A tale too long for a tail too short?

Identification of characteristics in pigs related to tailbiting and other oral manipulations directed at conspecifics

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Voor mijn ouders
Abstract


Tail biting in pigs, i.e. the chewing on and biting in tails of conspecifics, is a multifactorial problem leading to impaired pig welfare and health and economic losses in pig farming. In many countries tail docking is used as a preventive measure, but there is increased societal concern about this practice. Therefore, there is an urgent need to understand, prevent, and reduce tail biting and other damaging behaviours directed at pen mates. The main aim of this thesis was to identify biological characteristics of barren and enriched housed pigs that relate to their tendency to develop these damaging oral manipulative behaviours. Tail biting started already early in life and pigs that displayed tail biting post-weaning seemed to stem from litters in which tail biting behaviour was already present. The onset of tail biting behaviour was different for individual pigs, and many pigs were not consistently tail biters throughout different phases of life. It was difficult to predict which pigs would develop tail biting based on their individual behaviour. Groups of pigs with tail biting problems were, however, more easy to identify by increased activity, and increased levels of pig and pen-directed oral manipulative behaviours. Subjecting pigs to an individual behavioural test showed that tail biters may be more fearful. Fearfulness in pigs appeared related to measures of the brain and blood serotonergic system. Moreover, measures of the blood serotonergic system seemed temporarily altered in tail biting pigs mainly during the phase of life in which they displayed this behaviour. Additionally, (tail) biting behaviour may be associated with higher (phenotypic and genotypic) production, such as higher growth. Growth of individual pigs can be affected by the other pigs in a pen. The heritable effect of one pig on the growth of another group member is referred to as an indirect genetic effect. Pigs with a relatively negative indirect genetic effect for growth displayed more biting behaviours, caused more tail damage and destroyed more of the available jute sacks. The presence of straw-bedding or jute sacks as enrichment materials for rooting and chewing largely reduced damaging biting behaviours and, consequently, tail damage. Pigs that still develop tail biting behaviour in an enriched environment likely do so due to a (temporary) physiological problem, whereas in barren housed pigs the lack of
suitable rooting and chewing material plays a large role. Tail biting behaviour in pigs thus seems to be caused by a variety of temporary states and more stable traits that influence their motivation to display foraging and exploratory behaviours. Therefore, the tale of (tail) biting behaviours in pigs needs a better understanding of underlying physiological processes. Preventing and reducing damaging biting behaviours in pigs requires a joint effort of science, industry and society to optimize housing conditions, feeding, management and breeding of pigs.
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General introduction
General introduction

Animal welfare is an important topic on the national (Dijksma, 2014) and international agenda (EC, 2012). During the past decades animal production was mainly focused on increasing quantity and efficiency (Bos et al., 2008). However, these economical improvements seem accompanied by the development of some of today’s animal health and welfare problems (Beattie et al., 2000; Broom, 2006) and consequently the development of (new) economic losses (Bracke et al., 2004; Edwards, 2006). Some undesired, injurious oral manipulative behaviours observed in (intensive) pig husbandry systems are biting behaviours directed at pen mates, such as tail biting, ear biting, paw biting and flank biting (e.g. Zonderland, 2010). Especially tail biting (Figure 1) - which seems to stem from discomfort in the pig (Fritschen and Hogg, 1983; Wood-Gush and Vestergaard, 1989) - is a common problem that can result in severe and painful tail damage in the recipient pig (Fritschen and Hogg, 1983). This tail damage can, furthermore, lead to infections to internal organs (Munsterhjelm et al., 2013) and severe injuries can result in the death of the pig (Fritschen and Hogg, 1983). In several EU countries, including the Netherlands, tail docking (i.e. removing the tail partly or almost completely) of young piglets is common practice to prevent tail damage at later age (Sutherland and Tucker, 2011) (Figures 2A, 2B). Tail docking is a painful procedure and may lead to prolonged pain (see review of Sutherland and Tucker, 2011), and targets the consequences rather than the underlying cause of tail biting. Therefore, the procedure of tail docking has been criticised by society (Boogaard et al., 2011; Noonan et al., 1994). In fact, EU legislation states that routine tail docking is prohibited unless injuries due to tail biting remain present after trying other prevention methods (EC, 2008). Consequently, in several EU countries tail docking has been restricted or banned (e.g. Finland) (EFSA, 2007) which means that in some countries many pigs are kept with intact tails (Figure 2C), although tail biting remains a problem (Valros et al., 2012). In the Netherlands, tail docking is not (yet) banned, but recently a declaration was signed by the Dutch Federation of Agriculture and Horticulture, the Dutch pig farmers’ union, and Dutch Society for the Protection of Animals in which they share their ambition to gradually reduce and if possible to quit the practice of tail docking (Dalfsen Declaration, 2013). This declaration was supported by a diverse group of Declaration Partners (i.e. companies and societies) (Dalfsen Declaration, 2013) and the Dutch Ministry (Dijksma, 2014).
Both tail biting and tail docking may thus lead to welfare problems in pigs, but it is important to realize that tail biting very likely also reflects an underlying welfare problem in the pig displaying this behaviour. Previously, it has been found that tail biting can largely be prevented by adequate housing and management (e.g. Zonderland, 2010), but for many farmers the measures that should be taken (e.g. provision of adequate enrichment; EFSA, 2007) are difficult to implement. Therefore, tail docking seems for most farmers the best strategy to prevent tail damage. However, already in 1959 it was questioned whether such procedures are adequate against tail biting (cited in Van den Berg, 1982). Up until today, tail docking has not fully prevented tail biting behaviour and consequently tail damage. In spite of the fact that over 99 % of pigs in The Netherlands are tail docked, problems with tail biting still have a prevalence of ~1-2 % on Dutch conventional farms according to farmers and only 35-50 % of the farmers reported that they had never problems with tail biting (Bracke et al., 2013). Consequently, economical losses due to tail biting are large and for pig husbandry in The Netherlands were estimated to be over € 8 million per year (Zonderland et al., 2011a). Altogether, there is still a need to better understand tail biting and prevent pigs from displaying this damaging behaviour.

**Figure 1.** Fattening pig that bites in the tail of a pen mate.
Figure 2. Pig tails from shortly docked to undocked. A: Recently docked tail of a young piglet, B: Half docked tail of a sow, C: Intact tail of a recently weaned piglet.

Figure 3. Tail wounds. A: Shortly docked pig with scabs and small wound, B: Shortly docked pig with largely damaged and swollen tail, but showing signs of healing, C: Pig with intact tail with wound, treated with tar.
The definition of tail biting

Tail biting is the behaviour of a pig when it holds the tail of another pig, usually sideways, in its mouth and bites or chews on it (EFSA, 2007; Schröder-Petersen and Simonsen, 2001; Van Putten, 1969). Pigs that perform such behaviour are generally called ‘tail biters’ (e.g. Taylor, et al., 2010; Zonderland et al., 2011c). Tail biting behaviour can vary in frequency and intensity (i.e. how vigorously is bitten) (Brunberg et al., 2011). Some tail biters will gently hold and chew the tail of a recipient pig with or without causing damage, while other tail biters grab it and yank at it which most likely will result in damage (see for a review Taylor et al., 2010). The period in which tail biting is displayed but remains harmless is called the first or ‘pre-injury’ stage. The period when tail damage starts to develop is called the second or ‘injury stage’ (Fraser, 1987). Tail biting can eventually result in cannibalism and at that time the tail is usually grabbed lengthwise (Van Putten, 1969). Additionally, tail biting behaviour can exponentially spread amongst pigs in a group (Zonderland et al., 2011b). The way in which tail biters are identified varies between scientific studies. For example, some authors identified pigs as tail biters when they repeatedly showed tail biting behaviour (e.g. Zupan et al., 2012) and others chose only those pigs that displayed the behaviour most frequently (Zonderland et al., 2011c). Furthermore, the bitten pig is generally called the ‘victim’ (e.g. Zonderland et al., 2011c) and also identifying victims can be done in various ways. Victims have been identified on the basis of e.g. receiving the highest number of tail bites during behavioural observations (Zonderland et al., 2011c) or the presence of tail wounds (Sinisalo et al., 2012) (see Figure 3). This variety in which pigs were considered to be tail biters and victims highlights the difficulty of understanding the tail biting problem and when to consider animals as ‘problem’ animals. Additionally, information about tail biting behaviour on the long-term lacks. Therefore, in the current thesis, tail biting behaviour will be studied longitudinally and approached in various ways, i.e. at the individual and at pen level and with or without subdivision (medium or high frequency) in the level of tail biting behaviour displayed.
When and why do pigs start tail biting?

Tail biting behaviour is usually considered a post-weaning problem (i.e. often called a ‘tail biting outbreak’) when tail damage is observed (e.g. Zonderland et al., 2008). The onset of tail biting behaviour is unclear and, according to literature, can lie between 0-144 days post-weaning (Blackshaw, 1981; Moinard et al., 2003), which covers approximately the pig’s whole life post-weaning until slaughter. Nevertheless, tail biting may start with tail-in-mouth behaviour already before weaning (Schrøder-Petersen et al., 2003) and some authors have indeed reported the presence of some tail biting behaviour when piglets were still in the farrowing pen (Cox and Cooper, 2001), but it was not reported if this tail biting behaviour resulted in tail damage in the victim piglet. Consequently, it remains unclear at what age tail biting behaviour including tail damage develops.

Tail biting (and possibly also ear, paw and flank biting) is likely a redirected way of exploring and foraging (reviewed in Zonderland, 2010). Exploration and foraging behaviours are behaviours such as rooting, sniffing/nosing, touching, nibbling, and chewing or biting (e.g. Bolhuis et al., 2010; Day et al., 1996) directed to e.g. flooring, substrates or objects. By displaying such behaviours, a pig can gain information about its surroundings in order to find for instance food or a suitable nesting site (i.e. extrinsic exploration), and it can investigate for instance a novel object without having a direct goal (i.e. intrinsic exploration) (Wood-Gush and Vestergaard, 1989). Goal seeking behaviour (e.g. foraging) can be called ‘appetitive’ behaviour and precedes the action directly related to the goal (e.g. feed intake), i.e. ‘consummatory’ behaviour (e.g. De Jonge et al., 2008; reviewed in Hughes and Duncan, 1988). Opportunities to display appetitive foraging activities like rooting and chewing are often limited in intensive husbandry systems, where pigs are generally kept under barren, stimulus-poor conditions. The absence of suitable substrate or other enrichment material for rooting and chewing seems the major risk factor for the development of tail biting (Beattie et al., 2001; EFSA, 2007; Zonderland et al., 2008).

Tailbiting behaviour is generally considered to be a multifactorial problem (Bracke et al., 2004; Taylor et al., 2010; Van Putten, 1969) and it has been postulated that anything in a pig’s surrounding that can cause discomfort may eventually result in tail biting behaviour (Fritschen and Hogg, 1983). Environmental, or extrinsic, factors that have been associated with tail biting are, apart from a lack of enrichment materials, a suboptimal climate such as poor ventilation, heat
stress, or presence of draught (e.g. Sällvik and Walberg, 1984; Van Putten, 1969), imbalanced feed with respect to e.g. minerals (e.g. Fritschen and Hogg, 1983), or an inadequate feeding system, such as the presence of only a single feeder in a group of pigs that makes access to the feed difficult for some individuals (e.g. Hansen et al., 1982). Apart from extrinsic factors, intrinsic, pig-related factors may also play a role in tail biting. Intrinsic factors suggested to be associated with tail biting are sex, as female pigs are often found to be more prone to develop tail biting behaviour than males (e.g. Penny et al., 1981; Zonderland et al., 2010a), breed, as for instance Duroc pigs have been reported to display more biting behaviours directed at pen mates than Large White and Landrace pigs (Breuer et al., 2003), personality traits such as a bold coping strategy and nervousness (e.g. Korte et al., 2009; Van Putten et al., 1969), and poor health (Moinard et al., 2003). Altogether, tail biting seems to stem from different environmental and pig-related factors. This may explain why farmers and scientists pinpoint to different factors as being the main cause of tail biting; many (Dutch) farmers blame the climate (Bracke et al., 2013), whereas many scientists blame the lack of enrichment materials for chewing and rooting (EFSA, 2007). Both factors indeed seem to play a role in the development of the injurious behaviour and possibly many risk factors together result in the problem of tail biting (Taylor et al., 2012). The lack of enrichment materials may be the largest risk factor in the development of tail biting (EFSA, 2007), but other, possibly smaller, risk factors simultaneously present may eventually lead to a tail biting outbreak (Zonderland et al., 2010b). Zonderland and coworkers (2010b) used a metaphorical bucket (according to a Dutch saying) filled with possible risk factors to visualize that all risk factors together can result in tail biting if the edge of the bucket is reached (i.e. ‘the straw that breaks the camel’s back’). Bracke et al. (2012) adjusted this metaphor to show that once the threshold is reached and a tail biting outbreak has started, it can be difficult or even impossible to stop the outbreak (Figure 4).

