnormal behaviour and aggression in pigs with a high or low social breeding value and does the environment (barren vs. enriched) affect this?*

In this report, the following chapter contains a literature study on social breeding value, housing, serotonin and N/L ratio. Chapter 3 describes the materials and methods used in the experiment and chapter 4 presents the results of this experiment. In chapter 5 the results are discussed. The report ends with concluding remarks in the final chapter.

2. Literature study

2.1 Social breeding value

Interactions amongst pigs affect their performance and welfare. Injuries caused by manipulative and excessive aggressive behaviour can negatively affect the welfare of the receiving animals, whereas a social relationship without these excessive behaviours can positively affect them. Currently, breeding programmes target the genetic potential of an individual animal for production traits. It has been suggested that this selection for individual performance may favour selection of pigs more competitive towards pen mates (Muir and Schinckel 2002, Chen et al. 2007).

Social or associative genetic effects reflect the genetic effects of individuals on traits of their group members and can be expressed in a social breeding value (SBV). In pigs, social genetic effects for weight gain during the fattening period (from 25 kg until slaughter) have been estimated to contribute substantially to the genetic variance in growth (Bergsma et al., 2008). The formula for social breeding value can be included in the formula currently used as a base for breeding value estimation. This current formula only describes direct effects of the individual on its phenotype and the corresponding non-heritable direct effect of the environment (A_{Di} and E_{Di} respectively, shown in the formula underneath). Social breeding value is added as the heritable social effect of pen mate j on the phenotype of individual i (A_{Sj}), and the corresponding non-heritable social effects (E_{Sj}).

$$P_i = A_{D_i} + E_{D_i} + \sum_{i \neq j}^{n-1} (A_{S_j} + E_{S_j}) \quad \textit{(Bergsma et al., 2008)}$$

This social breeding value is calculated by using the pedigree and production information of pen mates of family members, where the effect of these family members on their pen mates is calculated. Therefore the SBV becomes more reliable when an animal has more offspring, because this produces more information about the effects on pen mates of this specific family.

Although social genetic effects on growth have been demonstrated (Canario et al., 2009, Chen et al., 2009 and Bergsma et al., 2008), it is still unclear how pigs with diverging SBV differ from each other. For instance, it is not known if and which social factors are limiting the growth rate in groups of pigs (Rodenburg, 2010). Also the effect of the environment, like enrichment, on pigs diverging in SBV is not yet known. Housing has been known to influence animal behaviour, and could therefore interact with the social breeding value of an animal, i.e. animals in barren housing could react differently to stressors and towards pen mates than animals in enriched environments.

2.2 Housing

Part of the species-specific behaviour of pigs is to root and nose the environment. The animals will show this foraging and explorative behaviour, even if their nutritional needs are met (Beattie and O'Connell, 2002, Munsterhjelm et al., 2009). In current production systems, pigs are often housed in barren pens to minimize rearing expenses and optimize husbandry efficiency. These barren conditions do not enable the animals to show foraging and explorative behaviour, which may then be redirected towards the pen itself or towards pen mates (McKinnon et al., 1989, Munsterhjelm, 2009).

In many studies, barren environments are compared with enriched environments, where enrichment can usually be defined as adding substrates such as straw. The results of this research indicate that enrichment increases activity, and herewith foraging and exploratory behaviour, and decreases pig or pen directed behaviour , such as tail biting and ear biting (Kelly et al., 2000, Beattie et al., 2000, Bolhuis, 2006, Burbridge et al., 1994, Spoolder et al., 1995).

In a preference test performed with 74 different kinds of enrichment materials, it appeared that the most important characteristics of enrichment for pigs are that it should be chewable, deformable, destructible, odorous and ingestible (van de Weerd et al., 2003). Straw possesses all of these characteristics, and is therefore chosen in many studies as an environmental enrichment.

Internal processes might be related to straw and the state of an animal. Walsh et al. (2010) performed a cognitive bias test with pigs. A cognitive bias occurs when the affective state of an animal influences its judgement of an event. Walsh et al. (2010) found that the provision of straw was associated with an optimistic bias, and barren housing with a pessimistic bias. Also, when the animals housed in barren pens received straw, they switched to an optimistic bias during the test and vice versa. This indicates that enrichment can cause a change in the animal's psychology (Walsh et al., 2010). As will be explained in the following paragraph, serotonin has been found to influence an animal's impulsiveness, thereby potentially influencing the animal's proneness to show excessive aggressive and abnormal behaviour. Enrichment could reduce or enhance these behaviours, interacting with the animal's physiology.

2.3 Serotonin

Serotonin (5-HT) is a biogenic amine, which means it is a neurotransmitter derived from an amino acid, in the case of serotonin the amino acid tryptophan. Serotonin is released in many sites in the central nervous system (CNS) where it affects, amongst others things; sleep, mood, attention, anxiety and learning (Campbell and Reece, 2005). Serotonin is synthesized in the serotonergic neurons, which cell bodies are localized in the brainstem, projecting to the forebrain, hindbrain and spinal cord (Molliver, 1987). The main function of serotonin in the CNS is as an inhibitory neuron (Moreno et al., 2005). But, depending on the site of action and the receptor, serotonin also acts by increasing the activity of neurons and by stimulating production of corticotrophin-releasing hormone. With this last action serotonin activates the Hypothalamus-Pituitary-Adrenal (HPA) axis (Best et al., 2010). The HPA axis is involved in, amongst others, the behavioural reaction to stress via the release of cortisol (Campbell and Reese, 2005). Three receptor subtypes have been distinguished for 5-HT:

1. 5-HT1 receptors mediate the inhibition of the release of multiple neurotransmitters and influence smooth muscle contraction and relaxation and some behavioural actions like hypothermia, anxiolytic and anti-aggressive actions
2. 5-HT2 receptors have been shown to mediate behavioural compulsive disorders, smooth muscle contractions and platelet aggregation
3. 5-HT3 receptors mediate anxiety-effects and gastric emptying (Mylecharane, 1989)

A key regulator of serotonin neurotransmission is the 5-HT transporter. This transports 5-HT from the synaptic cleft of the neuron back into the presynaptic terminal, which terminates the action of serotonin (Bolhuis et al., 2009). Information about the velocity of serotonin uptake indicates the amount of neurotransmission that can occur. Another important regulator of serotonin is MAO which is an enzyme that catalyses the inactivation of monoamines like serotonin. MAO can occur in two isoforms, of which MAO-A metabolized serotonin (Bach et al., 1988, Jia, 2010, Fišar, 2010).

Besides in the CNS, serotonin also exists in the periphery, here it is synthesized, stored and released separate from the brain. This is caused by the fact that serotonin cannot pass the blood-brain barrier. Blood serotonin is synthesized in the intestines and stored in blood platelets (Moreno et al., 2005). Uptake, storage and release functions are comparable in the CNS and periphery. As the study of 5-HT uptake in the brain is a more invasive procedure, blood platelet uptake is often used in research. The transporter consists of the same proteins and is encoded by the same gene in CNS and periphery, and both have been found to respond similarly to the inhibition of serotonin uptake by antidepressants (Maitre et al. 1980). For these reasons platelet serotonin uptake has been used as a model for brain serotonin uptake in mammals and humans (Bolhuis et al., 2009)

In past studies, it has been found that serotonin may be related to both aggression and abnormal behaviour, because it is associated with the inhibition of behaviour. When serotonin neurotransmission is blocked, a shift in behaviour occurs towards the enabling of a response (Soubrié, 1986). This enabling of a response makes the animal more impulsive, by altering their perception of risk and reward and by affecting their decisions and actions (Long et al., 2009). The term impulsiveness describes multiple, often maladaptive, behaviours (Evenden, 1999). Impulsive animals react with abnormal and/or aggressive behaviour sooner, which is stimulated by a lower level of serotonin neurotransmission in the forebrain, as has been found in rats (Daruna and Kent, 1976). This function of serotonin is underlined by selective serotonin inhibitors (SSRI), which are used in humans, and decrease brain serotonin uptake velocity. By doing so, they are used to treat anxiety, obsessive-compulsive behaviours and hyper-aggressive behaviours (Soubrié, 1986). This means that the decrease in uptake velocity, and therefore increase in serotonin neurotransmission, may result in less abnormal and aggressive behaviour. However, serotonin does not predispose animals to show aggressive behaviour, but when the animal already receives ‘aggressive impulses’, it is more likely to show aggression if it has a lower serotonin level (Krakowski, 2003).

Anxiety in animals and humans has been found to be related to serotonin as well. This can be illustrated by Clomipramine, which is a serotonin-norepinephrine reuptake inhibitor used as an antidepressant in humans and dogs. Clomipramine has been found to work better in treating depression than other antidepressants due to its greater serotonin uptake inhibitory action (Jones and Blackburn, 2002). In panic disorders, the precursor of serotonin tryptophan is used as treatment and has shown to be effective (Kahn et al., 1987). This indicates that a higher level of serotonin, and a lower level of serotonin uptake, is related to less anxiety.

Even though many studies have found a relation between aggression, manipulative behaviour and serotonin level or uptake, the serotonin system is very complicated. Many factors influence serotonin level and efficiency, such as different receptor types, MOA-A and other hormones. Therefore findings in research are often contradictory, because not all (physiological) circumstances can be kept equal.

2.4 Neutrophil to lymphocyte ratio

Neutrophils and lymphocytes are both white blood cells. Lymphocytes are responsible for mounting the acquired immune response, both antibody and cell mediated. Neutrophils are part of the innate immune system. They develop in the bone marrow where after they migrate into the bloodstream and move into tissues approximately 12 hours later. A difference between neutrophils and lymphocytes is that neutrophils can capture and destroy foreign particles by phagocytosis (Tizard, 2004).

The ratio between neutrophils and lymphocytes is found to be influenced by stress and has been found to be correlated to weight gain, social status and anxiety (Hjarvard et al., 2009, Sutherland et al., 2006, Campo et al., 2008). In a study by Wallgren et al (1994), high concentrations of cortisol induced an increase in the number of neutrophils (through migration to organs) and a decrease in the number of lymphocytes. Therefore, changes in Neutrophil to Lymphocyte (N/L) ratio may be related to short term elevations in cortisol levels caused by stress (Moore et al., 1994, Wallgren et al., 1994). Some examples of stressors that have been found to raise N/L ratio are transporting and weaning (Puppe et al., 1997) and regrouping in pigs (De Jong et al., 1999, Leek et al., 2004). Both studies showed that when fattening pigs were regrouped, their N/L ratio as measured on the day of regrouping increased.

Related to stress is the social status of pigs, which has been found to be related to N/L ratio. In a study by Hjarvard et al. in 2009, subordinate pigs had a significantly higher N/L ratio than dominant pigs kept in stable groups. The same study showed a significant negative relation between N/L ratio and weight (Hjarvard et al., 2009). An explanation for the finding that subordinate pigs may experience more stress, and thus a higher N/L ratio, is the level of environmental controllability. Animals with a high social rank have gained this position because they are able to prevent or end agonistic interactions (Hjarvard et al., 2009). Another environmental factor possibly affecting N/L ration is straw. As is discussed in the paragraph ‘housing’, pigs that live in a barren environment can experience stress from being hampered to show species-specific behaviour like exploring or foraging. This stress could influence the immune system, like the N/L ratio.