The existence of so many risk factors that can lead to tail biting behaviour suggests that not all tail biting is alike. Taylor et al. (2010) distinguished in their review three different types of tail biting behaviour. The first one was called ‘two-stage’ tail biting and covers the pre-injury and injury stages as described above (Fraser, 1987). Pigs that follow this pattern may develop tail biting behaviour from their need to explore and forage and may display the behaviour chronically (Taylor et al., 2010). The second behavioural type identified by Taylor et al. (2010) was called ‘sudden-forceful’ and relates to the forceful biting or yanking at the
Figure 4. Metaphorical bucket filled with risk factors of tail biting that should be in balance. The factor boredom is, here, suggested as largest risk factor and relates to the unfulfilled need to explore and forage. The presence of too many risk factors will lead to the threshold and causes the bucket to tip over and results in a tail biting outbreak (Bracke et al., 2012).

tail resulting in tissue damage or even in biting the tip of the tail off. This type of tail biting is generally not preceded by gentle manipulations and it may be related to attempting to gain access to a resource such as a food trough (Taylor et al., 2010). The third and final behavioural type was called ‘obsessive tail biting’. Pigs that display this type of tail biting also uses forceful biting and yanking which results in tail damage, but in a more fixated and persistent way (i.e. they search for tails) (Taylor et al., 2010). The motivation behind obsessive biting remains unclear and this type of biting may develop once tail damage is caused due to one of the first two behavioural types described, or this type of biting may be related to accessing a resource. However, obsessive tail biting may also be consummatory in itself rather than appetitive (Taylor et al., 2010).

In pigs in barren housing systems the unfulfilled motivation to explore and forage is likely a major cause of tail biting problems and occurrence of other oral manipulative behaviours directed at pen mates, such as ear biting. In enriched housing systems tail biting and tail damage are largely reduced (Van de Weerd et al., 2006; Zonderland et al., 2008) but even the provision of ample enrichment materials does not guarantee the absence of tail biting (e.g. Van de
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Weerd et al., 2006). In these enriched systems, other factors and motivations may play a role. It can be speculated that two-stage tail biting may be most prominent in barren housing systems, whereas in enriched housing systems – providing that fresh substrate is given regularly - one or both other behavioural types of tail biting (i.e. sudden forceful or obsessive) may be most prominent. To sum up, tail biting behaviour most likely stems from a heightened motivation to explore and forage, albeit in pigs kept in different circumstances possibly stimulated through a different set of potential risk factors.

Personal pig characteristics

Tail biting may relate to many risk factors (e.g. Taylor et al., 2012), and not all pigs kept under the same circumstances will develop tail biting behaviour. Therefore, personality may also be an important risk factor in the development of tail biting. The way in which animals cope with challenges such as novelty may depend on the animal's underlying personality (Koolhaas et al., 1999; Korte et al., 2009). Animals can vary largely in their behaviours displayed during novelty tests, which may depend on an animal’s coping strategy (i.e. acting bold or shy) (Koolhaas et al., 1999; Reimert et al., 2014), their level of sociality (Van Reenen et al., 2005) and fearfulness (Reimert et al., 2014). In pigs, both their coping strategy (Koolhaas et al., 2007) and the level of nervousness (cited in Van Putten, 1969) have been suggested to relate to tail biting behaviour. Pigs acting more in a bold way or being more nervous may be more likely to develop tail biting behaviour. However, to the best of our knowledge, at the onset of the current study no other studies with pigs tested these hypotheses.

Personality traits are generally associated with certain neurophysiological patterns observed in, for instance, functioning of the serotonergic system (Carver and Miller, 2006). Serotonin (5-HT) is a neurotransmitter associated with for instance mood (Hensler, 2010) and links have been found between the functioning of the serotonergic system and mental disorders in humans such as obsessive compulsive disorders (e.g. Delorme et al., 2005), and aberrant behaviours in animals such as feather pecking in laying hens (Van Hierden et al., 2004). Feather pecking shows strong similarities with tail biting in pigs as both behaviours are considered to be redirected foraging behaviours and are strongly reduced by the presence of foraging substrates (Rodenburg et al., 2007).
It has been proposed that the serotonergic system may also be involved in the tendency to develop tail biting behaviour (Edwards, 2006; Korte et al., 2009), but this remains to be studied. If tail biting is indeed associated with the 5-HT system functioning of pigs and their personality characteristics, early identification of animals that are predisposed to develop tail biting may be possible.

The invention of today’s pig

Pigs are used for many of today’s human products; e.g. shampoos, the hairs on paint brushes, candles and of course as food and feed (Meindertsma, 2008). Therefore, pig farming has largely intensified throughout the years (Robert and Martineau, 1994) and, currently, the EU pig industry produces over 22 million tonnes of pork meat (Eurostat, 2014). Selection in livestock farming largely focused on improving production levels with keeping feed intake at a relatively low level (see review of Rauw et al., 1998). However, there is a growing body of evidence that selection for increased productivity is associated with increased display of harmful behaviours in animals, such as tail biting in pigs (Breuer et al., 2005; Rauw et al., 1998; Turner, 2011). Selection against such injurious behaviours is up until now difficult if not impossible. However, there may be a selection method available that indirectly targets harmful biting behaviours in pigs. Recently, estimation methods were developed that can be integrated in breeding programmes and aim to account for the heritable effect of one individual on the performance (e.g. growth) of other individuals (Bijma et al., 2007a, b; Muir, 2005). Pigs seem to have a heritable effect on the growth of their pen mates, and it can be speculated that this effect results from their (anti)social behaviours (Rodenburg et al., 2010), although it is yet unknown which behaviours are targeted by such selection methods. It is known, though, that tail damage, caused by tail biting, can negatively affect the growth of pigs (Smulders et al., 2006; Wallgren and Lindahl, 1996), and recently it has been shown that pigs receiving a lot of oral manipulative behaviours, even without visible wounds, show a poor growth rate (Camerlink et al., 2012). It can thus be hypothesized that selection for pigs with a heritable relatively beneficial effect on the growth of their group members will yield animals with relatively low levels of harmful biting behaviours. If so, future selection can simultaneously improve production such as growth and reduce undesired oral manipulative behaviours directed at pen mates, such as tail biting.
Aims and outline of the thesis

The main aim of this thesis is to identify characteristics of pigs that relate to the development of oral manipulative behaviours directed to conspecifics, such as tail biting, under different housing conditions. Gaining more insight in the development of (tail) biting behaviours is highly important when developing strategies to prevent and reduce such behaviours. This thesis contains 7 chapters and describes tail biting in pigs and its relationship with several other behaviours, (neuro)physiological measures, and production characteristics. Chapter two examines the consistency in tail biting behaviour in different phases of life and whether it can be predicted by other behaviours at the individual and at the pen level. In this chapter, pigs were either housed in barren or straw-enriched pens. It was hypothesized that tail biting pigs may differ in neurophysiology of the serotonergic system compared to non-tail biting pigs. Studying tail biting behaviour requires a large number of pigs due to its unpredictable nature, but brain research is a delicate process and consequently impractical on a large scale. Some aspects of brain 5-HT functioning appear to be reflected in peripheral (blood) 5-HT measures. Therefore, in Chapter three possible relationships between brain and blood measures of 5-HT, and behavioural responses to novelty were studied on a small-scale. As some associations between blood 5-HT and brain 5-HT activity and behavioural responses to novelty were found, Chapter four examined whether both blood 5-HT measures and behavioural responses to novelty can be associated with tail biting behaviour in pigs kept in either barren or straw-bedded pens. In Chapter five, indirect genetic effects (IGE) on the growth of a pig's pen mates were studied in relation to oral manipulations directed at pen mates and general behaviour in barren or enriched pens. Chapters 2 and 5 confirmed that straw-bedding largely reduces tail biting and tail damage, but as straw-bedding is in many systems difficult to implement, an alternative enrichment material was also assessed. In Chapter six, the practicality of jute sacks in reducing oral manipulative behaviours directed at pen mates was studied in breeding gilts on a commercial farm. Additionally, the association between genetic and phenotypic production characteristics and biting behaviours was explored. In Chapter seven the results of the previous chapters are discussed and placed in a broader perspective which leads to a conceptual framework of biting behaviours.
References


General introduction


Tail biting behaviour and tail damage in pigs and the relationship with general behaviour: Predicting the inevitable?

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Abstract

Tail biting behaviour in pigs is a common problem in conventional housing systems. Our study examined the consistency over time in tail biting and tail damage, and it explored the predictive value of general behaviours observed in individual pigs and in pens as a whole.

Pigs (n = 480), reared in conventional farrowing pens with a sow crate, were followed from pre-weaning to slaughter (23 weeks). Post-weaning, piglets were housed barren (B) or enriched (E). Behaviours were observed pre-weaning (averaged per litter) and post-weaning in three phases (weaner, grower, finisher) (averaged per pig/phase). Tail damage of individual pigs was scored weekly from weaning (4 weeks) onwards (averaged per phase). Relationships between tail biting and tail damage with behaviour were investigated both at the individual and at pen level using mixed or generalized linear mixed models and Spearman’s rank correlations, respectively.

Tail biting and tail damage (2.1 ± 0.05, 1 = no tail damage, 4 = tail wound) were already observed pre-weaning. Post-weaning, tail biting and tail damage were less prevalent in E compared to B housing (P < 0.001). Tail biting behaviour in individual pigs was not consistently observed over time, i.e. none of the pigs was tail biter in all three phases, so new tail biters were found in later phases and some of the already identified tail biters stopped tail biting completely or temporarily. In B housing 38.3 % and in E housing 5.6 % of pigs was identified as tail biter in at least one phase post-weaning. B housed tail biters in different phases were likely to originate from litters with a relatively high level of tail biting behaviour pre-weaning (P < 0.05-0.01). Generally, post-weaning victims were likely to be a victim again in successive phases of life (B: P < 0.10-0.001; E: P < 0.01). Tail biting and tail damage were best predicted by behaviours at pen level and less by behaviours at the individual level: a higher activity, and more pig and pen-directed manipulative behaviours were observed in pens with high levels of tail biting. Particularly higher levels of chewing or consuming objects such as jute sacks could be useful in predicting tail bite outbreaks. To conclude, tail biting in pigs starts early in life and is difficult to predict due to its inconsistency, although tail damage is more consistent throughout life. Especially behaviour observed at litter or pen level is a promising tool in predicting tail biting and tail damage.

Keywords: Domestic pigs, piglets, tail biting, tail bite victim, behavioural predictors, environmental enrichment
1. Introduction

Tail biting is a problem in pig production (EFSA, 2007) from both an animal welfare and economical point of view (Blokhuis et al., 2000; Bracke et al., 2004a, b; Smulders et al., 2008). Tail biting by a pig can be described as grabbing “a tail transversely in its mouth” (Van Putten, 1969) while chewing on it (Taylor et al., 2010) and thereby likely inflicting mild to severe damage to the tail (Keeling et al., 2012; Zonderland et al., 2009). Tail wounds may cause infections to internal organs (Huey, 1996; Munsterhjelm et al., 2013) and, when severely bitten, pigs may become paralyzed and die (EFSA, 2007; Fritschen and Hogg, 1983). To reduce tail biting and tail damage in pigs, tail docking was introduced in several countries many years ago. Nevertheless, in late 1950s the effect of tail docking was already questioned (reviewed in Van den Berg, 1982) and nowadays tail biting and tail damage still occurs, also in docked pigs (see review of Sutherland and Tucker, 2011). As long as tail biting occurs, the need to understand underlying causes of tail biting and the need to prevent the damaging behaviour remain also present.

Tail biting has a multifactorial background (Bracke et al., 2004a; Taylor et al., 2010; Van Putten, 1969) which relates to environmental risk factors and biological characteristics of the pigs. Environmental risk factors include for instance a lack of suitable rooting substrate (Hunter et al., 2001; Van Putten, 1969; Zonderland et al., 2008), a deficiency in nutrients (Fraser, 1987b) or fibre (Pütz et al., 2011), an inadequate feeding system (Jaeger, 2013; Moinard et al., 2003), a high stocking density (Moinard et al., 2003), and a suboptimal air quality (Sällvik and Walberg, 1984; Van Putten, 1969). Biological characteristics of the pigs that may be involved in tail biting are breed (Breuer et al., 2003; Turner, 2011) and sex (Penny et al., 1981; Zonderland et al., 2010), but also individual pig characteristics in terms of underlying personality traits such as nervousness (Van Putten, 1969), fearfulness (Zupan et al., 2012), or coping strategies (Korte et al., 2009). Although many risk factors are known, tail biting remains a rather unpredictable behaviour which is performed by some but not all pigs kept under the same circumstances (Beattie et al., 2005). Providing the pigs with enrichment substrates to be able to satisfy the need to forage and explore (Bracke et al., 1999) is no guarantee for total absence of tail biting (e.g. Munsterhjelm et al., 2009; Van de Weerd et al., 2006; Zonderland et al., 2008). This suggests that other motivations than the need to forage and explore, may also underlie tail biting (Taylor et al., 2010). If tail biting behaviour in different
pigs is indeed caused by different motivations or underlying (behavioural) needs, it may be associated with other behaviours as well.

Although tail biting has been studied for many years (e.g. Fraser, 1987a; Van Putten, 1969; Zonderland, 2010), only few studies presented experiments to identify associations between tail biting and other (behavioural) pig characteristics (e.g. Beattie et al., 2005; Statham et al., 2009; Zonderland et al., 2009). The search for predictors of tail biting would benefit from a study that follows pigs from the pre-weaning to the finisher phase and, thereby, assesses not only biting incidences and tail damage, but also other behaviours of the pigs. The first aim of this study was to examine whether individual differences in tail biting behaviour and in tail damage are consistent over time. The second aim was to explore relationships between tail biting behaviour and tail damage with general behaviours both at the individual pig and at pen level. As housing likely affects the prevalence of tail biting behaviour and we assume that different underlying motivations for tail biting (as pointed out by Taylor et al., 2010) are involved in different housing systems, we chose to use both barren and enriched pens in our study.

2. Materials and methods

The experimental protocol followed during this study was approved by the Animal Care and Use Committee of the University of Groningen and of Wageningen University, The Netherlands.

2.1. Animals and housing

2.1.1. Pre-weaning

Piglets (n = 1210) were born in five rounds from 80 litters (Tempo × Topigs 20) at the experimental farm of TOPIGS Research Center IPG in Beilen, The Netherlands. Litters diverged in estimated Indirect Genetic Effects (IGE) for growth (Camerlink et al., 2013). IGE effects are not discussed in this paper, but will be presented elsewhere (Camerlink et al., accepted). Teeth and tails were kept intact, but males were castrated in the first week after birth. Piglets were further subjected to standard procedures on farm. Housing consisted of conventional farrowing pens (3.8 m², 53 % slats) with farrowing crates. Piglets were fed
commercial diets, starting with a crumbled pre-starter at seven days of age (or, when needed, replacement milk and then crumbled feed) which was replaced by a weaner pellet at three weeks of age. Piglets continuously had access to water by one drinking nipple. The first week a heating lamp with yellow lighting was present. Throughout the pre-weaning phase mean ambient temperature in the farrowing units was 25 °C. Lighting was dependent on day length, but lamps were on from 7.00 h until usually 16.00 h.

2.1.2. Post-weaning

Piglets (n = 480) were weaned at four weeks of age and transferred to the experimental farm “De Haar” in Wageningen, The Netherlands. A minimum of two and a maximum of eight healthy piglets per litter were selected. Allocation of pigs at weaning to B or E pens was balanced for litter and weaning weight. All groups (n = 80) consisted of six unacquainted pigs (i.e. from different litters) from one IGE class and were balanced for sex (1:1 ratio) and back test classification (LR:HR ratio in accordance with the whole tested population) (see also Bolhuis et al., 2003; Melotti et al., 2011; Reimert et al., 2013). All pens (± 6.7 m²) had a chain with ball, and once the pigs were eight weeks of age, a jute sack (round 1: 50 x 82 cm, other rounds: 60 x 103 cm) was attached to the pen wall (throughout the article ‘objects’ is used to refer to ‘chain with ball and jute sacks’). The jute sack was replaced by a new one if more than two-thirds of the sack was ‘consumed’ (i.e. pigs shortened the sack by chewing and tearing). B pens had barren concrete flooring with ± 47 % metal slats, and received two handfuls of wood shavings per day from six weeks of age onwards. E pens were filled with wood shavings (12 kg at start, 3 kg added daily) and straw (1.5 kg daily). All pens were cleaned daily, but in E pens only manure and soiled straw was removed. Pigs had continuously access to one drinking nipple and were fed ad libitum with a single feeder accessible for one pig, except during the first two weeks after arrival when three feeding places were available in a single feeder for piglets. Pigs received commercial diets corresponding to production stage: weaner pellets, starter pellets, and finisher pellets. Ambient temperature was set at 25 °C at time of weaning and temperature was then gradually decreased to a fixed level of 20 °C reached at eight weeks of age. The first week post-weaning a heating lamp with red light was provided. Pigs were slaughtered at 23 weeks of age. Lights were on between 7.00 h until 19.00 h.
2.2. Home pen observations

Home pen behaviour (Table 1) was observed by use of a Psion Workabout with Observer software (Noldus Information Technology, Wageningen, The Netherlands). Instantaneous scan sampling with an interval of two min was used (30 samples/h). At three weeks of age (i.e. pre-weaning), individual piglets (n = 987) were observed for 6 h (180 samples per piglet) split in two consecutive days (i.e. one morning and one afternoon observation of 3 h each). Post-weaning, individual pigs (n = 480) were observed during six observation days (at 4, 5, 8, 11, 16, and 21 weeks of age) of 6 h each (1080 samples per pig). All observations were conducted between 8.00 h and 17.30 h with the same time schedule used pre- and post-weaning. To be able to score in a similar way, observers were well trained by the same persons before onset of behavioural observations. Behaviours were expressed in percentages of observed time. Pigs were identified by stock marker sprayed on their back.

2.3. Tail damage

Tail scores recorded were tail damage (1. No damage, 2. Hair removed, 3. Bite marks, and 4. Wound), and tail blood freshness (1. No blood, 2. Dried blood, 3. Sticky blood, and 4. Fresh blood) (adapted from Zonderland et al., 2008). The tail scores were recorded at weaning and thereafter once a week. At time of weaning, piglets were lifted from the farrowing pen and tail scores were recorded from selected piglets (n = 480) only. Piglets that had severe tail wounds at weaning, i.e. shortened or swollen tail (which were only few piglets), were not transferred to the experimental facilities in Wageningen post-weaning, but replaced by another piglet. Post-weaning, tail posture was recorded before entering the pen and after entrance tail scores were recorded while tails were kept between the fingers of one hand. When needed for appropriate judgement of the tail’s condition, tails were cleaned gently with some water and a tissue. We also scored tail posture (1. Curly, 2. Hanging and straight, and 3. Tucked between legs) (adapted from Kleinbeck and McGlone, 1993, and Zonderland et al., 2009) once a week post-weaning, except for round one where we started at eight weeks of age. Before onset of tail scoring, observers were trained by the same persons to score in a similar way. Tail wounds were treated with CTC spray in case of open wounds, and a repulsive substance was smeared on the tail daily (PBH spray or Stockholm
Tail biting, tail damage and general behaviour

tar, alternated), until the wound showed sufficient signs of healing. Pigs were removed from the pens and consequently from the experiment when tails were shortened due to being bitten (i.e. a severe tail wound).

Table 1. Ethogram used for home pen observations pre- and post-weaning.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre- and post-weaning</strong></td>
<td></td>
</tr>
<tr>
<td>Tail biting</td>
<td>Nibbling, sucking or chewing at the tail of a pen mate*</td>
</tr>
<tr>
<td>Ear biting</td>
<td>Nibbling, sucking or chewing at the ear of a pen mate*</td>
</tr>
<tr>
<td>Other manipulations</td>
<td>Nibbling, sucking or chewing part of the body other than the tail or ear of a pen mate*</td>
</tr>
<tr>
<td>Belly nosing</td>
<td>Massaging, nosing, or rooting the belly of a pen mate*</td>
</tr>
<tr>
<td>Fighting</td>
<td>Aggressively ramming, pushing, head knocking, or biting a pen mate*</td>
</tr>
<tr>
<td>Mounting</td>
<td>Standing on hind legs while having front legs on another pig's back*</td>
</tr>
<tr>
<td>Exploration</td>
<td>Exploring the (substrate on the) floor or objects in the pen above floor level by sniffing, nosing, rubbing, licking, rooting or chewing it or scraping the floor with a leg</td>
</tr>
<tr>
<td>Social</td>
<td>Touching or sniffing any part of a pen mate without manipulative behaviours or massaging the udder*</td>
</tr>
<tr>
<td>Playing</td>
<td>Group wise or individual gamboling and pivoting, sometimes with gently nudging of pen mates, and playing with objects or substrate</td>
</tr>
<tr>
<td>Eating and drinking</td>
<td>Eating feed from feeder or spilled (sow) feed, and drinking water from nipple</td>
</tr>
<tr>
<td>Walking</td>
<td>Walking or running without performing any other behaviour listed here</td>
</tr>
<tr>
<td>Standing</td>
<td>Standing with four paws on the floor without performing any other behaviour listed here</td>
</tr>
<tr>
<td>Inactive</td>
<td>Sitting, kneeling or lying without performing any other behaviour listed here</td>
</tr>
<tr>
<td><strong>Pre-weaning</strong></td>
<td></td>
</tr>
<tr>
<td>Massaging udder</td>
<td>Massaging the udder of the sow with the rooting disc</td>
</tr>
<tr>
<td>Suckling</td>
<td>Drinking milk from the sow. All piglets stopped massaging the udder and performed soft suckling noises</td>
</tr>
<tr>
<td>Manipulating sow</td>
<td>Nibbling, sucking or chewing part of the body of the sow, including ears and tail</td>
</tr>
<tr>
<td><strong>Post-weaning</strong></td>
<td></td>
</tr>
<tr>
<td>Chewing objects</td>
<td>Chewing or tearing chain, ball or jute sack**</td>
</tr>
</tbody>
</table>

* Excluding behaviours directed towards the sow.
** Chewing objects was included in the category ‘Exploration’, but was also analyzed separately.
Chapter 2

2.4. Statistical analysis

SAS version 9.2 (Statistical Analysis System Institute and Inc., 2002-2008) was used for all statistical analyses. Four phases of life were distinguished: pre-weaning, and phase 1-3 post-weaning (roughly according to general production stages: piglet, weaner, grower, finisher). The pre-weaning phase comprised home pen observations of life week 3 and tail scores at time of weaning. In the farrowing pens, piglets could easily be distinguished from each other (i.e. if a litter consisted of e.g. 10 piglets, we scored each of the 10 piglets individually), but we were not always perfectly able to link the corresponding ID number to each piglet. Therefore, pre-weaning behaviours of piglets were averaged for litters. Post-weaning, both home pen observations and tail scores were (per pig and per pen) averaged per phase. Phase 1 post-weaning, comprised home pen observations of life week 4 (three days post-weaning) and 5, and tail scores of life week 5-7. Phase 2 post-weaning contained home pen observations of life week 8 and 11, and tail scores recorded in life weeks 8-15. Phase 3 post-weaning included home pen observations of life week 16 and 21, and tail scores of life weeks 16-23. Data are presented as means ± SEM. P-values < 0.05 are considered statistically significant. For brevity, tendencies (P < 0.10) of relations between ‘other’ behaviours and tail biting/tail damage or classification in tail biters/victims are not given in the text but can be found in the accompanying figures.

2.4.1. Correlations between tail damage, tail blood freshness and tail posture scores

To determine possible relationships between tail damage, tail blood freshness, and tail posture scores of individual pigs within each life stage (weaning and phases 1-3), a general linear model per type of housing (B/E) with round (1-5) as fixed effect was run on raw data to obtain residuals. As not all residuals approached normality, Spearman’s rank correlation coefficients were calculated by type of housing.

2.4.2. Tail biting and tail damage, and the effect of housing

To test the effect of housing and phase (1-3) post-weaning on tail biting behaviour or tail damage, a mixed model was used with fixed effects of phase, housing (B/E), their interaction and round (1-5), and random effects of pen (nested within housing and round) and pig (nested within pen, housing, and round). The Kenward Roger degrees of freedom approximation was used for
significance of the fixed effects in this and subsequent models. A similar model, but without the effect of phase and the random pig and pen effects, was used to test whether piglets were not unevenly distributed across the different housing types with respect to tail damage at time of weaning. Tail biting (proportion of observations) was first arcsine square root transformed to obtain normality of residuals.

Pigs were also categorized as non-tail biters or as tail biters per phase, where being a tail biter was defined as performing tail biting more than once during observations in that phase (0/1 or binary variable ‘tail biter’). Similarly, per phase each pig was characterized either as a non-victim if it never had a tail wound (i.e. tail damage score 4), or otherwise as a victim (0/1 or binary variable ‘victim’). Effects of housing on these classifications were analyzed with a generalized linear mixed model specifying a binary distribution and logit link function. The fixed effect of round (1-5), and random effect of pen (nested within housing and round) were also included in the model.

2.4.3. Association between tail biters and victims

To test whether classification as tail biter and victim within the same phase were associated, a generalized linear mixed model with binary distribution and logit link function was used. The dependent variable was the binary variable ‘victim’, the binary variable ‘tail biter’ and round (1-5) were introduced as fixed effects. Pen (nested within housing and round) was considered as random effect. Odds ratios (OR) were estimated to assess whether the likelihood of being classified as a victim was either higher or lower given the classification of tail biter in the same phase. All models were run by type of housing (B/E).

2.4.4. Individual tail biting and tail damage as predictors

The same procedure as used for the association between tail biter and victim was applied to test whether classification as tail biter in one phase predicted being classified as tail biter in another phase. The binary variable ‘tail biter’ of one phase was the dependent variable, and the binary variable ‘tail biter’ of the earlier phase was introduced as fixed effect. This method was also applied for the analysis of the classification as a victim. Preliminary analyses showed no effect of sow/litter or sex on being a tail biter or victim post-weaning, and, therefore, we excluded these effects from the models.
2.4.5. *Behaviour of the litter pre-weaning as predictor*

A similar procedure was applied as the one used for assessing the association between tail biter and victim to test the effect of average litter behaviours observed pre-weaning on being classified as tail biter or victim. The dependent variable was again the binary variable ‘tail biter’ or ‘victim’. However, the difference was that average litter behaviour (one behaviour at a time) was included in the model as covariate instead of the fixed effect of being classified as tail biter.