3. Materials and methods

3.1 Animals and housing

In this experiment crossbred pigs, offspring of Tempo boars and Topigs 20 sows were used (n=192). The pigs were born at a farm of the Institute for Pig Genetics (IPG) located in Beilen, the Netherlands. Piglets originated from 32 litters in total. Half of the piglets had an estimated high SBV with on average positive estimated effect of +2.72 grams on the daily weight gain (ADG) of their pen mates during the fattening period (from approximately 25 kg until slaughter). The other half had a low SBV and was estimated to have a negative genetic effect on the ADG of their pen mates of on average -1.5 grams. Pre-weaning, the piglets were housed in a partially slatted barren farrowing crate of 3.8m^2 together with their sow or a foster sow. A piglet was cross fostered only (within SBV group) when the original litter size was larger than 14 piglets to standardise the amount of piglets per sow to a maximum of 14 piglets. Males were castrated at 3 days of age. None of the animals were tail docked or tooth clipped.

Following weaning at 4 weeks of age, the animals were transported to the experimental farm 'de Haar' of Wageningen University. At de Haar, the weaned pigs were housed in pens of 6-7 m^2, in one room containing 16 pens, of which eight were enriched with sawdust and straw and eight kept barren. The animals were housed in groups of six per pen, half male and half female. Half of the pens contained animals with a high SBV only, and the other half of animals with a low SBV only. The floor of the barren pens was partially slatted, in enriched pens no part of the floor was slatted. To create a deep-litter system, sawdust (2.9 kg) and straw (1.8 kg) was added to the enriched pens daily. Until 17.5 weeks of age for batch 1 and 11.5 weeks for batch 2 soiled substrate was removed daily. After this age, it was performed weekly. The pens contained one drinking nipple providing the pigs with ad libitum water. Moreover, one feeding trough per pen with ad libitum feed was present. Lights were on from 07:00 until 19:00 h.

The experiment was carried out in two batches of 96 animals each, which were born approximately four weeks after each other. Eight pigs died before the end of the experiment, but their data was used in the data analysis. After weaning, the experiment was set up as a 2x2 arrangement, with Social Breeding Value (SBV) and housing (barren or enriched) as factors. Animals were provided with an extra earmark when they were weaned, this earmark contained their pen and animal number (1-16 and 1-6 respectively). To be able to easily recognise the pigs during behavioural observations, the number of the animal was sprayed on the pig's back with blue marking spray.

3.2 Tests

3.2.1 Behavioural observations

Pigs were observed at 8 ,10 and 21 weeks of age using 2-min instantaneous scan sampling for six hours on one day (see Appendix I for the ethogram). Behaviour was scored during live observations using a hand-held computer using Observer 9.0. The observation hours started at 8.00, 9.15, 10.30, 14.00, 15.15 and 16.30 h.

3.2.2 Regrouping test

At 9-10 weeks of age, animals were subjected to a regrouping test. During the regrouping test, animals were mixed with unfamiliar pigs within the same SBV x housing treatment combination. Mixed groups were formed by placing three pairs of pen mates in an unfamiliar pen similar in size as their home pen. After 24 hours the animals were placed back in their original pen. During mixing, observations were carried out for 3 h using the behavioural observation method mentioned in paragraph 3.2.1. The animals were regrouped at 12:00 and observations started at 14:00. In addition, skin lesions were scored (see 3.2.3) at 24 h after mixing. The animals from batch 1 were quite heavy at mixing, resulting in some lame pigs after regrouping. Therefore, it was decided to regroup pigs of batch 2 one week earlier. This did not affect the lesion score, however, as no significant difference in lesion scoring was found just after regrouping between batches. For brevity, in the text, regrouping will be referred to as if in week 10 for both batches.

3.2.3. Lesion score

Body lesions were scored as a measure of given and received aggression in week 9-10, before the day of regrouping, at 24 h after regrouping and under stable social conditions in week 17. Lesion scores were conducted by counting the number of lesions per body region using a method from Turner et al., (2006). The pig's body was divided into three regions; front middle and rear to be able to discriminate the sort of lesion. Turner et al (2009) found that front and middle lesions indicate mutual fighting and rear lesions indicate that the animal only received aggression (Figure 1).

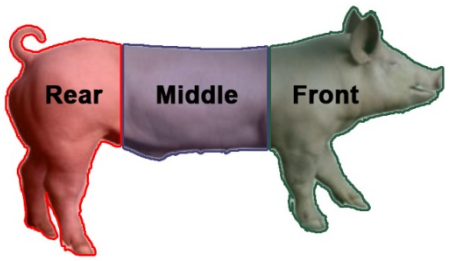


Figure 1: Dividing the body for lesion scoring

3.2.4 Tail bite score

Tail bite scores were recorded at 8, 11 and 22 weeks of age using the method described by (Zonderland, 2008) (see Appendix 2 for pictures). There were 4 classes of tail damage:

1. No tail damage visible
2. The tail lacks hair partially or completely
3. Small damage/bite marks in the size of a pinhead are visible
4. There is a clearly visible wound

3.2.5 Blood sampling

Before regrouping, at 8 weeks of age, three days after regrouping at 10 weeks of age, and at 22 weeks of age, blood samples were drawn from the jugular vein. During sampling, pigs were either restrained on their backs (first two samples) or by using a nose sling (last sample).

From the samples taken at 22 weeks of age from each animal, serotonin uptake in blood platelets was determined in the lab within 2 h after blood collection. Platelet rich plasma (PRP) was gained by low speed centrifugation for 10 min at 160 g after which PRP was collected and platelets were counted for each sample. Serotonin uptake in platelets was determined using the method described by Bolhuis et al. (2009). Briefly, three samples of 100 µl PRP were incubated in phosphate buffer without $CaC1^2$ at a concentration of 5 µM5-HT in the presence of [^{14}C]-5-HT for 1 h at 37 °C. Passive diffusion of [^{14}C]-5-HT was determined by incubating blanks for 1 h at 0 °C and subtracted from the total uptake values at 37 °C to yield the values of active uptake. Uptake velocity was expressed as pmol 5-HT per platelet per min.

To determine the N/L ratio, blood smears were made and stained with the Giemsa method (Cramer et al., 1973), using a rapid red and blue stain. In each smear 100 white blood cells were counted consisting of lymphocytes, monocytes, neutrophils, basophils and eosinophils. The N/L ratio was calculated by dividing the number of neutrophils by the number of lymphocytes.

3.3.6 Statistical analysis

SAS 9.2 was used for all statistical analyses. Variables with skewed distributions were logarithmic (log) or arcsine square root transformed to obtain homogeneity of variance.

For the statistical analysis of the behavioural observations, the following behaviours were grouped (see appendix I for the explanation of these behaviours):

- Social behaviour: gambolling, nosing body, nose contact and mounting
- Explorative: nosing, nosing object, rooting, rooting object, chewing, chewing object and chewing toy
- Manipulative: tail biting, ear biting, manipulating other and belly nosing
- Aggressive: fighting, head knocking, biting and fighting at the feeder
- Non-active: lying inactive and sleeping

These behaviours were chosen because, based on the literature study, they were expected to be related to SBV, and/or influenced by the environment the animals were kept in.

A mixed analysis of variance model was used to assess fixed effects of SBV, housing and their interaction on variables. The model also contained the effect of batch, and the random effect of pen within SBV, housing and batch. Correlations between behavioural observations, lesion scores, serotonin uptake, N/L ratio and tail bite scores were assessed using the Spearman's correlation test on the residuals of the data (measured data minus fitted spectrum).

To be able to determine if an animal was more aggressive than others, or was 'bullied' by pen mates, two extra classes of animals were formed (based on scatter plots which can be found in appendix III):

- Aggressors during stable social conditions: more than 8 lesions on the front of the body
- Receivers during stable social conditions: more than 6 lesions on the rear part of the body
- Aggressors during regrouping: more than 60 lesions on the front of the body
- Receivers during regrouping: more than 40 lesions on the rear part of the body

Animals that were identified to be aggressors and receivers were counted as aggressors only, because lesions on the front part of the body indicates that the animal has shown aggression which makes them an aggressor and not just a receiver. The data were analysed by adding aggressors (yes / no) or receivers (yes / no) as fixed effects to the mixed analysis of variance model, and checking for significant influences on behaviour, lesion score, blood analysis data and tail bite score. To check whether effects of being an aggressor and/or receiver were actually caused by housing or SBV, residuals were calculated of the variables and significant effects found in the mixed analysis of variance model earlier were run again with the residuals corrected for SBV and housing.
All data are presented as means ± SEM.

4. Results

4.1 Behavioural observations

Explorative behaviour was significantly influenced by housing. At 8 weeks of age (before regrouping), on the day of regrouping at 9-10 weeks of age, and at 21 weeks of age, the animals in enriched pens showed more explorative behaviour ($P < 0.05$, see Table 1). In stable social conditions (week 8 and 21), barren housed pigs were less active ($P < 0.05$). Animals in a barren environment showed more manipulative behaviour than animals housed in an enriched environment. At 10 and 21 weeks of age, this effect was significant ($P < 0.01$).

Social behaviour was not influenced by housing. SBV did not affect any of the behaviours shown, and no interaction between housing and SBV was found.

Table 1: Behaviour of pigs with high or low SBV in enriched or barren housing.