2.4.6. *Individual home pen behaviour post-weaning as predictor*

Considering post-weaning behaviours as possible predictors for being either a tail biter or a victim, the same procedure was applied. The binary variable ‘tail biter’ or ‘victim’ was the dependent variable. Individual pig behaviour as observed in the home pen was introduced in the model as covariate (one type of behaviour at a time). Also associations between behaviour and being classified as tail biter or victim in the same phase were explored (these associations may be predictors as well in phase 2 and 3, as these phases last 8 weeks).

2.4.7. *Correlations between tail biting and tail damage, and general behaviours at pen level post-weaning*

To test the predictive effect (or association) of post-weaning behaviours observed at pen level (pen averages) on tail biting or tail damage at pen level (pen averages), Spearman’s rank correlation coefficients on residuals of a general linear model per type of housing (B/E) with round (1-5) as fixed effect were determined.

2.4.8. *Loss of pigs*

In total, 33 pigs were lost during the experiment post-weaning. Pigs were removed due to being a victim of tail biting (n = 14) (i.e. shortened tail). In one case the tail biter was also removed from the experiment since no pen mates were left due to (suddenly) shortened tails. Other pigs died due to disease (n = 4), were euthanized (n = 8), or removed (n = 6) from the experiment and housed separately due to health problems. Consequently in one E pen and nine B pens, pigs were removed due to tail biting, and in total 13 B and 11 E pens had less than six pigs at time of slaughter. Results were also explored when tail damage data from removed victims were set at maximum (i.e. tail wound) and from one removed tail biter set at minimum (i.e. no tail wound), instead of missing values,
but this hardly affected the outcome of the analyses. Therefore, missing values were retained in all analyses.

3. Results

Tail damage scores of individual pigs were significantly positively correlated with tail blood freshness scores in both B and E pens recorded in the same life stage (at weaning and phase 1-3: $r = 0.39-0.92$, all $P < 0.001$) and with tail posture scores (phase 2-3: $r = 0.18-0.55$, with $P < 0.01-0.001$), except tail posture in phase 1. Therefore, only tail damage scores were considered in further results.

3.1. Tail biting and tail damage, and the effect of housing

3.1.1. Pre-weaning tail biting

As we were not always able to perfectly link behaviour of individual piglets to the corresponding ID number of each piglet, no analyses were performed on individual piglet behaviour (however, estimated average of individual tail biting behaviour in % of observations was: total: $0.25 \pm 0.01$, range: $0-3.37$, n = 987 piglets, B: $0.25 \pm 0.03$, n = 240 piglets, E: $0.25 \pm 0.03$, n = 240 piglets; estimated % of piglets identified as tail biters was: total: 9, B: 8.3, E: 9.6).

3.1.2. Pre-weaning tail damage

At time of weaning tail damage was present in some of the piglets that were chosen for the subsequent experiment (mean score: $2.1 \pm 0.05$, range: 1-4, with score 1 = 49.4 %, 2 = 4.6 %, 3 = 36.9 % and 4 = 9.2 % of piglets, n = 480 piglets). Average tail damage at weaning was not different between piglets allocated to either B or E housing (mean % of observations: B: $2.0 \pm 0.07$, E: $2.1 \pm 0.07$). However, more victims with a tail wound were, unintentionally, placed in E compared to B pens post-weaning (total: 9.2, B: 5.8, E: 12.5 %) ($P < 0.05$) (Figure 2).

3.1.3. Post-weaning tail biting

Phase post-weaning significantly affected tail biting behaviour observed ($P < 0.001$), with higher levels in phase 2 and 3 compared to phase 1. Tail biting in phase 2 and 3 did not differ. B housed pigs performed more tail biting behaviour (phase 1: $0.15 \pm 0.02$, phase 2: $0.17 \pm 0.02$, phase 3: $0.19 \pm 0.02$ % of observations)
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Figure 1. Percentage of pigs identified as (non) tail biter during phases 1-3 post-weaning, where pigs performing tail biting more than once during scan sampling in the particular phase were classified as tail biters. A: Barren housed pigs, B: Enriched housed pigs. Please note: some animals died or were removed during the experiment and therefore the number of pigs may slightly differ in one phase compared to the other, but one phase always adds up to 100 % pigs.

Figure 2. Percentage of pigs identified as (non) tail bite victim at time of weaning and phases 1-3 post-weaning, where pigs that had at least once a wound during the particular phase were classified as victims of tail biting. A: Barren housed pigs, B: Enriched housed pigs. Please note: some animals died or were removed during the experiment and therefore the number of pigs may slightly differ in one phase compared to the other, but one phase always adds up to 100 % pigs.
compared to E housed pigs (phase 1: 0.01 ± 0.004, phase 2: 0.05 ± 0.01, phase 3: 0.05 ± 0.01 %) in all three phases (P < 0.001). However, only during phase 3 significantly more B housed pigs (17.2 %) were classified as tail biters compared to E housed pigs (3.5 %) (P < 0.001). The percentage of tail biters in phase 2 was numerically (but not significantly) higher in B (16.6 %) compared to E pens (2.5 %). Phase 1 could not be tested due to a lack of tail biters identified in E housing (E: 0%, B: 12.1 %) (Figure 1).

3.1.4. Post-weaning tail damage

Tail damage score was affected by the interaction between housing and phase (P < 0.001). In B housed pigs the tail damage score increased significantly in every following phase (phase 1: 2.3 ± 0.05, phase 2: 2.5 ± 0.04, phase 3: 2.8 ± 0.05), whereas in E housed pigs, tail damage was lower in phase 2 compared to phase 1 and 3 (phase 1: 1.7 ± 0.04, phase 2: 1.5 ± 0.02, phase 3: 1.7 ± 0.04). In all three phases, tail damage scores were higher for B than for E pigs. In line with this, housing also affected the proportion of pigs with tail wounds, where in B housing more victims were found compared to E housing (all phases: P < 0.001) (Figure 2).

3.2. Association between tail biters and victims post-weaning

In B pens in phase 1 and 2, non-tail biters had a higher likelihood of being a victim (OR = 0.31 and 0.44, respectively, both P < 0.05). No such association was found in phase 3. The percentage of pigs that were exclusively observed as tail biter and not as victim, was 9.6, 9.2, and 5 % for phase 1-3, respectively. In phase 1-3, 29.2, 47.7, and 57 % of the B pigs were solely victims. However, 2.5, 7.5, 12.2 % of the pigs were both tail biter and victim in phase 1-3, respectively, and 58.8, 35.6, and 25.8 % were neither classified as tail biter nor as victim in these phases.

No association between tail biters and victims could be found for E housed pigs. In phase 1-3, 10.8, 11.8, and 22.5 % of the pigs, respectively, were identified as victims solely. In phase 2, 0.42 and in phase 3, 2.6 % of the pigs were identified both as victim and tail biter. The percentage of solely tail biters was low in all three phases, 0, 2.1, and 0.9 %, respectively, and 89.2, 85.7, and 74.1 % were neither tail biter nor victim in these phases.

Throughout life, 10.8 % of the B pigs was never identified as a victim (i.e. during all four phases) or tail biter (i.e. during all three phases), or both, and 52.8 % of the E pigs remained ‘neutral’ with respect to tail biting and tail damage.
3.3. Individual tail biting and tail damage as predictors

3.3.1. Barren housed tail biters

In total, 8.3% of B housed pigs were estimated to be tail biters pre-weaning, but as explained under section 2.4, this data was not further used for analysis. In phase 1, 12.1% of the piglets was identified as a tail biter. Being classified as a tail biter in phase 1 did not increase the likelihood of being a tail biter in phase 2 or 3, and neither was classification as tail biter in phase 2 predictive of being a tail biter in phase 3. Consequently, in phases 2 and 3, new tail biters were found in B pens, and some of the tail biters identified in previous phases stopped tail biting completely or temporarily. More than half of the B pigs (61.7%) were not identified as tail biter in any of the phases post-weaning.

3.3.2. Barren housed victims

In B housing, 5.8% of all piglets arrived with (mild) tail wounds (Figure 2A). Having a tail wound, i.e. the criterion for being classified as victim, at weaning was not predictive of having a tail wound in later phases, although piglets with a tail wound at weaning tended to be a victim again in phase 2 post-weaning when B housed (OR = 4.15, P < 0.10). In B pigs, victims of tail biting in phase 1, tended to be victims again in phase 2 (OR = 2.04, P < 0.10), and were likely to be victim again in phase 3 (OR = 4.61, P < 0.01) post-weaning. B housed victims in phase 2 were also likely to be victim in phase 3 again (OR = 9.65, P < 0.001) (Figure 4A). In total, 16.6% of the B pigs never suffered a tail wound throughout life.

3.3.3. Enriched housed tail biters

Almost 10% of E housed piglets were estimated to be tail biters at time of weaning, but this data was not further used for analysis. As none of the E pigs was identified as a tail biter in phase 1 post-weaning, it was not possible to use phase 1 as predictor of phase 2 and 3 tail biters (Figure 3B). In phase 2, 2.5% of all E housed pigs developed tail biting, but these pigs were not the tail biters in phase 3 (Figure 1B), and therefore, also phase 2 was not predictive of tail biters in phase 3 (prevalence was too low for statistical analysis). In total, 94.4% of the E housed pigs was never identified as a tail biter post-weaning.
Figure 3. Funnels presenting behaviours observed during the pre-weaning period (average of the whole litter) and phases 1-3 (individual pig), that are likely to predict tail biters in phase 1-3 post-weaning. Grey shapes indicate significant relationships, white shapes indicate tendencies, ‘+’ indicates a positive and ‘−’ indicates a negative relationship with being a tail biter. A: Tail biters kept in barren housing, B: Tail biters kept in enriched housing.
Figure 4. Funnels presenting behaviours observed during the pre-weaning period (average of the whole litter) and phases 1-3 (individual pig), that are likely to predict tail bite victims in phase 1-3 post-weaning. Pentagons indicate binary (0/1) variables. Grey shapes indicate significant relationships, white shapes indicate tendencies, ‘+’ indicates a positive and ‘-’ indicates a negative relationship with being a tail bite victim. A: Victims kept in barren housing, B: Victims kept in enriched housing.
3.3.4. Enriched housed victims

At weaning, 12.5% of piglets placed in E pens had a tail wound (Figure 2B). These E housed piglets were not likely to have a tail wound later in life again (concerning phase 1, variation was too low for statistical analysis). Pigs identified as victims in phase 1 were also not likely to be a victim again in phase 2, and they even had a smaller chance of developing a tail wound in phase 3 (OR = 0.11, P < 0.05). However, victims in phase 2 were likely to remain (or become again) a victim in phase 3 (OR = 4.84, P < 0.01) (Figure 4B). More than half of the enriched housed pigs did not develop a tail wound throughout their life (54.7%).

3.4. Behaviour of the litter pre-weaning as predictor

3.4.1. Barren housed tail biters

Pigs from litters with a relatively high level of tail biting behaviour pre-weaning were likely to be identified as tail biter in B pens during phase 1 (OR = 55.79, P < 0.01) and 3 (OR = 12.59, P < 0.05), but not during phase 2 post-weaning (Figure 3A). Time spent by litters on manipulating the sow increased the probability of being classified as a tail biter in B pens in phase 1 (OR = 7.37, P < 0.01). None of the other averaged litter behaviours was associated with being classified as a tail biter in phase 2. However, time spent by litters on social behaviour directed at litter mates decreased the likelihood of being classified a tail biter in phase 3 when housed in B pens (OR = 0.30, P < 0.01).

3.4.2. Barren housed victims

B housed pigs originating from litters with a relatively high level of tail biting pre-weaning tended to have a high likelihood of developing a tail wound in phase 2 post-weaning (OR = 7.27, P < 0.10), but not in phase 1 or 3 (Figure 4A). Time spent by litters on nursing (i.e. drinking sow milk) increased the probability of being classified as a victim in B pens during phase 1 (OR = 1.43, P < 0.05) and 3 (OR = 1.6, P < 0.01). Furthermore, spending relatively much time on massaging the sow’s udder (OR = 1.08, P < 0.05) and ear biting (OR = 4.52, P < 0.05), and spending relatively little time on inactive behaviours (i.e. mainly lying behaviour) (OR = 0.95, P < 0.05), increased the probability of being classified as a victim during phase 2 in B pens.
3.4.3. Enriched housed tail biters

None of the averaged litter behaviours observed pre-weaning could predict classification as tail biter in E housing in the three phases post-weaning (during phase 3, variation was too low for statistical analysis) (Figure 3B).

3.4.4. Enriched housed victims

Average litter behaviour pre-weaning could not predict victims in E pens during phase 1 post-weaning (Figure 4B). However, pigs from litters with a relatively high level of tail biting (OR = 16.47, P < 0.10) and ear biting (OR = 8.19, P < 0.05) behaviour (tended to) have a high probability of being classified as a victim during phase 2.

3.5. Individual home pen behaviour post-weaning as predictor

3.5.1. Barren housed tail biters

In B pens post-weaning, none of the behaviours of individual pigs were significantly predictive of being classified as a tail biter. However, time spent on manipulations directed at pen mates (OR = 2.98, P < 0.001) and exploring the environment (OR = 1.08, P < 0.05) performed by B housed pigs during phase 3, were associated with being classified as a tail biter during that phase (Figure 3A).