Week	Behaviour%	Enriched housing		Barren housing		Effects		
		High SBV	Low SBV	High SBV	Low SBV	H	S	H*S
8: Stable social conditions before regrouping	Social	1.4 ± 0.2	1.23 ± 0.1	1.36 ± 0.1	1.15 ± 0.1	ns	ns	ns
	Explorative	26.34 ± 1.3	24.98 ± 1.2	22.63 ± 1.2	24.07 ± 1.1	*	ns	ns
	Non-active	51.72 ± 1.5	53.65 ± 1.5	57.48 ± 1.7	54.24 ± 1.4	*	ns	ns
	Aggressive	0.25 ± 0.1	0.50 ± 0.1	0.31 ± 0.1	0.29 ± 0.1	ns	ns	ns
	Manipulative	1.13 ± 0.2	1.14 ± 0.1	1.00 ± 0.2	1.16 ± 0.2	ns	ns	ns
10: Regrouping	Social	1.66 ± 0.1	1.46 ± 0.2	1.56 ± 0.2	1.56 ± 0.2	ns	ns	ns
	Explorative	17.95 ± 1.1	14.11 ± 1.0	12.96 ± 0.8	11.97 ± 0.7	*	ns	ns
	Non-active	55.61 ± 2.1	61.25 ± 2.1	61.8 ± 1.5	62.72 ± 1.6	ns	ns	ns
	Aggressive	1.13 ± 0.2	0.89 ± 0.1	1.05 ± 0.2	0.88 ± 0.1	ns	ns	ns
	Manipulative	1.9 ± 0.2	1.69 ± 0.1	2.37 ± 0.2	2.38 ± 0.2	**	ns	ns
21: Stable social conditions after regrouping	Social	0.93 ± 0.13	0.63 ± 0.1	0.85 ± 0.9	0.93 ± 0.2	ns	ns	ns
	Explorative	16.94 ± 1.1	15.83 ± 1.0	12.64 ± 0.9	14.23 ± 1.0	*	ns	ns
	Non-active	67.43 ± 1.3	68.37 ± 1.5	73.61 ± 1.4	70.58 ± 1.3	*	ns	ns
	Aggressive	0.1 ± 0.1	0.08 ± 0.1	0.11 ± 0.1	0.06 ± 1.0	ns	ns	ns
	Manipulative	0.5 ± 0.2	0.16 ± 0.1	0.41 ± 0.1	0.49 ± 0.1	**	ns	ns

*Significances of effects of housing (H), SBV (S) and their interaction (H*S) are indicated in the following way:*

ns = non-significant

** = P < 0.05*

*** = P < 0.01*

4.2 Lesion score

Under stable social conditions, before regrouping at week 10, lesion scores of animals housed in a barren environment were lower than those of animals housed in an enriched environment (Table 2). During the stable social conditions after regrouping, at 17 weeks of age, animals housed in a barren environment had fewer lesions on the front ($P < 0.01$) and rear ($P < 0.05$) part of their body and tended to have fewer lesions at the middle part ($P < 0.10$).

SBV did not affect lesion scores before regrouping, but just after regrouping, animals with a high SBV tended to have more lesions at the middle part of their body than animals with a low SBV ($P < 0.10$). Lesion scores after regrouping were not affected by housing or SBV, except that lesion scores at the middle part of the body tended to be higher for high SBV than for low SBV animals ($P < 0.10$). There were no interactions found between housing and SBV for any of the lesion scores.

Table 2: Lesions scored at the front, middle and rear of the body of pigs with high and low SBV in enriched or barren housing

Week	Lesion score	Enriched		Barren		Effects		
		High SBV	Low SBV	High SBV	Low SBV	H	S	H*S
10: Stable social conditions before regrouping	Front	5.29 ± 0.7	6 ± 0.9	2.55 ± 0.4	2.58 ± 0.3	***	ns	ns
	Middle	6.00 ± 1.0	4.65 ± 0.9	2.5 ± 0.5	2.02 ± 0.4	***	ns	ns
	Rear	2.08 ± 0.3	1.88 ± 0.3	1.09 ± 0.3	0.58 ± 0.2	***	ns	ns
	Total	13.38 ± 1.7	12.5 ± 1.9	5.9 ± 0.9	5.19 ± 0.7	***	ns	ns
10: Regrouping	Front	34.48 ± 4.4	33.06 ± 3.9	35.98 ± 3.8	39.04 ± 4.8	ns	ns	ns
	Middle	35.78 ± 5.4	28.29 ± 3.6	30.75 ± 4.3	26.32 ± 4.1	ns	†	ns
	Rear	14.58 ± 1.9	15.25 ± 2.0	14.9 ± 2.4	15.35 ± 3.0	ns	ns	ns
	Total	84.83 ± 10.5	76.6 ± 8.0	81.6 ± 9.1	80.7 ± 10.5	ns	ns	ns
17: Stable social conditions after regrouping	Front	4.22 ± 0.6	4.13 ± 0.9	2.52 ± 0.4	2.4 ± 0.7	***	ns	ns
	Middle	3.42 ± 0.6	3.44 ± 0.7	1.9 ± 3.6	1.75 ± 0.5	†	ns	ns
	Rear	2.43 ± 0.4	2.13 ± 0.4	1.7 ± 0.3	1.25 ± 0.4	*	†	ns
	Total	10.29 ± 1.4	9.69 ± 1.7	6.1 ± 1.0	5.4 ± 1.4	***	ns	ns

*Significances of effects of housing (H), SBV (S) and their interaction (H*S) are indicated in the following way:*
ns = non-significant
† = P < 0.10
** = P < 0.05*
**** = P < 0.001*

4.3 Blood analysis

4.3.1 Platelet serotonin uptake

Serotonin uptake velocity by platelets at 21 weeks of age was lower for high SBV than for low SBV animals ($P < 0.05$). Housing or its interaction with SBV did not affect serotonin uptake velocity.

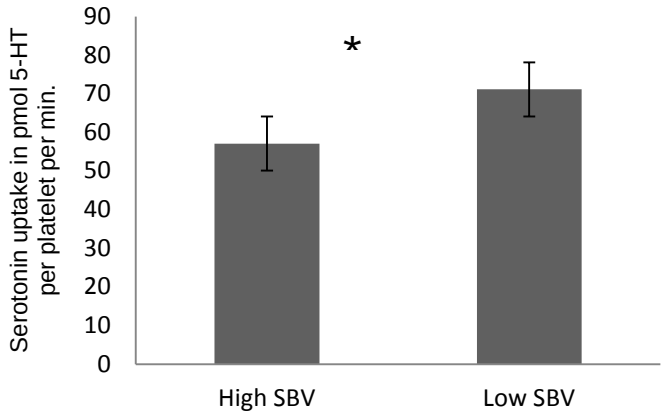


*Figure 1: Serotonin uptake in animals with a high and a low SBV. * = P < 0.05.*

4.3.2 N/L ratio

N/L ratio in animals with a high SBV during the stable social conditions before regrouping was lower than in animals with a low SBV (Table 3). The change of N/L ratio in the stable social conditions to regrouping was also influenced by SBV, with a larger decrease in animals with a low SBV than in animals with a high SBV. Housing influenced N/L ratio at the age of 8 weeks, where animals housed barren had a higher N/L ratio than animals in an enriched housing. After regrouping, barren-housed pigs tended to have a lower N/L ratio than enriched housed pigs ($P < 0.10$). No other effects of SBV, housing and their interactions on N/L ratios were found.

Table 3: N/L ratio of high and low SBV pigs in enriched or barren housing, before regrouping, 3 days after regrouping at 10 weeks of age and at 17 weeks of age under stable social conditions

Week	**Enriched housing**		**Barren housing**		**Effects**		
	High SBV	Low SBV	High SBV	Low SBV	H	S	H*S
8: Stable social conditions before regrouping	0.69 ± 0.1	0.83 ± 0.1	0.83 ± 0.1	0.89 ± 0.1	*	*	ns
10: Regrouping	0.61 ± 0.06	0.56 ± 0.1	0.72 ± 0.1	0.63 ± 0.2	†	ns	ns
17: Stable social conditions after regrouping	0.9 ± 0.1	0.87 ± 0.1	0.83 ± 0.1	0.86 ± 0.1	ns	ns	ns
Δ 8 - 10	-0.08 ± 0.1	-0.28 ± 0.1	-0.1 ± 0.1	-0.26 ± 0.1	ns	*	ns
Δ 10 - 17	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.23 ± 0.1	ns	ns	ns
Δ 8 - 17	0.21 ± 0.1	0.04 ± 0.1	0 ± 0.1	-0.03 ± 0.1	ns	ns	ns

*Significances of effects of housing (H), SBV (S) and their interaction (H*S) are indicated in the following way:*
ns = non-significant
† = P < 0.10
** = P < 0.05*
*** = P < 0.01*
**** = P < 0.001*

4.4 Tail bite scores

Tail bite scores were significantly influenced by housing (P < 0.01, see Table 4). During all stages, pigs housed barren had more tail damage than animals housed in enriched pens. No significant effects of SBV were found, except that during the steady stage before regrouping (at 8 weeks of age), animals with a high SBV tended to have a higher tail damage score than animals with a low SBV (P < 0.10). No interaction between housing and SBV on tail bite score was found.

Table 4: Mean tail bite scores of animals with a high or low SBV, in enriched or barren housing

Week	Enriched housing		Barren housing		Effects		
	High SBV	Low SBV	High SBV	Low SBV	H	S	H*S
8: Stable social conditions before regrouping	1.52 ± 0.1	1.4 ± 0.1	2.38 ± 0.2	1.79 ± 0.1	**	†	ns
11: Regrouping	1.65 ± 0.1	1.6 ± 0.1	2.38 ± 0.1	2.34 ± 0.1	***	ns	ns
22: Stable social conditions after regrouping	1.9 ± 0.2	1.82 ± 0.1	2.67 ± 0.17	2.57 ± 0.2	**	ns	ns

*Significances of effects of housing (H), SBV (S) and their interaction (H*S) are indicated in the following way:*
ns = non-significant
** = *P < 0.01*
*** = *P < 0.001*

4.5 Aggressor and receiver

N/L ratio was not significantly different in aggressors or receivers during this study. Serotonin uptake in blood platelets was significantly higher in receivers than in non-receivers (P < 0.001, see Table 6). Aggressors tended to be more active at 8 weeks (P < 0.10), but significantly less active at 10 weeks, just after regrouping (P < 0.01). Aggressors showed significantly less explorative behaviour than non-aggressors during regrouping. When using the residuals of the variables in the mixed model, corrected for housing and SBV effects, the effects of tail damage on receivers during regrouping, explorative behaviour on aggressors and serotonin on receivers after regrouping were still present.

Table 4: NL-ratio, 5-HT uptake, explorative behaviour, non-active behaviour and tail damage of aggressors and non-aggressors, receivers and non-receivers

Date	Variable					**Effects**	
		Receiver	Non-receiver	Aggressor	Non-aggressor	R	A
Stable social conditions before regrouping	N/L ratio	0.67 ± 0.3	0,81 ± 0.1	0.78 ± 0.1	0.81 ± 0.1	ns	ns
	Explorative	52.5 ± 6.7	44.26 ± 1.6	20.24 ± 1.0	26.79 ± 2.0	ns	ns
	Non active	89.79 ± 9.0	97.68 ± 2.1	98.36 ± 1.6	98.62 ± 1.6	ns	†
	Tail damage	1.06 ± 0.5	1.81 ± 0.1	1.73 ± 0.2	1.80 ± 0.1	†	ns
Regrouping	N/L ratio	0.94 ± 0.3	0.63 ± 0.1	0.61 ± 0.1	0.64 ± 0.1	ns	ns
	Explorative	35.00 ± 2.0	25.43 ± 0.9	20.21 ± 2.0	26.79 ± 1.0	ns	**
	Non active	81.37 ± 2.0	108.19 ± 1.5	117.76 ± 3.0	105.65 ±1.6	ns	**
	Tail damage	2.50 ± 0.8	1.97 ± 0.1	2.04 ± 0.1	1.96 ± 0.1	ns	ns
Stable social conditions after regrouping	N/L ratio	0.65 ± 0.2	0.88 ± 0.1	0.76 ± 0.2	0.87 ± 0.1	ns	ns
	5ht uptake	156.5 ± 23	60.78 ± 5.1	63.99 ± 18.5	60.37 ± 5.2	***	ns
	Explorative	32.00 ± 4.0	27.1 ± 1.2	29.00 ± 3.3	27.24 ± 1.2	ns	ns
	Non active	127.00 ± 6.8	124.00 ± 2.0	118.00 ± 5.0	124.37 ± 2.1	ns	ns
	Tail damage	2.1 ± 0.4	2.2 ± 0.1	2.7 ± 0.3	2.17 ± 0.1	ns	*

*Explorative behaviour and non-active behaviour are shown in frequencies per 6 hours (i.e. number of scans per 180 scans). Significances of effects of housing (H), SBV (S) and their interaction (H*S) are indicated in the following way:*

ns = non-significant

† = P < 0.10

** = P < 0.05*

*** = P < 0.01*

**** = P < 0.001*

4.5 Correlations

In the following paragraphs, correlations between all variables with lesion score, N/L ratio, serotonin uptake and tail bite score are presented. The correlation table of all data can be found as appendix IV.