3.5.2. Barren housed victims

Time spent on social behaviour in phase 1 (OR = 1.73, P < 0.01) and chewing objects in phase 2 increased the probability of being a victim of tail biting in phase 2 (OR = 2.01, P < 0.05) (Figure 4A). Time spent on standing (OR = 1.36, P < 0.05) and ear biting (OR = 3.50, P < 0.05) in phase 1, and chewing objects in phase 2 (OR = 2.86, P < 0.01) increased the probability of being classified as a victim in phase 3. Time spent on belly nosing (OR = 0.68, P < 0.05) and walking (OR = 0.68, P < 0.05) observed in phase 2, decreased the probability of being classified as a victim in phase 3.

3.5.3. Enriched housed tail biters

During phase 1, no tail biters were identified in E housing, consequently behaviours observed in phase 1 could not be related to tail biters in the same phase. A relatively high level of fighting behaviour during phase 2 (OR = 4.09, P < 0.05) was associated with being classified as a tail biter in the same phase (Figure
Phase 3 tail biters could not be related to behaviours observed in phase 1-3 as variation was too low for statistical analysis.

3.5.4. Enriched housed victims

In E pens, behaviours observed during phase 1 could not be related to being classified as victims in the same phase as variation was too low for statistical analysis. Standing behaviour as observed during phase 1 increased the probability of being a victim during the next phase (OR = 1.57, P < 0.05) (Figure 4B). Much time spent on standing during phase 1 (OR = 1.59, P < 0.05), inactivity in phase 2 (OR = 1.07, P < 0.05), and little time spent on eating and drinking (OR = 0.82, P < 0.05) during phase 2, increased the likelihood of being a victim in E pens during phase 3.

3.6. Correlations between tail biting and tail damage at pen level post-weaning

3.6.1. Barren pens

Time spent on tail biting behaviour at pen level (average of all pigs in a pen) in B housing was not significantly correlated between the three phases post-weaning (Table 2). However, average tail damage scores per pen were correlated between successive phases (Table 3). Tail biting behaviour and tail damage observed in pens were only correlated in phase 1.

3.6.2. Enriched pens

Time spent on tail biting behaviour in E pens was not significantly correlated between the three phases post-weaning (Table 4). Tail damage as observed in E pens during phase 1 was not correlated with tail damage during phase 2 and 3, but phase 2 and 3 tail damage were correlated (Table 5). Tail damage in E pens observed in phase 1 was negatively correlated with tail biting behaviour in phase 1 and 3. However, tail damage observed in phase 3 was positively correlated with tail biting behaviour in phase 3.
Table 2. Correlations between average tail biting observed in barren pens and general activity of the pens in phase 1-3 post-weaning.

<table>
<thead>
<tr>
<th></th>
<th>Phase 1 Tail biting</th>
<th>Phase 2 Tail biting</th>
<th>Phase 3 Tail biting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail biting</td>
<td>-0.01</td>
<td>-0.25</td>
<td>-0.13</td>
</tr>
<tr>
<td>Eating and drinking</td>
<td>-0.24</td>
<td>0.31+</td>
<td>0.07</td>
</tr>
<tr>
<td>Walking</td>
<td>-0.06</td>
<td>-0.31+</td>
<td>0.04</td>
</tr>
<tr>
<td>Standing</td>
<td>-0.06</td>
<td>-0.20</td>
<td>0.09</td>
</tr>
<tr>
<td>Inactive</td>
<td>0.17</td>
<td>-0.10</td>
<td>-0.01</td>
</tr>
<tr>
<td>Fighting</td>
<td>0.17</td>
<td>-0.04</td>
<td>0.20</td>
</tr>
<tr>
<td>Ear biting</td>
<td>0.11</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>Belly nosing</td>
<td>0.03</td>
<td>-0.13</td>
<td>-0.27+</td>
</tr>
<tr>
<td>Other manipulation</td>
<td>0.07</td>
<td>0.01</td>
<td>-0.09</td>
</tr>
<tr>
<td>Mounting</td>
<td>0.10</td>
<td>-0.35+</td>
<td>0.08</td>
</tr>
<tr>
<td>Exploration</td>
<td>-0.18</td>
<td>0.18</td>
<td>0.13</td>
</tr>
<tr>
<td>Chewing objects</td>
<td>0.09</td>
<td>-0.16</td>
<td>0.09</td>
</tr>
<tr>
<td>Social</td>
<td>-0.02</td>
<td>-0.24</td>
<td>-0.11</td>
</tr>
<tr>
<td>Playing</td>
<td>-0.16</td>
<td>0.19</td>
<td>0.10</td>
</tr>
</tbody>
</table>

* Please note: the correlation is based on infrequent behaviour (< 0.05 % of observations).
+ P < 0.10, * P < 0.05, ** P < 0.01, *** P < 0.001.
Table 3. Correlations between average tail damage observed in barren pens and general activity of the pens in phase 1-3 post-weaning.

<table>
<thead>
<tr>
<th></th>
<th>Phase 1 Tail damage</th>
<th>Phase 2 Tail damage</th>
<th>Phase 3 Tail damage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ph. 1</td>
<td>Ph. 2</td>
<td>Ph. 3</td>
</tr>
<tr>
<td>Tail damage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail biting</td>
<td>0.31*</td>
<td>0.23</td>
<td>-0.15</td>
</tr>
<tr>
<td>Eating and drinking</td>
<td>0.38*</td>
<td>0.30+</td>
<td>0.08</td>
</tr>
<tr>
<td>Walking</td>
<td>-0.10</td>
<td>-0.32*</td>
<td>0.04</td>
</tr>
<tr>
<td>Standing</td>
<td>-0.13</td>
<td>-0.12</td>
<td>-0.24</td>
</tr>
<tr>
<td>Inactive</td>
<td>-0.06</td>
<td>-0.16</td>
<td>-0.08</td>
</tr>
<tr>
<td>Fighting</td>
<td>0.32*</td>
<td>0.21</td>
<td>0.34*</td>
</tr>
<tr>
<td>Ear biting</td>
<td>-0.05</td>
<td>0.07</td>
<td>-0.13</td>
</tr>
<tr>
<td>Belly nosing</td>
<td>-0.29</td>
<td>0.09</td>
<td>-0.04</td>
</tr>
<tr>
<td>Other manipulation</td>
<td>-0.09</td>
<td>0.24</td>
<td>-0.22</td>
</tr>
<tr>
<td>Mounting</td>
<td>-0.13</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Exploration</td>
<td>0.02</td>
<td>0.16</td>
<td>0.20</td>
</tr>
<tr>
<td>Chewing objects</td>
<td>0.32*</td>
<td>0.18</td>
<td>0.10</td>
</tr>
<tr>
<td>Social</td>
<td>-0.02</td>
<td>-0.17</td>
<td>0.05</td>
</tr>
<tr>
<td>Playing</td>
<td>-0.01</td>
<td>-0.13</td>
<td>-0.16</td>
</tr>
</tbody>
</table>

+ P < 0.10, * P < 0.05, ** P < 0.01.
3.7. Correlations between tail biting and tail damage, and general behaviours at pen level

3.7.1. Barren pens
None of the average pen behaviours observed during phase 1 post-weaning was associated with average tail biting behaviour performed in B pens during that phase (Table 2). Time spent on eating and drinking behaviour, exploration and more specifically chewing objects as observed in B pens during phase 1, and other manipulative behaviours and chewing objects during phase 2 were positively correlated with tail biting behaviour as observed during phase 2. Inactivity in phase 1 was negatively correlated with phase 2 tail biting behaviour. Walking, standing and playing behaviour in B pens during phase 2 were positively correlated with tail biting behaviour in phase 3, while ear biting during phase 2 was negatively correlated with tail biting in phase 3. Eating and drinking behaviour, fighting, and chewing objects as observed in B pens in phase 1 post-weaning, were positively correlated with average tail damage as observed in these pens during that phase (Table 3). Chewing objects during phase 1 was positively correlated with tail damage observed in phase 2. Furthermore, time spent on standing during phase 2 was negatively correlated with level of tail damage measured in phase 2. Walking and fighting during phase 1, chewing objects during phase 1 and 2, and other types of manipulations during phase 2 were positively correlated with tail damage in phase 3.

3.7.2. Enriched pens
None of the average pen behaviours observed during phase 1 post-weaning was associated with average tail biting behaviour performed in E pens during that phase (Table 4). Time spent on fighting, other types of manipulations and social behaviour in E pens during phase 1 were positively correlated with tail biting in E pens during phase 2. Furthermore, other types of manipulations observed during phase 2 and play behaviour observed in phase 3 were positively correlated with tail biting behaviour as observed in E pens during phase 3. Explorative behaviours during phase 1, and play behaviour during phase 2 correlated positively and inactivity during phase 2 correlated negatively with tail damage during phase 2 at pen level in E housing (Table 5). Time spent on play behaviour as observed in E pens during phase 3 was positively, but other types of manipulative behaviours as observed in phase 3 were negatively correlated with tail damage measured in E pens during the final phase post-weaning.
Table 4. Correlations between average tail biting observed in enriched pens and general activity of pens in phase 1-3 post-weaning.

<table>
<thead>
<tr>
<th></th>
<th>Phase 1 Tail biting</th>
<th>Phase 2 Tail biting</th>
<th>Phase 3 Tail biting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phase 1</td>
<td>Phase 2</td>
<td>Phase 3</td>
</tr>
<tr>
<td>Tail biting</td>
<td>-0.20</td>
<td>0.08</td>
<td>0.14</td>
</tr>
<tr>
<td>Eating and drinking</td>
<td>0.01</td>
<td>0.03</td>
<td>0.21</td>
</tr>
<tr>
<td>Walking</td>
<td>0.12</td>
<td>0.21</td>
<td>0.17</td>
</tr>
<tr>
<td>Standing</td>
<td>0.17</td>
<td>0.16</td>
<td>0.04</td>
</tr>
<tr>
<td>Inactive</td>
<td>-0.19</td>
<td>-0.06</td>
<td>-0.05</td>
</tr>
<tr>
<td>Fighting</td>
<td>-0.12</td>
<td>0.04</td>
<td>0.30+</td>
</tr>
<tr>
<td>Ear biting</td>
<td>-0.21</td>
<td>0.10</td>
<td>0.04</td>
</tr>
<tr>
<td>Belly nosing</td>
<td>-0.01</td>
<td>-0.07</td>
<td>0.06</td>
</tr>
<tr>
<td>Other manipulation</td>
<td>-0.22</td>
<td>0.37*</td>
<td>-0.10</td>
</tr>
<tr>
<td>Mounting</td>
<td>0.10</td>
<td>-0.04</td>
<td>0.00</td>
</tr>
<tr>
<td>Exploration</td>
<td>0.19</td>
<td>-0.02</td>
<td>-0.06</td>
</tr>
<tr>
<td>Chewing objects</td>
<td>-0.04</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Social</td>
<td>-0.17</td>
<td>0.11</td>
<td>0.10</td>
</tr>
<tr>
<td>Playing</td>
<td>0.24</td>
<td>0.03</td>
<td>-0.09</td>
</tr>
</tbody>
</table>

+ P < 0.10, * P < 0.05.
Table 5. Correlations between average tail damage observed in enriched pens and general activity of pens in phase 1-3 post-weaning.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Phase 1 Tail damage</th>
<th>Phase 2 Tail damage</th>
<th>Phase 3 Tail damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail damage</td>
<td>0.06</td>
<td>-0.05</td>
<td>0.32*</td>
</tr>
<tr>
<td>Tail biting</td>
<td>-0.33*</td>
<td>0.03</td>
<td>-0.37*</td>
</tr>
<tr>
<td>Eating and drinking</td>
<td>0.07</td>
<td>-0.32*</td>
<td>-0.09</td>
</tr>
<tr>
<td>Walking</td>
<td>0.26</td>
<td>-0.13</td>
<td>-0.07</td>
</tr>
<tr>
<td>Standing</td>
<td>-0.04</td>
<td>-0.03</td>
<td>-0.11</td>
</tr>
<tr>
<td>Inactive</td>
<td>0.02</td>
<td>0.15</td>
<td>0.08</td>
</tr>
<tr>
<td>Fighting</td>
<td>-0.12</td>
<td>-0.27*</td>
<td>-0.04</td>
</tr>
<tr>
<td>Ear biting</td>
<td>-0.01</td>
<td>0.03</td>
<td>-0.10</td>
</tr>
<tr>
<td>Belly nosing</td>
<td>-0.05</td>
<td>0.06</td>
<td>0.07</td>
</tr>
<tr>
<td>Other manipulation</td>
<td>0.28*</td>
<td>0.01</td>
<td>0.17</td>
</tr>
<tr>
<td>Mounting</td>
<td>-0.24</td>
<td>0.09</td>
<td>0.08</td>
</tr>
<tr>
<td>Exploration</td>
<td>-0.03</td>
<td>-0.05</td>
<td>-0.03</td>
</tr>
<tr>
<td>Chewing objects</td>
<td>0.05</td>
<td>0.08</td>
<td>0.04</td>
</tr>
<tr>
<td>Social</td>
<td>0.20</td>
<td>0.00</td>
<td>-0.31*</td>
</tr>
<tr>
<td>Playing</td>
<td>-0.31*</td>
<td>-0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* Please note: the correlation is based on infrequent behaviour (< 0.05 % of observations).
+ P < 0.10, * P < 0.05, ** P < 0.01, *** P < 0.001.
4. Discussion

4.1. Interrelations between tail damage, blood freshness and tail posture scores

The positive correlations between tail damage and tail posture in several phases during this study are in line with previous studies where (low) tail postures were predictive of tail damage (Statham et al., 2009; Zonderland et al., 2009). As expected, we also found positive correlations between tail damage scores and blood freshness, the more damage, the higher the likelihood of observing blood. Blood freshness is mainly valuable for determining the age of a wound (i.e. is it fresh or not) (Keeling et al., 2012), observing the process of healing and possible efficacy of curative treatments (Zonderland et al., 2008).