4.5.1 Lesion score

Total lesion score at 24 h after regrouping was negatively correlated with explorative behaviour and social behaviour on the day of regrouping and positively with time spent on non-active behaviour (P < 0.01). Lesion scores measured 24 h after regrouping and at 17 weeks of age, correlated positively and negatively respectively with manipulative behaviour measured at 8 weeks of age.

Table 5: Spearman correlations on the residuals of the lesion scoring with N/L ratio, behaviours and 5-HTuptake

Week	Variable	LS1	LS2	LS3
Stable social conditions before regrouping	N/L ratio	-0.03	-0.09	-0.02
	Explorative	-0.02	0.02	0.07
	Social	0.03	-0.05	0.07
	Manipulative	-0.05	0.15*	-0.17*
	Non-active	-0.001	0.02	-0.05
Regrouping	N/L ratio	-0.03	-0.03	0.12
	Explorative	0.11	-0.22**	0.06
	Social	0.04	-0.18*	0.02
	Manipulative	-0.09	-0.08	-0.05
	Non-active	0.01	0.25***	-0.04
Stable social conditions after regrouping	N/L ratio	-0.01	-0.04	-0.10
	5-HT uptake	-0.11	0.06	0.07
	Explorative	-0.14	-0.03	0.12
	Social	0.06	-0.04	0.07
	Manipulative	-0.02	0.00	0.04
	Non-active	0.00	0.06	-0.11

LS1 = total lesion score stable social conditions before regrouping, LS2 = total lesion score 24h after regrouping and LS3 = total lesion score stable social conditions after regrouping. Black values indicate that the correlation was calculated between data collected at approximately the same day or week, grey values are correlation between variables assessed at different days or weeks.

† = P < 0.10

** = P < 0.05*

*** = P < 0.01*

**** = P < 0.001*

4.5.2 Blood analysis

Pigs with a higher N/L ratio on day 3 after regrouping were more active during regrouping (P< 0.001). Before and after regrouping, N/L ratio was not correlated with any of the variables. Explorative behaviour shown at 21 weeks of age was negatively correlated with the N/L ratio measured at 8 and 10 weeks of age.

Table 6: Spearman correlations on the residuals of N/L ratios with lesion scores, behaviours, tail damage and 5-HT uptake

Moment	Variable	N/L ratio1	N/L ratio 2	N/L ratio 3
Stable social conditions before regrouping	Lesion score	-0.03	-0.04	-0.01
	Explorative	0.23	-0.27	0.11
	Social	0.04	-0.01	-0.09
	Manipulative	0.05	0.21	0.05
	Non-active	-0.001	-0.08	-0.13†
	Tail damage	0.06	-0.01	0.09
Regrouping	Lesion score	-0.09	-0.03	-0.04
	Explorative	-0.09	0.06	0.04
	Social	0.00	0.1	0.02
	Manipulative	0.07	0.1	0.02
	Non-active	-0.08	-0.35***	-0.03
	Tail damage	0.00	-0.09	0.11
Stable social conditions after regrouping	Lesion score	-0.02	0.12†	-0.10
	Explorative	0.16*	0.22**	0.05
	Social	0.09	0.10	0.06
	Manipulative	0.01	0.09	-0.08

N/L ratio1 = stable social conditions before regrouping, N/L ratio 2 = regrouping and N/L ratio 3 = stable social conditions after regrouping. Black values indicate that the correlation was calculated between data collected at approximately the same day or week, grey values are correlation between variables assessed at different days or weeks.

† = P < 0.10

** = P < 0.05*

**** = P < 0.001*

Serotonin uptake

In the stable social conditions after regrouping, serotonin uptake negatively correlated with manipulative behaviour (P < 0.01, see Table 9). No other significant correlations between serotonin uptake and behaviour were found.

Table 7: Spearman correlations on the residuals of serotonin uptake with behaviour

Moment	Variables	5-HTuptake
Stable social conditions before regrouping	Explorative	0.02
	Aggressive	-0.07
	Social	-0.08
	Manipulative	0.08
	Non-active	0.04
	Tail damage	-0.1
Regrouping	Explorative	-0.12
	Aggressive	0.02
	Social	0.03
	Manipulative	0.07
	Non-active	0.13†
	Tail damage	0.03
Stable social conditions after regrouping	Explorative	-0.1
	Aggressive	-0.01
	Social	0.05
	Manipulative	-0.19**
	Non-active	0.09
	Tail damage	-0.17

† = P < 0.10

** = P < 0.05*

*** = P < 0.01*

4.5.3 Tail bite scores

Tail bite score during the stable social conditions before regrouping correlated positively with manipulative behaviour (P < 0.01, see Table 10). During regrouping, tail damage correlated negatively with lesion scoring measured 24 h after regrouping (P < 0.05).Total lesion score (the sum of lesion scores at all three body parts) measured at 17 weeks of age correlated positively with tail damage measured at 8 and 11 weeks of age.

Table 8: Spearman correlations on the residuals of tail bite scores with lesion score and behaviours

Observation day	Variables	Tail damage 1	Tail damage 2	Tail damage 3
Stable social conditions before regrouping	Lesion score	0.07	-0.04	-0.14
	Explorative	-0.05	-0.08	-0.15*
	Aggressive	0.09	0.03	-0.20**
	Social	0.08	-0.01	-0.06
	Manipulative	0.23**	0.04	-0.13†
	Non-active	0.01	-0.03	0.07
Regrouping	Lesion score	0.02	0.17*	-0.06
	Explorative	-0.09	-0.09	-0.05
	Aggressive	-0.04	0.03	0.04
	Social	-0.03	-0.08	-0.1
	Manipulative	0.12†	0.1	0.09
	Non-active	0.1	0.03	0.02
Stable social conditions after regrouping	5-HT uptake	-0.1	-0.15*	-0.17
	Lesion score	0.14*	0.18*	-0.05
	Explorative	0.07	-0.08	-0.04
	Aggressive	-0.04	0.11	-0.05
	Social	0.04	-0.07	-0.07
	Manipulative	-0.07	-0.01	-0.12
	non-active	-0.04	-0.18*	0.08

Tail damage 1 = stable social conditions before regrouping, Tail damage 2 = regrouping and Tail damage 3 = stable social conditions after regrouping. Black values indicate that the correlation was calculated between data collected at approximately the same day or week, grey values are correlation between variables assessed at different days or weeks.

† = P < 0.10

** = P < 0.05*

*** = P < 0.01*

5. Discussion

In this chapter, the results of this study are discussed and compared with previously performed research. Behavioural observations are discussed first, followed by the results of the lesion score, blood analysis and tail bite score.

5.1 Behavioural observations

Housing, (i.e. in this study absence or presence of straw bedding) did not significantly influence aggressive behaviour under stable social conditions during any of the observation days. This is in accordance with findings in earlier studies (Arey et al., 1998, Waran and Broom, 1993). When enrichment was found to influence aggressive behaviour, this was in combination with extra space allowance for animals housed enriched (Beattie et al., 2000). Like found in numerous other studies (for instance Kelly et al., 2000, Burbridge et al., 1991 and Spoolder et al., 1995), explorative behaviour and active behaviour in this study was shown more by animals housed enriched. Also, manipulative behaviour was influenced by housing during this experiment. When housed in a barren environment, the animals showed more manipulative behaviour. This is in accordance with findings in other studies where it is concluded that straw provision helps decreasing manipulative behaviour directed towards pen mates (Fraser et al., 1999, Burbridge et al., 1994 and Bolhuis et al., 2005).

SBV had no significant effects on the behaviours that were observed. This is contradictory to the expected results where the heritable effects of pigs on the traits of their pen mates were expected to be expressed in behavioural differences. This was expected because SBV is determined by heritable weight gain effects of pigs on their pen mates, which could most likely be explained by behavioural differences. In a study by de Vries (cited in Rodenburg, 2010), animals with a high SBV showed more aggression during regrouping. Canario et al (2009) did a similar study and found similar results, but in the current study no such result was found. The difference between the studies by Canario et al. (2009), de Vries (cited in Rodenburg, 2010) and the current study is that their experiments did not include barren vs. enriched housing. In the current study animals with a high and low SBV did not cope with the environment in a different way, this suggests that the environmental effect ruled out the SBV effect and that in this study, enrichment by straw was a more potent solution for manipulative behaviour than SBV. It could also be that there was a behavioural difference, but that by grouping behaviours, the scan sampling method or simply not including that behaviour in the ethogram, this behavioural difference was not recorded. It could be that the animals did not show a difference in agonistic behaviour but that they were more or less anxious, causing a more or less nervous atmosphere in the pens. Thus behaviour or tests reflecting nervousness and anxiety could perhaps be included in the future. The finding that SBV did significantly influence the internal parameters, i.e. N/L ratio and serotonin, underlines this. It could also be true that the animals are more prone to show more manipulative or aggressive behaviour when provoked to do so, but that the current environments did not stimulate them enough to show this difference. Another explanation is that the number of observations, or the number of animals was not sufficient to identify a distinct difference in behaviour

between the two SBV groups. For instance Canario et al. (2009), used 1440 pigs in his study, in this study 192 pigs were used.