4.2. Tail biting, tail damage and behaviour pre-weaning

It was expected that at time of weaning, piglets would have undamaged tails as tail biting is generally thought to be a health and welfare problem in weaners and finishing pigs (e.g. Munsterhjelm et al., 2013; Wallgren and Lindahl, 1996; Zonderland et al., 2011b). It has been suggested, though, that pigs may develop tail biting at a young age through, non-damaging, tail-in-mouth behaviour (Schrøder-Petersen et al., 2003). Behavioural observations of the piglets pre-weaning were limited in our study, but tail biting was evidently present and consequently part of the piglets had, at time of weaning, tail damage varying from minor damage to (small) wounds. Some studies describe the time spent on manipulations directed at litter mates in piglets pre-weaning (e.g. Cox and Cooper, 2001; Oostindjer et al., 2011; Vanheukelom et al., 2011), but, to our knowledge, information about tail damage in young piglets is lacking. Tail biting in piglets may go unnoticed as piglets are small and tail damage is, for the human eye, consequently also small and probably not perceived as a problem. Pigs originating from litters with a relatively high level of tail biting and oral manipulation of the sow pre-weaning were more likely to become tail biters in later life when placed in B pens. This suggests that the impact of pre-weaning behavioural development on tail biting in later life is worthwhile to explore in future studies.

It has been suggested that tail wounds in piglets may increase the risk of being a victim of tail biting post-weaning as tail wounds may attract other pigs (Fraser, 1987a). We found, however, that tail wounds at time of weaning were
Chapter 2

not predictive of tail wounds in later life. At time of weaning victimized piglets and their initial tail biters were not placed in the same environment and possible habituation to chew on the tail of particular individuals was therefore disrupted. This separation of tail biters and victims may have caused the non-predictive value of tail wounds at time of weaning for being victimized again post-weaning.

Piglets originating from litters with higher levels of teat-directed behaviours pre-weaning were, when placed in B pens, more likely to become victims of tail biting in later life. This suggests a role for feeding behaviour in becoming victimized and may be in line with the thought that victims of tail biting are the pigs that feed more frequently prior to observed tail bite problems (Wallenbeck and Keeling, 2013), although cause and effect remains debatable.

4.3. Tail biting, tail damage and behaviour in E and B housing post-weaning at the individual level

Housing was expected to profoundly affect tail biting and tail damage in pigs (e.g. Beattie et al., 1995; Van de Weerd et al., 2006; Zonderland et al., 2008). In our study, E housing post-weaning strongly reduced tail biting behaviour and tail damage indeed, and generally also the number of tail biters and victims, compared to B housing. These results support the idea that tail biting is largely related to a lack of exploration possibilities (Anonymous, 2001; Feddes et al., 1993; Schröder-Petersen and Simonsen, 2001). However, in agreement with other studies (Munsterhjelm et al., 2009; Van de Weerd et al., 2006; Zonderland et al., 2008), still some tail biting occurred in E pigs. All piglets used in the current study were, pre-weaning, kept in a conventional barren farrowing system which likely increased the time spent on manipulative behaviours directed at litter mates (Cox and Cooper, 2001; Oostindjer et al., 2011). Consequently, a considerable proportion of the piglets, used in our further study post-weaning, possibly already learned (see review of Schröder-Petersen and Simonsen, 2001) to direct their biting and chewing behaviour towards pen mates pre-weaning. However, enrichment materials have a large immediate effect on damaging behaviour (Bolhuis et al., 2006; Van de Weerd et al., 2005), which may have overruled these pre-weaning effects. Nevertheless, in E housed pigs a lack of exploration possibilities may have developed as well due to the decreasing space availability of the pigs during the fattening period, even though the quality of the environment with respect to rooting materials is thought to be more important than space
availability (Beattie et al., 1996; Rodenburg and Koene, 2007). We expected that other, individual, factors may also be relevant in the development of tail biting in relatively enriched environments. In this study, however, the (rare) tail biters identified in E pens in the different phases were mostly different individuals, and were hard to characterize by other behaviours.

Also in B pens, where more tail biters were identified, individual tail biters were not consistent over post-weaning phases, even though high pre-weaning levels of tail biting at the litter level increased the risk of becoming a tail biter in later life. Tail biting post-weaning was, at the individual level, also not consistently related to other behaviours. Once pigs in our study started tail biting, some tail biters continued tail biting, but others seemed to have given up on tail biting temporarily or completely. Motivations to perform tail biting may be different, and time of onset may, hence, also be different. It has been suggested that three types of tail biters can be distinguished: two-stage (likely related to foraging and exploration), sudden forceful (likely related to gaining access to a resource) and obsessive (or ‘fanatical’) tail biters (Taylor et al., 2010). Furthermore, fanatical tail biters may be specialized in all kinds of biting behaviour and pigs performing little, but some, tail biting behaviour performed all kinds of abnormal behaviours (Brunberg et al., 2011). In our study, we have likely observed the ‘two-stage’ tail biters mostly, and only one ‘obsessive’ tail biter. Consistency (or non-consistency) in tail biting may, therefore, be related to the type of tail biter observed, but it may also be related to its epidemic nature as tail biting can spread exponentially among pigs in a pen (Zonderland et al., 2011a). Tail biting may be socially facilitated (reviewed in Schröder-Petersen and Simonsen, 2001), pigs may be attracted to damaged (bloody) tails (Fraser, 1987a), or another underlying cause may trigger the behaviour and hold for the whole pen (e.g. problems with air quality; Sällvik and Walberg, 1984; Van Putten, 1969).

Post-weaning, victims were likely to remain victims in successive phases, however, in E housing this was only true from the grower to the finisher phase. Pigs may have a preference for nose contact directed towards a specific individual (Camerlink and Turner, 2013), and we may hypothesize that this is also true for tail biting behaviour. The consistency in being victimized may stem from the scabs or wounds that attract other pigs, but victims may also behave in such a way that they are interesting for tail biters. Pigs with health problems such as lameness may for instance be easy targets for tail biting (although tail damage can also be the cause of health problems) (Valros et al., 2012), or
victims may have given up retreating from the tail biters (reviewed in Schrøder-Petersen and Simonsen, 2001). In pigs no longitudinal studies with respect to victimization due to tail biting were found, but in humans these studies exist. Bullying in humans is related to several underlying factors of which boredom may be one (Yoneyama and Naito, 2003). Boredom may also be related to tail biting in pigs (Zonderland et al., 2008), and therefore, bullying and tail biting may have a (partly) common motivational background. Human victims of bullying are known to have a high probability of being bullied again later in life, by the same or other bullies (Bernstein and Watson, 1997; Wolke et al., 2009). Post-weaning tail damage seems related to a higher level of activity in individual pigs, and to pig- and pen directed manipulative behaviours other than tail biting, i.e. ear biting and chewing objects (i.e. chain with ball and jute sack). The increase in tail damage over time in our pigs is in line with a previous study (Schmolke et al., 2003) and was most likely caused by the increase in tail biting and tail biters. However, the increase in percentage of tail biters was much lower compared to the increase in percentage of victims, thus the intensity and force of tail biting likely increased over time as well.

4.4. Tail biting and tail damage and their association with behaviour at pen level

At pen level post-weaning, a similar pattern was found as for individual pigs where tail biting was not consistently observed throughout time, but tail damage was observed to remain present in successive phases. However, in E housing, tail damage at pen level was, again, only consistent from the grower to the finisher phase. Furthermore, tail biting and tail damage were not always positively correlated as we would have expected. This lack of correlations or even negative correlations between tail biting and tail damage at pen level may have several reasons. First, we may have missed some tail biting events. Second, the intensity and force of tail biting may differ in time and, finally, the tail damage in phase 1, particular in E housing, may partially result from the pre-weaning phase and therefore is not (yet) a reflection of the level of tail biting in the pens. Even though we may have underestimated the time spent on tail biting and the number of tail biters due to the scan sampling observation method used (Martin and Bateson, 1993), several correlations between tail biting and behaviours at pen level, were also found between tail damage (which is a more reliable measure
than tail biting behaviour) and these behaviours at pen level. In B pens postweaning, and to a lesser extent also in E pens, with higher levels of tail biting and tail damage a generally higher activity was observed. Although the behaviours as eating and drinking, walking, standing (which is likely a reflection of arousal (Elliott et al., 2005)), fighting, playing (which may increase arousal (LaFreniere, 2013) and/or reflect increased arousal), and inactivity were independently not always consistently related to tail biting or tail damage, they altogether suggest a higher level of activity in the pens. Higher activity levels before a tail bite outbreak were also found by others (Statham et al., 2009; Wood-Gush and Beilharz, 1983; Zonderland et al., 2011b).

Although in E pens the general activity levels were not as clearly predictive of problems with tail biting compared to B pens, higher levels of exploration and manipulations directed at pen mates, other than tail biting or ear biting, were able to predict tail damage or tail biting behaviour in the successive phases postweaning. In B pens, especially the explorative behaviour ‘chewing objects’ was found to be most consistently related to tail biting and tail damage. This indicates that the available objects were used as a substitute for foraging substrate (Bracke et al., 2006), but that objects solely were not sufficient enough to prevent tail biting in B pens. Nevertheless, higher levels of tail biting behaviour, other manipulative behaviours, and chewing objects observed in pens, may indicate that in some pens a higher need for exploration is present. Therefore, in conventional housing systems, a higher level of chewing objects, especially on pen level, is likely an important predictor of problems with tail biting in the (near) future. As a more practical tool, the level of wear or ‘consumption’ of objects such as jute sacks may be used to indicate the level of chewing on objects and thereby predicting tail biting behaviour.

5. Conclusions

Tail biting and tail damage is a problem that already starts in piglets pre-weaning when kept in conventional farrowing systems. Tail biters in barren housing post-weaning, were more likely to stem from litters that display higher levels of tail biting behaviour. This indicates that the pre-weaning conditions warrant more attention to gain a better understanding of the development of tail biting and to identify early factors or events involved in order to prevent
the damaging behaviour. Post-weaning housing with straw bedding largely reduced tail biting and tail damage, but did not fully prevent the undesirable behaviour. Individual tail biters were not consistent throughout time and they were difficult to characterize by other behaviours observed. Victims of tail biting, however, were consistent over time and several other behaviours were predictive of victimization. Although it seems thus difficult to characterize future tail biters based on their behaviour, tail damage at pen level was quite consistent, particularly in barren housing, and pen-level observations were most valuable in predicting ‘problem pens’ with respect to tail biting. Pens with high levels of tail biting and/or tail damage were generally more active and engaged in higher levels of different types of pig- or pen directed manipulative behaviours. Higher levels of chewing objects such as jute sacks could be used as valuable tools in predicting tail bite outbreaks. Our results underline the complex background of tail biting, and further (early life) behavioural, but also physiological indicators need to be explored to gain more knowledge about the development of damaging behaviours in pigs.

Acknowledgement

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Chapter 2


Tail biting, tail damage and general behaviour


Chapter 2


Relations between peripheral and brain serotonin measures and behavioural responses in a novelty test in pigs

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Abstract

Pigs differ in their behavioural responses towards environmental challenges. Individual variation in maladaptive responses such as tail biting, may partly originate from underlying biological characteristics related to (emotional) reactivity to challenges and serotonergic system functioning. Assessing relations between behavioural responses and brain and blood serotonin parameters may help in understanding susceptibility to the development of maladaptive responses. The objective of the current study was, therefore, to assess the relationship between the pigs' serotonergic parameters measured in both blood and brain, and the behaviour of pigs during a novelty test.

Pigs (n = 31) were subjected to a novelty test at 11 weeks of age, consisting of 5-min novel environment exposure after which a novel object (a bucket) was introduced for 5 min. Whole blood serotonin, platelet serotonin level, and platelet serotonin uptake were determined at 13 weeks of age. Levels of serotonin, its metabolite and serotonin turnover were determined at 19 weeks of age in the frontal cortex, hypothalamus and hippocampus.

The behaviour of the pigs was different during exposure to a novel object compared to the novel environment only, with more fear-related behaviours exhibited during novel object exposure. Platelet serotonin level and brain serotonergic parameters in the hippocampus were interrelated. Notably, the time spent exploring the test arena was significantly correlated with both platelet serotonin level and right hippocampal serotonin activity (turnover and concentration). In conclusion, the existence of an underlying biological trait - possibly fearfulness - may be involved in the pig's behavioural responses toward environmental challenges, and this is also reflected in serotonergic parameters.