5.2 Lesion score

Animals housed in a barren environment had fewer lesions during the stable social conditions before regrouping. During regrouping, no significant effects of housing were found but after regrouping, animals housed barren had fewer lesions again. These findings are not supported by the behavioural observation data, as no significant influence of housing on aggression was found. This is contrary to other research, where the number of lesions was positively correlated to the amount of aggressive behaviour shown during regrouping (Turner et al., 2006, Barnett et al., 1992). In earlier studies by Arey in 1998 and Waran and Broom in 1993, no influence of straw on aggression was found. In studies that did find animals in enriched pens to show more aggressive behaviour, straw provision was limited (Morgan et al., 1998), or the animals were floor-fed (Whittaker et al., 1999). This was not the case during the current study. It could be that the scan sampling method used to measure behaviour was not suitable to record aggressive behaviour. In other research, continuous observations are often used to measure aggressive behaviour combined with lesion scoring (e.g. Barnett et al., 1996, Turner al., 2006, O'Connel and Beattie, 1999). When using scan sampling with a 2 minute interval, aggressive behaviour could be missed. As the amount of aggressive behaviour did not seem to be influenced by the environment, the intensity may differ between housing or other causes may underlie this difference in lesion score. In literature, some studies have not found a difference in lesion scoring between barren and straw-enriched housing system (Scott et al., 2006; 2007). Munsterhjelm et al.(2009) found a higher lesion score in straw enriched environments than in a barren environment during the nursery phase. Their explanation is that the lesions could be caused by the fact that the animals in an enriched environment were more active. During the current study, this could be the true as well.

During regrouping, no effect of housing was found. This is in line with earlier findings (Waran and Broom, 1993; and Arey and Franklin, 1995) where pigs were used that were housed in a barren farrowing pen, just like the pigs in the current study. But by starting observations during regrouping two hours after the event, the most serious fights could have been missed and instead most of the behaviour seen was due to exhaustion from fighting. In earlier studies (e.g. Erhard et al., 1997) the first two hours after mixing were the target-hours to observe, and it is concluded that the majority of fighting actively occurs the first two hours after regrouping (Luescher et al., 1990; Tan and Shackleton, 1990). Results that were found 2 h after regrouping showed that explorative behaviour and lesion scoring were strongly negatively correlated and non-active behaviour and lesion scoring were positively correlated. These results both indicate that animals that were less active during regrouping had more lesions in this study. This might be due to the intense fighting the animals showed during regrouping, which may have resulted in exhaustion and consequently in non-active behaviour between two consecutive fights.

SBV did not influence lesion scoring during this experiment. This is contradictory to the expected results, and earlier findings. In an experiment by De Vries (cited in Rodenburg, 2010), 12 groups of pigs were formed with a high social breeding value and 12 groups with a low social breeding value. In this study, pigs with a high social breeding value had higher lesion scores, suggesting that they were involved in more and more severe regrouping fights, while they fought less when the dominance order had been established at 6 weeks post-mixing. Canario *et al*. (2010) did a similar experiment, but they formed mixed groups of high and low SBV's. They found similar results with the addition that animals with a high SBV had more lesions to the rear part of their body three weeks post-mixing. This indicates that animals with a high SBV in these studies fought more during regrouping, but less under socially stable conditions indicating that their social hierarchy was more stable and therefore needed less aggressive behaviour to secure under socially stable conditions. The study of Canario et al. (2010) indicated that animals with a high SBV were bullied more often in socially stable conditions. The difference in experimental set-up between the study of de Vries and Canario is that Canario mixed high and low SBV, while de Vries separated them. This suggests that animals with a high SBV will retreat from a fight, while animals with a low SBV do not and keep biting the back side of the high SBV animals. These conclusions were not found during the current study, neither in the behavioural observation data nor in the lesion scoring data. This could mean that the influence of housing overruled the influence of SBV, as in the studies of de Vries and Canario no difference in environmental enrichment between the treatment groups was present.

5.3 Blood analysis

5.3.1 Platelet serotonin uptake

The results of the current study show that housing did not influence platelet serotonin uptake. This is as hypothesized, because housing was not suspected to influence serotonin uptake itself. Serotonin uptake in animals was expected to make animals more prone to show certain behaviour during stressful events, which may be evoked by the barren environment. Other aspects of the serotonergic system like receptors and serotonin level may be influenced by housing, but serotonin uptake in this study was not influenced by environment, only by genetics.

The uptake of serotonin in blood platelets was significantly influenced by SBV. Animals with a high SBV had a lower serotonin uptake than animals with a low SBV. The method used during this experiment was to measure serotonin uptake in platelets and to assess the relationships of serotonin on behaviour. The most common used approach is to measure the level of serotonin in the CNS. But there are restrictions on this method, for instance by the more invasive character of measuring brain serotonin level compared to blood serotonin level. Therefore, in multiple studies blood serotonin levels were used. In these studies significant correlations between blood serotonin level and aggression, psychiatric disorders and other behavioural disfunctioning was found (Banki, 1978; Mann et al., 1992; Quintana, 1992; Moffitt et al., 1998). This could be caused by the similar uptake in brain and blood serotonin, which could mean that when uptake velocity is high in blood platelets, it is high in the brain as well. This is underlined by the finding that alterations in blood serotonin have been found to positively correlate with brain serotonin levels measured in rats (Malyszko et al., 1993) and the finding

that in mammals the brain serotonin transporter is identical to the blood platelet transporter (Lesch et al., 1993). Also, studies have shown that the protein accountable for the uptake in the CNS, and in the blood is synthesized by the same gene (Moreno et al., 2005). Therefore blood serotonin and its uptake can be seen as a good biological marker for brain serotonin (Barrett et al., 2006), when comparing blood uptake and behaviour.

In a study by Bolhuis et al., performed in 2009, chickens from a low mortality line were selected which showed less feather pecking. These animals had a lower serotonin uptake and showed less anxiety than control animals. This indicates that animals with a low SBV in this study may be more anxious as well, and would react so when provoked to. A study by Oehrberg and Christiansen in 1995 showed that treatment with paroxetine, which is a selective serotonin uptake inhibitor, significantly reduced the amount of panic attacks shown by patients. This underlines the anxiety reducing properties of a lower serotonin and with this a higher serotonin action. In platelet serotonin uptake, a higher serotonin uptake in suicidal depressed and non-suicidal depressed patients was found when compared to healthy controls (Roggenbach et al., 2006). The conclusion that could be drawn from this is that the behaviour measured in this study was not affected by SBV, but based on the difference in 5-HT uptake the animals with a low SBV might be more prone to show manipulative and aggressive behaviour and to experience anxiety.

5.3.2 N/L ratio

Housing significantly influenced N/L ratio during life week 8, where animals in enriched housing had a lower N/L ratio than animals in barren housing. This is as hypothesized and can be explained by a lower stress level in animals housed in enriched environments. Even though N/L ratio is often used as a measurement of acute stress, studies have indicated that chronic stress like social stress influences N/L ratio as well (Hjarvard et al., 2009, Sutherland et al., 2006). In a study done by Nazar & Marin (2011), Japanese quail exposed to a chronic stressor showed a significantly higher H/L ratio (N/L ratio in birds) than animals that were not exposed to a chronic stressor. This indicated that N/L ratio is influenced by chronic stress. Chronic stress induced by immobilisation for three hours a day during 11 days has been shown to decrease the number of lymphocytes and increase the neutrophil count in rats (Steplewski et al., 1986). If this also holds for pigs, then the difference in N/L ratio, as mentioned in paragraphs 2.2 and 5.1, could be caused by the fact that straw provided an outlet for highly motivated foraging and exploration behaviour and prevented the redirection of this behaviour towards pen mates. Studies have shown that the absence of enrichment is related to behavioural signs of stress (Munstjerhelm et al., 2009). Animals housed barren have been found to experience more stress when exposed to a stressor (de Jonge et al., 1996) and inability to show foraging and explorative behaviour is experienced as a stressor for pigs expressed by the increase in abnormal behaviour (Beattie et al., 2000, Zonderland et al., 2008, Munsterhjelm et al., 2009) which is shown in this study as well. Therefore it works both ways; the animals themselves experience less stress because they can show certain behaviour, which prevents them from manipulating other animals, who would have been stressed by this attention.

During this experiment, animals with a high SBV had a lower N/L ratio before regrouping than animals with a low SBV. As mentioned above, chronic stress has been found to increase N/L ratio, this could mean that animals with a low SBV during this study experienced more chronic stress than animals with a high SBV. This was not directly supported by the behavioural observations, lesion scoring or tail damage data, as SBV did not influence any of these variables. It must be noted though that N/L ratio, like serotonin and all other physiological aspects, is a very complicated factor. It is influenced by hormones and other parts of the immune system, making it difficult to draw a conclusion from the results found.

De Jong and Leek (2004) found that N/L ratio increased when the animals were regrouped. In this study, the opposite result was found as the N/L ratio decreased from before regrouping to regrouping and rose again after regrouping. In earlier studies, it was indicated that higher N/L ratio was correlated to acute stress (Wallgren et al., 1994, Puppe et al., 1997, de Jong et al., 1999 and Leek et al., 2004). The difference with the current research and the previously mentioned studies is that they measured N/L ratio on the day of the stressor. Puppe et al. (1997) measured N/L ratio 1 day before, on the day of and 4 days after weaning. They found that 4 days after weaning the N/L ratio was returned to its normal state as measured one day before weaning. Therefore the effect of regrouping on the animals might be missed by measuring 3 days after the event. In the study mentioned earlier by Steplewski et al.(1986), where chronic stress was inflicted on mice, it was found that 12 days after the stress period the percentage of lymphocytes had increased above normal values and neutrophils significantly decreased. This could be an explanation for the fact that N/L ratio was found to be lower 3 days after regrouping; an overcompensating process of increasing lymphocytes and decreasing neutrophils might have been measured. Therefore, in future research it will be important to sample the blood on the same day of regrouping to be able to measure the exact effect. The change in N/L ratio is significantly influenced by SBV when looking at stable social conditions before regrouping to regrouping. The difference in N/L ratio during the stable social conditions before regrouping and during regrouping was greatest in animals with a low SBV. Therefore it seems likely that the regrouping test had a greater impact on animals with a low SBV than on animals with a high SBV. When looking at the different batches, a distinct difference in N/L ratio's during regrouping was visible. The N/L ratio of batch 1 was higher than batch 2 (data not shown). This could be due to the fact that the animals from batch 1 received antibiotics for respiratory problems in their ninth week of life, in between the first two blood sampling moments for respiratory problems. Another cause could be that animals in batch 1 were older when they were regrouped, causing them to be heavier, and fight more intensely which caused some pigs to become lame. Another explanation could be a subclinical infection of the lesions due to regrouping; this has been known to cause more inactive behaviour (sickness behaviour, reviewed by Dantzer et al., 2004).

A positive correlation was found between non active behaviour measured during regrouping and N/L ratio at day 3 after regrouping. A conclusion could be that animals that were less active during regrouping experienced more stress until 3 days after regrouping. But related to the finding that lesion score and non-active behaviour were positively correlated during regrouping could mean they fought

more during regrouping, could have caused this positive correlation between non active behaviour and N/L ratio.

5.4 Tail bite scores

The tail bite score differed between barren and enriched housing during this experiment. In enriched housing, the mean tail damage score was lower than in barren housing. This is supported by literature, where enrichment like straw serves as an outlet for exploratory behaviour. When not given the opportunity to forage and root, animals can become frustrated and redirect this behaviour towards pen mates (Young et al., 1994, Day et al., 2002, Beattie and O'Connell, 2002, Munsterhjelm et al., 2009 and McKinnon et al., 1989).

During regrouping, tail damage was negatively correlated with lesion scoring. This indicates that the animals that received a lot of aggressive behaviour (which could be due to mutual fighting), received less tail biting behaviour. Lesion score measured at 17 weeks of age correlated positively with tail damage measured at 8 and 11 weeks of age. This could mean that aggression increases together with manipulative behaviour. From these findings it should not be concluded that barren environments cause more pigs to show tail biting behaviour, as one pig can cause a lot of tail damage (Zonderland et al., 2008). Therefore the conclusion should be that barren housing is related to more tail damage.

SBV did not significantly influence tail damage score. This is in accordance to the finding that manipulative behaviour was not influenced by SBV. As mentioned earlier, SBV did not affect any behaviour, which is in line with these findings. An interaction between housing and SBV was not found either, which could be caused by the finding that SBV did not influence tail damage and therefore there was no effect to interact with.

5.5 Aggressors and receivers

When looking at the characteristics of aggressors and receivers, N/L ratio did not differ significantly. Serotonin uptake was significantly higher at 17 weeks of age in receivers than in non-receivers of aggression. Soubrié (1986) reviewed that blocking the serotonin transmission was related to more impulsive animals that act with aggression earlier. As receivers showed less aggression, possibly related with their lower serotonin uptake, this could mean they retreated from a fight more than non-receivers did and got bitten in the rear part of their body causing lesions there. Tail damage was lower in receivers than in non-receivers, which indicates that animals that received a lot of aggression had less tail damage. This could mean that the animals avoid pen mates, do not lay in close proximity to pen mates which means their tail will get bitten less as well.

Just after regrouping aggressors showed significantly less explorative behaviour than non-aggressors. As has been shown in the lesion score results, non-active behaviour and less explorative behaviour was related to higher levels of aggression. Therefore the finding that aggressors showed less explorative behaviour could be due to the animals fighting more, which exhausts them and decreases the amount of explorative behaviour shown.

6. Conclusion

The main conclusion that can be drawn from this study is that animals diverging in SBV did not significantly differ in home pen behaviour, lesion scores or tail damage. Pigs with a relatively high vs. low SBV did differ, however, in platelet serotonin uptake and N/L ratios. It is possible that the sample size was too low to reveal behavioural differences in the high and low SBV pigs, but it could also be that the behavioural difference was not recorded. Therefore in future research an anxiety test could be useful to test whether the behavioural difference lies in anxiety. This anxiety test could best be done in the home pen of the animals, to not only examine the individual reactions of the animals but their reaction as a group as well. The anxiety test could be performed by simply letting someone walk into the home pen of the animals and then recording their reactions like vocalisations and behaviour.

Housing did, as expected, highly influence behaviour where enrichment largely prevented tail damage and manipulative behaviour and stimulated active and explorative behaviour. Aggressive behaviour under stable social conditions, as measured by lesion scores was influenced by housing as well, but unlike expected, animals in barren housed had less lesions than animals in enriched housing

Housing was found to have a greater impact on behaviour during this experiment than SBV. This indicates that, supported by the absence of interactions between SBV and housing, high and low SBV animals did not cope with a barren of enriched environment differently.

References

Andersen, I. L., E. Nævdal, M. Bakken, and K. E. Bøe. 2004. Aggression and group size in domesticated pigs, sus scrofa: 'when the winner takes it all and the loser is standing small'. Animal Behaviour 68: 965-975.

Arey, D.S., Edwards, S.A. 1998. Factors influencing aggression between sows after mixing and the consequences for welfare and production. Livestock Production Science 56: 61-70.

Arey, D.S., Franklin, M.F. 1995. Effects of straw and unfamiliarity on fighting between newly mixed growing pigs. Applied Animal Behaviour Science 45: 23-30.

Bach, A. W. J. et al. 1988. DNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. Proceedings of the National Academy of Sciences 85: 4934–4938.

Banki, M. C. 1978. Serotonin concentration and monoamine oxidase activity in the blood of patients with endogenous psychoses. ORV. HETIL. 119: 2245-2248.

Barnett, J.L., Hemsworth, P.H., Cronin, G.M., Newman, E.A., McCallum, T.H., Chilton, D. 1992. Effects of pen size, partial stalls and method of feeding on welfare-related behavioural and physiological responses of group-housed pigs. Applied Animal Behaviour Science 34: 207-220

Barrett, G. M., M. Bardi, A. K. Z. Guillén, A. Mori, and K. Shimizu. 2006. Regulation of sexual behaviour in male macaques by sex steroid modulation of the serotonergic system. Experimental Physiology 91: 445-456.

Beattie, V. E., and N. E. O'Connell. 2002. Relationship between rooting behaviour and foraging in growing pigs. Animal Welfare 11: 295-303.

Beattie, V.E., O'Connell, N.E., Moss B.W. 2000. Influence of environmental enrichment on the behaviour, performance and meat quality of domestic pigs. Livestock Production Science 65: 71–79

Bergsma, R., Kanis, E., Knol, E.F. and Bijma, P. 2008. The contribution of social effects to heritable variation in finishing traits of domestic pigs (*sus scrofa*). Genetics 178: 1559-1570.

Best, J., H. F. Nijhout, and M. Reed. 2010. Serotonin synthesis, release and reuptake in terminals: A mathematical model. Theor. Biol. Med. Model. 7.

Bolhuis J.E., Schouten, W.G.P., Schrama, J.W., Wiegant V.M. 2006. Effects of rearing and housing environment on pigs with different coping characteristics. Applied Animal Behaviour Science 101:68-85.

Bolhuis, J. E. et al. 2009. Effects of genetic group selection against mortality on behavior and peripheral serotonin in domestic laying hens with trimmed and intact beaks. Physiology & Behavior 97: 470-475.

Burbidge, J.A., Spoolder, H.A.M., Lawrence, A.B., Simmins, P.H., Edwards, S.A. 1994. The effect of feeding regime and the provision of a foraging substrate on the development of behaviours in group-housed sows. Applied Animal Behaviour Science 40: 72

Campbell, N. A., and J. A. Reece. 2005. Biology. 7th ed. Benjamin Cummings.

Campo, J. L., Prieto, M. T., Dávila S. G. 2008. Effects of housing system and cold stress on heterophil-to-lymphocyte ratio,fluctuating asymmetry, and tonic immobility duration of chickens. Poultry Science 87:621–626.

Canario, L. et al. 2009. Genetic associations of group effects for growth, estimated using a cooperation model, with post-mixing agonistic behaviours, skin lesions and activity in pigs. In: UFAW International Symposium: Darwinian selection, selective breeding and the welfare of animals, Bristol, UK

Chen, C. Y. et al. 2009. Influence of heritable social status on daily gain and feeding pattern in pigs. Journal of Animal Breeding and Genetics 127: 107-112.

Chen, C. Y., Johnson, R. K., Newman,S., Van Vleck, L.D. 2007. A general review of genetic competition effects with an emphasis on swine breeding. Genetics and Molecular Research 6: 594-606.

Cramer, A.D., Rogers, E.R., Parker, J.W., Lukes, R.J. 1973. The Giemsa stain for tissue sections: an improved method. American Journal of Clinical Pathology 60: 148-156

Daruna, J.H. and Kent, E.W. 1976. Comparison of regional serotonin levels and turnover in the brain of naturally high and low aggressive rats. Brain Research 101: 489-501.

Day, J. E. L. et al. 2002. The effects of prior experience of straw and the level of straw provision on the behaviour of growing pigs. Applied Animal Behaviour Science 76: 189-202.

De Jong, I. C., E. Lambooij, S. M. Korte, H. J. Blokhuis, and J. M. Koolhaas. 1999. Mixing induces long-term hyperthermia in growing pigs. Animal Science 69: 601-605.

Erhard, H.W., Mendl, M., Ashley, D.D., 1996. How information about individual pigs can help to reduce aggression and injuries after mixing. Animal Science 62: 623–624.

Evenden, J. L. 1999. The pharmacology of impulsive behaviour in rats VII: The effects of serotonergic agonists and antagonists on responding under a discrimination task using unreliable visual stimuli. Psychopharmacology 146: 422-431.

Fišar, Z. 2010. Inhibition of monoamine oxidase activity by cannabinoids. Naunyn-Schmiedeberg's Archives of Pharmacology 381: 563-572.

Fraser, D., Phillips, P.A., Thompson, B.K., Tennessen,T.1991.Effect of straw on the behaviour of growing pigs. Applied Animal Behaviour Science 30: 307-318.

Heinrichs, S.C., Richard, D. 1999. The role of corticotropin-releasing factor and urocortin in the modulation of ingestive behavior. Neuropeptides 33: 350–359.

Hjarvard, B. M., O. N. Larsen, H. R. Juul-Madsen, E. Jørgensen, and K. H. Jensen. 2009. Social Rank Influences the Distribution of Blood Leukocyte Subsets in Female Growing Pigs. Scandinavian Journal of Laboratory Animal Science 36: 309-320.

Jia, Z., and Q. Zhu. 2010. 'Click' assembly of selective inhibitors for mao-a. Bioorganic & Medicinal Chemistry Letters 20: 6222-6225.

Jones, B. J., and T. P. Blackburn. 2002. The medical benefit of 5-ht research. Pharmacology Biochemistry and Behavior 71: 555-568.

Dantzer, R., 2004. Cytokine-induced sickness behaviour: A neuroimmune response to activation of innate immunity. European Journal of Pharmacology 500: 399-411

De Jonge, F.H., Bokkers, E.A.M., Schouten, W.G.P., Helmond, F.A. 1996. Rearing piglets in a poor environment: Developmental aspects of social stress in pigs. Physiology and Behavior 60: 389-396

Kahn, R.S., Westenberg, H.G.M., Verhoeven, W.M.A., Gispen-de Wied, C.C., Kamerbeek, W.D.J. 1987. Effect of a serotonin precursor and uptake inhibitor in anxiety disorders: A double-blind comparison of 5-hydroxytryptophan, clomipramine and placebo, International Clinical Psychopharmacology 2 : 33.

Kelly, H. R. C., J. M. Bruce, P. R. English, V. R. Fowler, and S. A. Edwards. 2000. Behaviour of 3-week weaned pigs in Straw-Flow®, deep straw and flatdeck housing systems. Applied Animal Behaviour Science 68: 269-280.

Krakowski, M. 2003. Violence and Serotonin: Influence of Impulse Control, Affect Regulation, and Social Functioning. Journal of Neuropsychiatry and Clinical Neuroscience 15: 294-305.

Leek, A. B. G., B. T. Sweeney, P. Duffy, V. E. Beattie, and J. V. O'Doherty. 2004. The effect of stocking density and social regrouping stressors on growth performance, carcass characteristics, nutrient digestibility and physiological stress responses in pigs. Animal Science 79: 109-119.