Keywords: serotonin, blood, brain, pigs, behaviour, novelty
1. Introduction

Pigs in commercial pig husbandry are frequently kept in housing systems under rather barren circumstances where they lack the opportunity to sufficiently fulfil their (welfare) needs, such as foraging and exploration (Bracke, 2008; Bracke et al., 1999). Pigs often have difficulty in coping with the limitations and challenges of their living environment, both behaviourally and physiologically. This might be reflected in signs of maladaptation, for example in the development of aberrant behaviours (e.g. tail biting; Beattie et al., 2005; Edwards, 2006), an increased susceptibility to diseases (Prunier et al., 2010), or an impaired productivity (Bolhuis et al., 2006; Gentry et al., 2002). Although maladaptation is strongly affected by environmental factors, there is a large variation in responses of animals kept under similar circumstances. For instance, not all individual pigs develop behavioural problems such as tail biting under adverse conditions, and, conversely, tail biting can still be observed among pigs that are kept under relatively favourable conditions (Munsterhjelm et al., 2009; Van de Weerd et al., 2006; Zonderland et al., 2008). Apparently individual biological characteristics are involved too. The individual variation in the propensity to develop maladaptive responses may partly originate from underlying differences in functioning of neurotransmitter systems (Koolhaas et al., 2010). Individual differences in behaviour of animals during environmental challenges may covary with the animal’s serotonergic (5-HT, i.e. 5-hydroxytryptamine) system functioning in both brain and periphery. In laying hens, for example, a predisposition for high levels of fear or anxiety, stress sensitivity, and feather pecking - a behaviour similar to tail biting (Zupan et al., 2012) - were all associated with the 5-HT system (Bolhuis et al., 2009; Uitdehaag et al., 2011; Van Hierden et al., 2002). Furthermore, in pigs, brain 5-HT concentrations were found to be related to the pig’s way of adapting to environmental challenges (Adeola et al., 1993; Rosochacki et al., 2003). Accordingly, the 5-HT system has been associated with variation in affect, temperament and personality (Koolhaas et al., 2010). Mental disorders such as depression, excessive aggression and obsessive compulsive disorders in humans (e.g. Barton et al., 2008; Besiroglu et al., 2011; Korpi et al., 1986; Parsey et al., 2002), and maladaptive behaviours such as excessive aggression and depression-like behaviours in rodents (e.g. Caramaschi et al., 2007; Veenema et al., 2004; Weiss et al., 1981), and feather pecking in chickens (Bolhuis et al., 2009; Uitdehaag et al., 2011; Van Hierden et al., 2002) seem to be coupled with suboptimal 5-HT
functioning in the brain (i.e. either higher or lower 5-HT system activity). There are, however, limitations to studying 5-HT measures in the brain, and therefore alternative 5-HT measures have been explored as 5-HT is also present in the periphery (Chou et al., 1983; Mück-Šeler and Pivac, 2011; Rausch et al., 2005; Stahl, 1977). Peripheral 5-HT is synthesized in the gastrointestinal tract and then released into the blood where it can be stored in blood platelets (Mück-Šeler and Pivac, 2011). It has been suggested that 5-HT storage, uptake and release in blood platelets is similar to that in brain 5-HT neurons (see for reviews Mück-Šeler and Pivac, 2011 and Stahl, 1977). Uptake of 5-HT in blood platelets is mediated through the platelet 5-HT uptake transporter which shares genetic (Lesch et al., 1993) and kinetic properties (Rausch et al., 2005) with the 5-HT uptake transporter in brain neurons. Furthermore, it is known that (at least) one of the 5-HT receptors in the periphery, the 5-HT$_2$ receptor, has similar binding properties as the 5-HT$_2$ receptor in brain synaptosomes (Ostrowitzki et al., 1993). Remarkably, similar to brain 5-HT measures, peripheral 5-HT measures have been reported to relate to mental disorders and maladaptive behaviours in humans and other animals (e.g. Askenazy et al., 2000; Bakish et al., 1997; Bianchi et al., 2002). It is unknown whether peripheral 5-HT measures in pigs may likewise represent suitable markers for brain 5-HT measures and behavioural responses.

In both laying hens and pigs, the relation between behavioural responses in behavioural tests and maladaptive behaviours has been studied. Feather pecking behaviour in laying hens was associated with fearfulness expressed in an open field (Uitdehaag et al., 2008), and in pigs, tail biting was associated with behavioural (and physiological) responses in a novel arena and novel object test, i.e. tail biters seemed less fearful compared to victims of tail biting (Zupan et al., 2012). In humans and other animals, fear or anxiety expressed during environmental challenges can be related to both maladaptive behaviours (Inderbitzen and Hope, 1995; Uitdehaag et al., 2008; Zupan et al., 2012) and 5-HT system functioning (e.g. Barton et al., 2008; Uitdehaag et al., 2011). Hence, novelty tests (i.e. ‘open field’, ‘novel environment’, or ‘novel arena’, sometimes combined with a ‘novel object’) that are likely to generate fear or anxiety, might be predictive not only for maladaptive behaviours such as tail biting, but also for 5-HT system activity. Assessing relations between behavioural responses and activity of the serotonergic system could provide insight into the susceptibility of pigs for developing maladaptive behavioural or physiological responses. The occurrence of maladaptive responses on farms, such as tail biting (Smulders et al., 2008) or
other aberrant behaviours, is rather unpredictable and requires a relatively high number of animals. Brain collection is laborious and only provides retrospective information on 5-HT system activity. Therefore, potential correlates of central 5-HT functioning in blood or behaviour could support future large-scale research on predisposing characteristics for developing maladaptive behaviours.

The first aim of this study was to investigate whether blood (platelet) 5-HT measures in pigs are correlated with brain 5-HT measures. We focused on three brain areas, the hippocampus, the frontal cortex, and the hypothalamus. These brain areas are of specific interest regarding 5-HT system functioning since they are involved in cognition, mood, memory, fear or anxiety, and in mediating stress responses (e.g. Hashimoto et al., 1999; Korte, 2001; Oquendo and Mann 2001; Sandi and Pinelo-Nava, 2007). The second and final aim of this study was to assess the relationship between measures of brain and blood 5-HT activity and the behavioural responses of pigs in a challenging situation (a novelty test), and to assess if a biological (personality) trait may be involved.

2. Materials and methods

The experimental protocol followed during this study was approved by the Animal Care and Use Committee of Wageningen University.

2.1. Animals and housing

The pigs (n = 32) studied were Tempo × TOPIGS 30 pigs (TOPIGS, The Netherlands) born and reared at the experimental farm “De Haar” of Wageningen University, The Netherlands. The pigs entered this study at eight weeks of age and were previously used in other studies on pre- and post-weaning behaviour (Melotti et al., 2011; Oostindjer et al., 2011). Each pen consisted of four pigs (unfamiliar at weaning) with a 1:1 ratio of entire boars and gilts. Pigs were kept either in barren (B) or enriched (E) pens throughout their lives. B pens (4.2 m²) referred to conventional housing without enrichment substrate but with a chain; in addition to a chain, pigs housed in E pens (6.4 m²) had straw, peat, wood shavings, branches and extra space at their disposal (see Melotti et al., 2011 and Oostindjer et al., 2011 for details). Pens were cleaned daily. Pigs were fed ad libitum with commercial pig diets and had continuous access to drinking water.
The mean ambient temperature was 20 °C. Lights were on from 7.00 h until 19.00 h. Pigs were euthanized at 19 weeks of age.

2.2. Novelty test

At 11 weeks of age, pigs were individually subjected to a 10-min novelty test. Pigs were placed in a 5 × 5 m test arena with a grey coated concrete floor and hardboard walls, located in a room adjacent to their home pens. The first part of the test - novel environment exposure (NEe) - started when the door to the arena was closed after entrance of the pig. After 5 min, the second part of the test - novel object exposure (NOe) - started by lowering a novel object (a metal bucket) from the ceiling to the floor at the centre of the arena. The bucket touched the floor to create some noise, was then placed 30 cm above the floor (bottom of bucket-floor) and was left in that position for 5 min. Behavioural events were scored live using a Psion Workabout MX that contained the Observer 5.0 software package (Noldus Information Technology B.V., Wageningen, The Netherlands). The behavioural events were summarized into frequencies of ‘vocalizations’ (squeals, grunt squeals, short and long grunts) and ‘excretions’ (urinating and defecating). Other behaviours were scored from video using continuous recording with the Observer 5.0 (Noldus Information Technology B.V., Wageningen, The Netherlands). Two behavioural classes were used at the same time: ‘Postures and locomotion’ and ‘Other behavioural activities’, where the first category consisted of general activity and the second category consisted of the specific behaviours performed while performing the general activity. ‘Postures and locomotion’ contained percentage of time spent on ‘standing’ (all forms of standing), ‘lying and sitting or kneeling’, and ‘locomotion’ (running and walking). ‘Lying and sitting or kneeling’ were hardly seen during the novelty test and this behavioural category was therefore excluded from analysis. Only the behavioural category ‘locomotion’ (and therefore not ‘standing’) is presented in our results. ‘Other behavioural activities’ contained percentage of time spent on ‘exploration’ (sniffing and rooting floor and walls), ‘standing alert’ (standing immobile with raised head and ears pricked), ‘nosing novel object’ (nosing the bucket that was dropped from the ceiling), and ‘other’ (all other behaviours). Furthermore, the latency times to approach and touch the novel object were recorded. Every pig approached and touched the novel object at least one time. Prior to each novelty test faeces and urine were removed from the test arena and the arena was cleaned with water. Tests were carried out on two
consecutive days with half of the pigs tested on day one and the other half of the pigs tested on day two. The order of testing was balanced for housing condition and sex.

2.3. Blood collection

Blood samples of the pigs were taken at 13 weeks of age by venipuncture while pigs were fixed in a nose sling. Samples were collected in tubes containing Ethylenediaminetetraacetic acid (EDTA) and used to determine platelet 5-HT level, whole blood 5-HT, and platelet 5-HT uptake velocity.

2.3.1. Blood platelet serotonin level

Platelet 5-HT (i.e. 5-hydroxytryptamine) level was determined using a fluorimetric assay (Curzon and Green, 1970; Kluge et al., 1999). Briefly, platelet rich plasma (PRP) was extracted by centrifuging 6 ml of blood (160 g × 10 min) at room temperature (RT). The extracted PRP (1 ml) was centrifuged (16100 g × 15 min) at RT to obtain pellets. The washed (0.9% sodium chloride) and centrifuged (16100 g × 5 min) pellets were stored at -70 °C until analysis. After thawing, perchloric acid (HPClO₄) was added (500 µl). Supernatant was retrieved by centrifuging the samples (16100 g × 5 min). The supernatants (100 µl) were added in duplicate to a cysteine (1 ml) and ortho-phthalaldehyde (1 ml) solution and incubated in a water bath (80 °C × 20 min). After cooling down on ice (to 20 °C) fluorescence was measured at 360 nm excitation and 475 nm emission. The 5-HT level of the supernatants was determined by using a linear standard curve with known quantities of 5-HT. Platelet counts in whole blood and in PRP were determined with a Sysmex F-820 Counter. Serotonin in platelets represents > 95 % of 5-HT found in blood (Celada et al., 1994; Da Prada and Picotti, 1979; Pussard et al., 1996). Therefore, we multiplied platelet 5-HT level by the number of platelets counted in whole blood (10⁹ cells/l) to obtain whole blood 5-HT. Platelet 5-HT level was expressed in nmol/10⁹ platelets and whole blood 5-HT in nmol/ml.

2.3.2. Blood platelet serotonin uptake velocity

PRP samples were analysed for platelet 5-HT uptake velocity using an assay adapted from previous studies (Bolhuis et al., 2009; Butler et al., 1992; Franke et al., 2000; Thies-Flechtner et al., 1994). Briefly, triplicates of PRP samples (100 µl) were placed in Krebs-Ringer-Phosphate buffer lacking Calcium Chloride (CaCl₂) (pH = 7.4). They were incubated (37 °C × 1 h) at a concentration of 5 µM 5-HT in
the presence of Radiocarbon-5-hydroxytryptamine (\(^{14}\)C-5-HT). The 5-HT uptake was ended by adding ice cold buffer (2 ml) followed by filtration (Whatman GF-B filter) in a cell harvester (Tomtec). The filters were also flushed by ice cold buffer (2 ml) and dried. Counting for radioactivity was performed in a liquid scintillation counter. Blanks were incubated for 60 min at 0 °C to measure passive diffusion of \(^{14}\)C-5-HT and subtracted from the uptake values (means of the triplicates) gained at 37 °C to determine active uptake. Platelet 5-HT uptake velocity was expressed as pmol/10\(^9\) platelets/min.