Lesch, K.-P., Wolozin, B.L., Murphy, D.L., Riederer, P. 1993. Primary structure of the human platelet serotonin uptake site: Identity with the brain serotonin transporter. Journal of Neurochemistry 60: 2319-2322

Long, A.B., Kuhn, C.M., Platt, M.L. 2009. Serotonin shapes risky decision making in monkeys. Social Cognitive and Affective Neuroscience 4: 346-356

Luescher, U.A., Friendship, R.M., McKeown, D.B.1990. An evaluation of methods to reduce fighting among regrouped gilts. Journal of Animal Science 70: 363–370.

Maitre, L., Moser, P., Baumann, P.A., Waldmeier, P.C. 1980. Amine uptake inhibitors: Criteria of selectivity. Acta Psychiatrica Scandinavica Volume 61: 97-110

Malyszko, J. et al. 1993. Serotonergic measures in blood and brain and their correlations in rats treated with tranylcypromine, a monoamine oxidase inhibitor. Japanese Journal of Physiology 43: 613-626.
Mann, J. J. et al. 1992. Relationship between central and peripheral serotonin indexes in depressed and suicidal psychiatric inpatients. ARCH. GEN. PSYCHIATRY 49: 442-446.
McKinnon, A. J., S. A. Edwards, D. B. Stephens, and D. E. Walters. 1989. Behaviour of groups of weaner pigs in three different housing systems. Br. Vet. J. 145: 367-372.
Moffitt, T. E. et al. 1998. Whole blood-serotonin relates to violence in an epidemiological study. Biol. Psychiatry 43: 446-457.
Morgan, C.A., Deans, L.A., Lawrence, A.B., Nielsen, B.L., 1998. The effects of straw bedding on the feeding and social behaviour of growing pigs fed by means of single-space feeders. Applied Animal Behaviour Science 58: 23-33
Moore, A. S., H. W. Gonyou, J. M. Stookey, and D. G. McLaren. 1994. Effect of group composition and pen size on behavior, productivity and immune response of growing pigs. Applied Animal Behaviour Science 40: 13-30.
Moreno, J., M. G. Campos, C. Lara, and C. Torner. 2005. The platelet as biological marker of the neural serotonergic function. Salud Ment. 28: 79-87.
Muir, W. M. 2005. Incorporation of competitive effects in Forest Tree or animal breeding programs. Genetics 170: 1247-1259.
Muir, W. M. and A. Schinkel. 2002. Proceedings of the 7th World Congress on Genetics Applied to Livestock Production "Incorporation of competitive effects in breeding programs to improve productivity and animal well being". Communication No. 14-07. Montpellier, France.
Mulliver, M.E. 1987. Serotonergic neuronal systems: What their anatomic organization tells us about function. Journal of Clinical Psychopharmacology 7: 3-23
Munsterhjelm, C. et al. 2009. Experience of moderate bedding affects behaviour of growing pigs. Applied Animal Behaviour Science 118: 42-53.
Mylecharane, E. J. 1989. The classification of 5-hydroxytryptamine receptors. Clinical and Experimental Pharmacology and Physiology 16: 517-522.\
Nazar, F.N., Marin, R.H. 2011. Chronic stress and environmental enrichment as opposite factors affecting the immune response in Japanese quail (Coturnix coturnix japonica). Stress 14: 166-173
Oehrberg. S. and Christiansen, P. 1995. Paroxetine in the treatment of panic disorder. A randomised. double-blind, placebocontrolled study. Brain Journal of Psychiatry 167: 374-379.
Ostrowitzki, S., M. L. Rao, J. Redei, and A. H. Andres. 1993. Concurrence of cortex and platelet serotonin2 receptor binding characteristics in the individual and the putative regulation by serotonin. J. NEURAL TRANSM. GEN. SECT. 93: 27-35.
Puppe, B., M. Tuchscherer, and A. Tuchscherer. 1997. The effect of housing conditions and social environment immediately after weaning on the agonistic behaviour, neutrophil/lymphocyte ratio, and plasma glucose level in pigs. Livestock Production Science 48: 157-164.
Quintana, J. 1992. Platelet serotonin and plasma tryptophan decreases in endogenous depression. Clinical, therapeutic, and biological correlations. Journal of Affective Disorders 24: 55-62.
Richard, H. a. 1999. The role of corticotropin-releasing factor and urocortin in the modulation of ingestive behavior. Neuropeptides 33: 350–359.
Roggenbach, J., Müller-Oerlinghausen, B., Franke, L., Uebelhack, R., Blank, S., Ahrens, B. 2007. Peripheral serotonergic markers in acutely suicidal patients. 1. Comparison of serotonergic platelet measures between suicidal individuals, non-suicidal patients with major depression and healthy subjects. Journal of Neural Transmission 114: 479-487
Rodenburg, T. B. et al. 2010. Breeding amiable animals? Improving farm animal welfare by including social effects in breeding programmes. Animal Welfare 19: 77-82.
Scott, K., Chennells, D.J., Campbell, F.M., Hunt, B., Armstrong, D., Taylor, L., Gill, B.P., Edwards, S.A. 2006. The welfare of finishing pigs in two contrasting housing systems: Fully-slatted versus straw-bedded accommodation. Livestock Science 103: 104-115

Scott, K., Taylor, L., Gill, B.P., Edwards, S.A. 2007. Influence of different types of environmental enrichment on the behaviour of finishing pigs in two different housing systems. 2. Ratio of pigs to enrichment. Applied Animal Behaviour Science 105: 51-58.

Soubrié, P. 1986. Reconciling the role of central serotonin neurons in human and animal behavior. Behavioral Brain Science 9: 319–364.

Spoolder, H. A. M., J. A. Burbidge, S. A. Edwards, P. Howard Simmins, and A. B. Lawrence. 1995. Provision of straw as a foraging substrate reduces the development of excessive chain and bar manipulation in food restricted sows. Applied Animal Behaviour Science 43: 249-262.

Steplewski, Z., Wolfgang H. 1986. Total leukocytes, T cell subpopulation and natural killer (NK) cell activity in rats exposed to restraint stress. Vogel Life Sciences 38: 2419-2427

Tan, S.S.L., Shackleton, D.M. 1990. Effects of mixing unfamiliar individuals and of azaperone on the social behaviour of finishing pigs. Applied Animam Behaviour Science 26: 157–168

Tizard, I. A. 2004. Veterinary immunology, an introduction. seventh edition ed. Elsevier.

Turner, S.P., Farnworth, M.J., White, I.M.S., Brotherstone, S., Mendl, M., Knap, P., Penny, P., Lawrence, A.B. 2006. The accumulation of skin lesions and their use as a predictor of individual aggressiveness in pigs. Applied Animal Behaviour Science 96: 245-259.

Wallgren, P., I. L. Wilén, and C. Fossum. 1994. Influence of experimentally induced endogenous production of cortisol on the immune capacity in swine. Veterinary Immunology and Immunopathology 42: 301-316.

Walsh, C., C. Douglas, A. Bédué, M. Bateson, and S. Edwards. 2010. Proceeding of the UFAW Animal Welfare Conference "Tests of cognitive bias can inform on pigs subjective affective state". In: Recent advances in animal welfare science, York, U.K.

Waran, N.K. and Broom, D.M. 1993. The influence of a barrier on the behaviour and growth of early-weaned piglets. Animal Production 56: 115–119.

Wemelsfelder, F., I. Nevison, and A. B. Lawrence. 2009. The effect of perceived environmental background on qualitative assessments of pig behaviour. Animal Behaviour 78: 477-484.

Van De Weerd, H. A., C. M. Docking, J. E. L. Day, P. J. Avery, and S. A. Edwards. 2003. A systematic approach towards developing environmental enrichment for pigs. Applied Animal Behaviour Science 84: 101-118.

Whittaker, X., Edwards, S.A., Spoolder, H.A.M., Lawrence, A.B., Corning, S. 1999. Effects of straw bedding and high fibre diets on the behaviour of floor fed group-housed sows. Applied Animal Behaviour Science 63: 25-39

Winstanley, C. A., J. W. Dalley, D. E. H. Theobald, and T. W. Robbins. 2004. Fractioning impulsivity: Contrasting effects of central 5-HT depletion on different measures of impulsive behaviour. Neuropsychopharmacology 29: 1331-1343.

Young, R. J., J. Carruthers, and A. B. Lawrence. 1994. The effect of a foraging device (The 'Edinburgh Foodball') on the behaviour of pigs. Applied Animal Behaviour Science 39: 237-247.

Zonderland, J. J. et al. 2008. Prevention and treatment of tail biting in weaned piglets. Applied Animal Behaviour Science 110: 269-281.

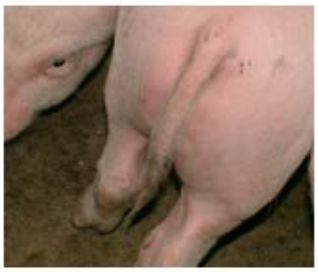
Appendix

I Ethogram scan sampling

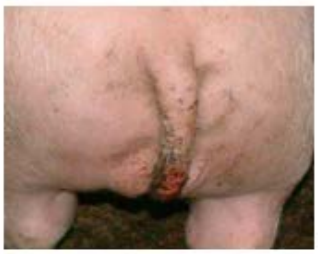
Code	Behaviour	Recipient	Description
	INACTIVE		
SS	Sleeping		Lying without performing any other described behaviour, eyes closed.
LL	Lying inactive		Lying without performing any other described behaviour, eyes opened.
	ACTIVE		
ST	Standing		Standing without performing any other described behaviour
WW	Locomotion		Walking or running without performing any other described behaviour
KK	Sitting		Sitting or kneeling without performing any other described behaviour
CB	Comfort behaviour		Rubbing body against objects or pen mate, scratching body with hind leg or stretching (part of) body.
PO	Urinate/defecate		Piglets urinates or defecates
	Feeding		
EE	Eating feeder		Eating at feeder
DD	Drinking		Drinking from drinking nipple
	Pen directed		
NN	Nosing floor		Sniffing, touching or scraping floor
NO	Nosing object		Nosing above floor level (e.g. walls)
RR	Rooting		Rooting pen floor or in straw
RO	Rooting object		Rooting above floor level (e.g. walls) or object
CH	Chewing		Non-feed chewing (e.g. air, dung) or chewing straw
CO	Chewing object		Chewing object or part of pen above floor level
CT	Chewing toy		Chewing toy (chain with ball or jute bag)
	SOCIAL		
	Aggressive		
FF	Fighting	# pig	Ramming or pushing a pen mate with or without biting the pen mate. Can be either mutual or individual.
HH	Head knock	# pig	Head knock given at place other than feeder
BB	Bite	# pig	Bite given at other place than feeder
FE	Fighting at feeder	# pig	Push, head knock or bite given at feeder
	Social		
GA	Play behaviour		Group wise gambolling, pivoting: running around the pen, sometimes with gently nudging of pen mates
PL	Play individually		Pivoting or gambolling without other pen mates
SP	Substrate play		Playing with substrate like straw
NB	Nosing head or	# pig	Touching/sniffing any part of a pen mate except nose
	body	# pig	Mutual nose contact
NC	Nose contact	# pig	Standing on hind legs while having front legs on other pig's
MO	Mounting		body
	Manipulative		
BN	Belly nosing	# pig	Rubbing belly of a pen mate with up and down snout movements
TB	Tail biting	# pig	Nibbling, sucking or chewing the tail of a pen mate
EB	Ear biting	# pig	Nibbling, sucking or chewing the ear of a pen mate
MM	Manipulating other	# pig	Nibbling, sucking or chewing part of the body of a pen mate
XX	*Extra*		Possible to fill in when piglet is missing

II Tail bite score

 = 1; No damage

 = 2; Hair removed

 = 3; Bite marks

 = 4; Wound

III Determining Aggressors and Receiver classes

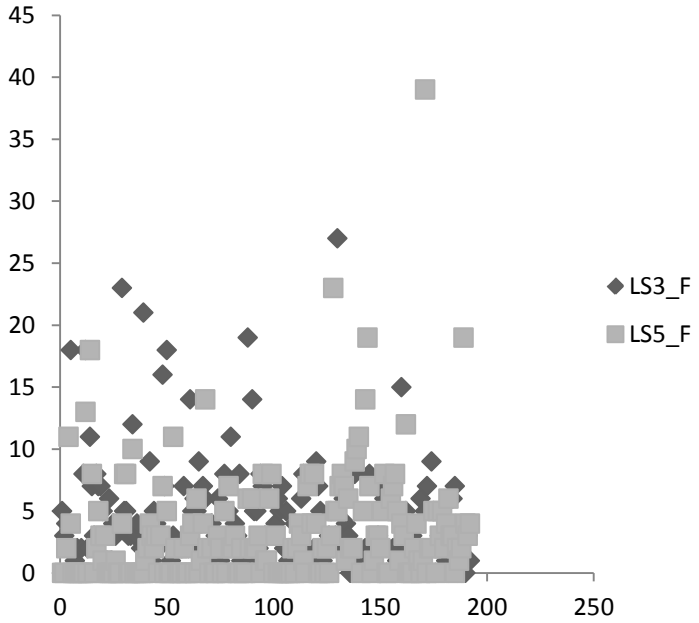


Figure A , lesion scores on the front part of the body

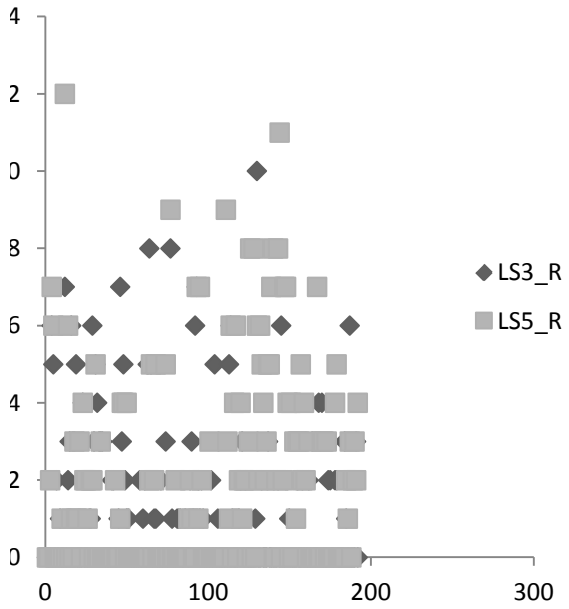


Figure B, lesion scores at the rear part of the body

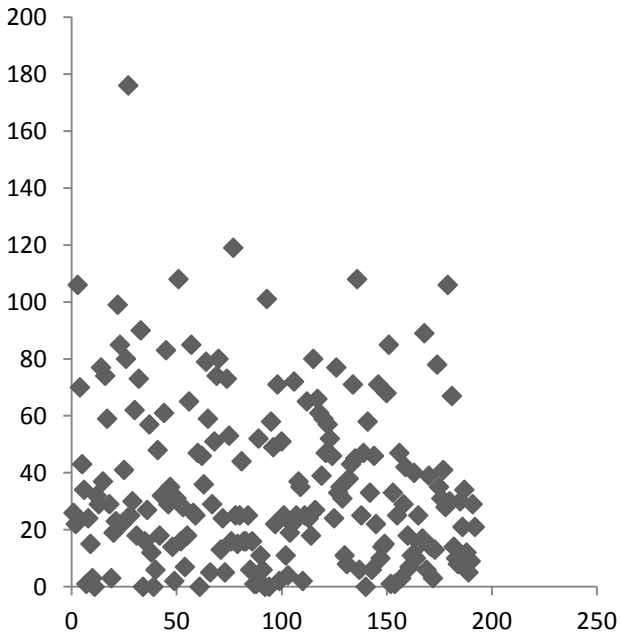


Figure C, lesion scores at the front part of the body

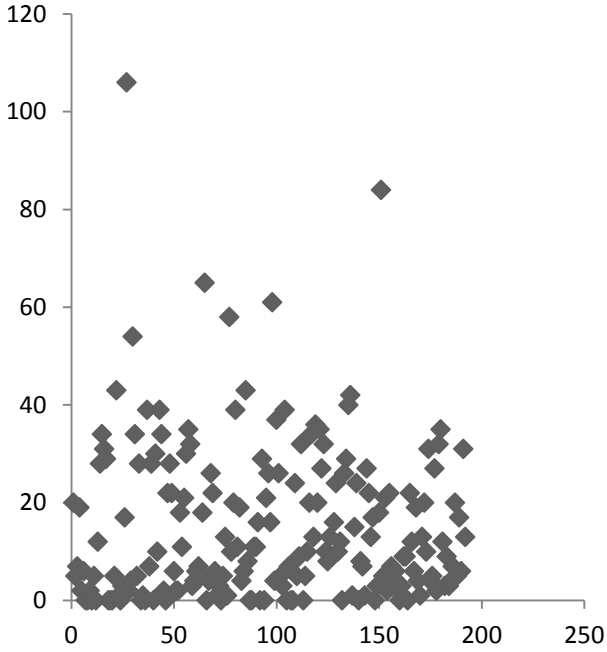


Figure D, lesion scores at the rear part of the body

Above, the scatter plots of the lesion score data can be seen. Figure A and B contain the lesion scores measured before and after regrouping, during the steady stages (LS3 and LS5). Figure C and D show data collected the day after regrouping. The following limits for aggressors and receivers were made based on the figures above:

- Aggressors during steady stages: more than 8 lesions on the front of the body
- Receivers during steady stages: more than 6 lesions on the rear part of the body
- Aggressors during regrouping: more than 60 lesions on the front of the body
- Receivers during regrouping: more than 40 lesions on the rear part of the body

IV Correlations table

	NL ratio1	nl ratio2	nl ratio3	LS3	LS4	LS5	explo1	explo2	explo3	aggr1	aggr2	aggr3	social1	social2	social3	man1	man2	man3	nonact1	nonact2	nonact3	tail dam1	tail dam2	tail dam3
5ht uptake	0.20**	0.25***	0.08	-0.11	0.06	0.07	-0.18*	-0.12	0.17*	-0.07	0.02	-0.01	-0.08	0.03	0.05	0.08	0.07	0.17*	0.20**	-0.17*	-0.11	-0.10	-0.15*	-0.168
nl ratio1		0.27***	-0.01	-0.13†	-0.09	-0.02	-0.29***	-0.09	0.16*	0.03	-0.04	0.03	-0.15*	0.00	0.09	0.05	0.07	0.00	0.27***	-0.08	-0.12†	0.00	0.00	0.04
nl ratio2			0.06	-0.24***	-0.03	0.12†	-0.27***	0.06	0.22**	-0.07	-0.03	-0.01	-0.01	0.10	0.10	0.21**	0.00	0.09	0.23**	-0.35***	-0.13†	-0.01	-0.09	-0.03
nl ratio3				-0.01	-0.04	-0.10	0.11	0.04	0.05	0.09	0.08	-0.01	-0.09	0.02	0.06	0.05	0.02	-0.08	-0.13†	-0.03	-0.07	0.09	0.11	0.03
LS3					0.02	0.20**	0.32***	0.11	-0.14†	0.08	0.07	-0.07	0.03	0.04	0.06	-0.05	-0.09	-0.02	-0.30***	0.01	0.00	-0.03	-0.04	-0.07
LS4						0.15*	0.02	-0.22**	-0.03	-0.03	0.09	-0.02	-0.05	-0.18*	-0.04	0.15*	-0.08	0.00	0.02	0.25***	0.06	0.02	0.11	-0.06
LS5							0.07	0.06	0.12	0.10	0.00	-0.02	0.07	0.02	0.15*	-0.17*	-0.05	0.15*	-0.05	-0.25***	-0.11	0.06	-0.03	-0.16*
explo1								0.35***	-0.18*	0.18*	0.10	-0.04	0.22**	0.10	-0.06	-0.13†	-0.05	-0.06	-0.90***	-0.03	0.11	-0.05	-0.08	-0.15*
explo2									-0.01	-0.04	-0.15*	0.11	0.11	0.37***	0.14†	-0.10	0.10	0.09	-0.30***	-0.67***	-0.07	-0.09	-0.18*	-0.05
explo3										0.01	-0.04	-0.02	-0.01	0.00	0.21**	-0.03	0.01	0.19*	0.21**	-0.14†	-0.79***	0.07	-0.08	-0.04
aggr1											0.06	0.01	0.00	-0.09	-0.03	0.07	-0.10	-0.06	-0.23**	0.07	0.02	0.09	0.03	-0.04
aggr2												-0.04	0.09	-0.03	-0.14†	0.10	0.05	-0.12	-0.16*	0.05	0.09	-0.04	0.03	0.04
aggr3													0.08	0.06	0.10	-0.05	0.02	0.07	0.01	-0.02	-0.13†	0.15*	0.11	-0.05
social1														0.06	0.02	0.22**	0.01	-0.01	-0.28***	-0.08	0.02	0.08	0.23**	-0.06
social2															0.11	-0.01	0.11	0.18*	-0.11	-0.40***	-0.05	-0.03	-0.08	-0.10
social3																0.06	0.00	0.35***	0.03	-0.19**	"-0.43***	-0.03	-0.05	-0.09
man1																	0.16*	-0.07	-0.03	-0.02	0.05	0.23**	0.28***	0.12
man2																		-0.04	0.02	-0.16*	0.03	0.18*	0.19*	0.09
man3																			0.10	-0.26***	-0.35***	-0.05	-0.12	-0.13†
nonact1																				0.02	-0.15*	0.01	-0.03	0.07
nonact2																					0.17*	0.10	0.21**	0.14†
nonact3																						-0.04	0.09	0.15*
tail dam1																							0.45***	0.14†
tail dam2																								0.13†

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