2.4. Brain tissue collection and HPLC analysis

In week 19 after birth, the pigs were euthanized for brain tissue collection at the experimental farm on three consecutive days between 8.00 and 14.20 h. Order of euthanizing was balanced for housing (per pen), and within pen for sex with pairs from one pen successively. Pairs of pigs from one pen were taken together to minimize stress responses. Pigs were restrained and anaesthetized with two i.m. injections in the buttocks with in total 2 mg/kg body weight (BW) xylazine hydrochloride (Sedamun, Eurovet Animal Health BV, The Netherlands) combined with 20 mg/kg BW of ketamine 100 INJ (AST Farma, The Netherlands). Within 30 min (14.63 ± 0.94) after sedation they were euthanized by an i.c. injection of 100 mg/kg BW of sodium pentobarbital (Euthasol 40 %, AST Farma BV, The Netherlands). Brains were removed from the skull, weighed and dissected using the stereotaxic atlas of the pig brain for anatomical landmarks (Félix et al., 1999). The frontal cortex samples were collected from the anterior part of the brain. First, a coronal section was made before the central sulcus so that the corpus callosum was not included. Then, the frontal cortex was separated into left and right at the midline. The hypothalamic samples were dissected from the ventral part of the midbrain, from the optic chiasm to the posterior border of the mammillary bodies. From the posterior part of the brain, the left and right hippocampus was dissected in its full length. The brains were stored in plastic containers and, at max 35 min post-mortem (18.45 ± 1.18), placed in liquid nitrogen and thereafter stored at -70 °C until further analyses.

Concentrations of 5-HT and its metabolite 5-hydroxyindole-3-acetic acid (5-HIAA) were measured in all five brain areas. Samples of 50-100 mg (73.82 ± 1.88) from each brain area were taken while keeping the samples frozen in liquid nitrogen. Samples where weighed, stored on dry ice and transported to
a laboratory of Utrecht University, The Netherlands, where 5-HT and 5-HIAA were measured as described previously (Barf et al., 1996). Briefly, tissue samples were homogenized in an ice-cold solution containing 5 µM clorgyline, 5µg/ml glutathione and 0.6 µM N-methylserotonin (NMET, internal standard) using sonication. To 100 µl homogenate, 25 µl 2 M HPClO$_4$ was added and mixed. Then 20 µl 2.5 M potassium acetate was added and again mixed. After 15 min in ice water, the homogenates were centrifuged (15000 $g \times 10$ min at 4 °C). The supernatants were diluted 10 times with water before high performance liquid chromatography (HPLC) analysis. The concentration of 5-HT and 5-HIAA in the tissue extracts were measured by HPLC with electrochemical detection. The mobile phase solution consisted of 50 mM citric acid, 50 mM phosphoric acid, 0.1 mM EDTA, 45 µL/L dibutylamine, 77 mg/L 1-octanesulfonic acid sodium salt, and 10 % methanol (buffer pH was adjusted to 3.4 with natriumhydroxide). Separation was performed at 45 ºC using a flow rate of 1.0 ml/min. The concentration of each compound was calculated by comparison with both the internal and the external standards. The limit of detection (signal/noise ratio 3:1) was 0.9 nM. Concentrations are expressed as nmol/g. The 5-HIAA/5-HT turnover was calculated as an index of activity of the 5-HT system (Korte-Bouws et al., 1996).

2.5. Statistical Analysis

SAS version 9.2 (Statistical Analysis System Institute and Inc., 2002-2008) was used for all statistical calculations. One pig was removed from the experiment and analysis due to a severe tail wound at 15 weeks of age. Number of pigs used in the statistical models ranged from 28 to 31 pigs depending on the number of missing values. A mixed model was used to determine the effect of test phase (NEe and NOe). Besides test phase, housing and the interaction between test phase and housing were added to the model. Random effects of pen (nested within housing), pig (nested within housing and pen) and date were included as well. Housing did not significantly affect serotonergic and behavioural measurements, although some tendencies were present (unpublished results). Therefore, pearson correlations between behavioural responses, blood 5-HT measures and brain 5-HT measures were calculated per test phase (NEe and NOe) by using the residuals of a general linear model (GLM) with housing (B versus E) as a fixed effect. In this way, correlations between variables were appropriately adjusted for the possible effect (tendencies) of housing and housing is not further
discussed in this study. Residuals were tested for normality and variables were, when necessary, (arcsin) square root or logarithmically transformed and the new variables were again subjected to a GLM to subtract normalized residuals.

3. Results

3.1. Correlations between brain and peripheral 5-HT

Mean values and SEM of blood and brain serotonergic measures are presented in Table 1. Whole blood 5-HT and platelet 5-HT levels were positively correlated (\( r = 0.71, P < 0.0001 \)). Platelet 5-HT uptake velocity was positively correlated to both whole blood 5-HT (\( r = 0.69, P < 0.0001 \)) and platelet 5-HT level (\( r = 0.46, P < 0.01 \)). Whole blood 5-HT of 13 week old pigs was not significantly correlated with any of the brain 5-HT measures determined at 19 weeks of age (see Table 2). Platelet 5-HT level correlated positively with 5-HT turnover in left and right hippocampus, and negatively with the 5-HT concentration in these brain areas. Furthermore, platelet 5-HT uptake velocity was negatively correlated with 5-HIAA concentration in the left frontal cortex.

3.2. Novelty test

Mean values and SEM of behaviours during NEe and NOe (i.e. the first and second half of the novelty test, respectively) are presented in Table 3. Behaviours significantly changed after introduction of the novel object (NOe), with number of vocalizations and excretions, and time spent on standing alert performed more, but time spent on locomotion and exploring the test arena performed less during NOe compared to NEe. Blood 5-HT measures were not significantly correlated with behaviour during NEe, except that pigs with a high platelet 5-HT uptake velocity vocalized more frequently (see Table 4). Brain 5-HT turnover levels were not correlated with behaviour during NEe. Levels of the 5-HT metabolite 5-HIAA in the left hippocampus and right frontal cortex were positively correlated with time spent on exploration and frequency of excretion, respectively.

During NOe, both whole blood 5-HT and platelet 5-HT level correlated positively with time spent on exploration (see Table 5). No other correlations between blood 5-HT measures and behaviour during NOe were found. Serotonin
turnovers in left and right hippocampus were negatively correlated with time spent standing alert. Furthermore, 5-HT concentration in the right hippocampus was positively correlated with time spent standing alert. Serotonin turnover in the right hippocampus correlated positively, and the 5-HT concentration in this brain area negatively with time spent exploring the test arena. Serotonin turnover in the right hippocampus and 5-HIAA in the hypothalamus were positively correlated with the time spent on locomotion.
Table 1. Mean values for pig blood and brain 5-HT measures taken at 13 and 19 weeks of age, respectively.

<table>
<thead>
<tr>
<th>Measures</th>
<th>N</th>
<th>Mean</th>
<th>SEM</th>
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</thead>
<tbody>
<tr>
<td><strong>Blood</strong></td>
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<tr>
<td>Whole blood 5-HT</td>
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<td>7.36</td>
<td>0.65</td>
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<tr>
<td>Platelet 5-HT level</td>
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<td>22.74</td>
<td>1.32</td>
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<tr>
<td>Platelet 5-HT uptake</td>
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<td>47.74</td>
<td>3.53</td>
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<tr>
<td><strong>Brain</strong></td>
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<tr>
<td><strong>Hippocampus</strong></td>
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<tr>
<td>Left</td>
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<td></td>
</tr>
<tr>
<td>5-HT turnover</td>
<td>30</td>
<td>0.51</td>
<td>0.02</td>
</tr>
<tr>
<td>5-HT</td>
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<td>1.41</td>
<td>0.11</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>30</td>
<td>0.66</td>
<td>0.04</td>
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<tr>
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<tr>
<td>5-HT</td>
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<td>0.75</td>
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<td>Left</td>
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<td>5-HT turnover</td>
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<td>5-HT</td>
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<td>5-HIAA</td>
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<tr>
<td>5-HT turnover</td>
<td>31</td>
<td>0.47</td>
<td>0.04</td>
</tr>
<tr>
<td>5-HT</td>
<td>31</td>
<td>1.43</td>
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<td>5-HIAA</td>
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<td><strong>Hypothalamus</strong></td>
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<td>3.24</td>
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<tr>
<td>5-HIAA</td>
<td>31</td>
<td>2.12</td>
<td>0.22</td>
</tr>
</tbody>
</table>

*Whole blood 5-HT level in nmol/ml; Platelet 5-HT level in nmol/platelet 10^9; Platelet 5-HT uptake velocity in pmol/platelet 10^9/min.*

*5-HT and 5-HIAA in nmol/g; 5-HT turnover as 5-HIAA/5-HT ratio.*
Table 2. Relations\(^a\) between pig blood and brain 5-HT parameters at 13 and 19 weeks of age respectively.

<table>
<thead>
<tr>
<th>Measures</th>
<th>Blood(^b)</th>
<th>Blood(^c)</th>
<th>Blood(^c)</th>
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<td>Platelet</td>
<td>Platelet</td>
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<tr>
<td></td>
<td>5-HT</td>
<td>5-HT level</td>
<td>5-HT uptake</td>
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<td><strong>Hippocampus</strong></td>
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<tr>
<td><em>Left</em></td>
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<tr>
<td>5-HT turnover</td>
<td>0.15</td>
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<tr>
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<td>-0.49**</td>
<td>-0.09</td>
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<tr>
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<td>-0.24</td>
<td>-0.22</td>
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<td><em>Right</em></td>
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<tr>
<td>5-HT turnover</td>
<td>0.24</td>
<td>0.54**</td>
<td>-0.20</td>
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<tr>
<td>5-HT</td>
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<td>-0.40*</td>
<td>0.01</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>-0.18</td>
<td>-0.02</td>
<td>-0.19</td>
</tr>
<tr>
<td><strong>Frontal cortex</strong></td>
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<td><em>Left</em></td>
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<tr>
<td>5-HT turnover</td>
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<td>-0.08</td>
<td>-0.23</td>
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<tr>
<td>5-HIAA</td>
<td>-0.33+</td>
<td>-0.20</td>
<td>-0.49**</td>
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<tr>
<td><em>Right</em></td>
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</tr>
<tr>
<td>5-HT turnover</td>
<td>0.19</td>
<td>0.02</td>
<td>0.22</td>
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<td>5-HT</td>
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<td>5-HIAA</td>
<td>0.17</td>
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<td><strong>Hypothalamus</strong></td>
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<tr>
<td>5-HT turnover</td>
<td>0.02</td>
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<td>-0.20</td>
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<td>5-HIAA</td>
<td>-0.23</td>
<td>-0.10</td>
<td>-0.34+</td>
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</table>

\(^a\) Pearson correlation coefficients of residuals.
\(^b\) 5-HT in nmol/g; 5-HIAA in nmol/g; 5-HT turnover as 5-HIAA/5-HT ratio.
\(^c\) Whole blood 5-HT level in nmol/ml; Platelet 5-HT level in nmol/platelet 10^9; Platelet 5-HT uptake velocity in pmol/platelet 10^9/min.

+ P < 0.10; * P < 0.05; ** P < 0.01.
Table 3. Mean values and effect of test phase for pig behaviour observed during novel environment exposure (NEe) and novel object exposure (NOe) performed at 11 weeks of age.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>NEe N</th>
<th>Mean</th>
<th>SEM</th>
<th>NOe Mean</th>
<th>SEM</th>
<th>Test phase</th>
</tr>
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<tbody>
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<td>Vocalizations</td>
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<td>30.26</td>
<td>5.68</td>
<td>68.13</td>
<td>8.55</td>
<td>***</td>
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<tr>
<td>Excretions</td>
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*a Vocalizations and excretions in freq; locomotion, exploration, standing alert and nosing NO in % of observations; LAT= Latency time in sec; NO = Novel Object.

* P < 0.01; ** P < 0.001; *** P < 0.0001.
### Table 4. Relations between pig blood-brain 5-HT parameters measured at 13 and 19 weeks of age respectively, and behaviour during exposure to the novel environment (NEe) at 11 weeks of age.

<table>
<thead>
<tr>
<th>Measures</th>
<th>Behavioura</th>
<th>Excretions</th>
<th>Locomotion</th>
<th>Exploration</th>
<th>Standing alert</th>
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</table>

a Pearson correlation coefficients of residuals.

b Whole blood 5-HT level in nmol/ml; Platelet 5-HT level in nmol/platelet $10^9$; Platelet 5-HT uptake velocity in pmol/platelet $10^9$/min.

c 5-HT in nmol/g; 5-HIAA in nmol/g; 5-HT turnover as 5-HIAA/5-HT ratio.

d Vocalizations and excretions in freq; locomotion, exploration, standing alert in % of observations.

+ P<0.10; * P<0.05.
Table 5 Relations between pig blood-brain 5-HT parameters measured at 13 and 19 weeks of age respectively, and behaviour during exposure to a novel object (NOe) at 11 weeks of age.

<table>
<thead>
<tr>
<th>Measures</th>
<th>Behaviour^d</th>
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<th>Locomotion</th>
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^a Pearson correlation coefficients of residuals.
^b Whole blood 5-HT level in nmol/ml; Platelet 5-HT level in nmol/platelet 10^9; Platelet 5-HT uptake velocity in pmol/platelet 10^9/min.
^c 5-HT in nmol/g; 5-HIAA in nmol/g; 5-HT turnover as 5-HIAA/5-HT ratio.
^d Vocalizations and excretions in freq, locomotion, exploration, standing alert and nosing NO in % of observation; LAT= Latency time in sec; NO = Novel Object.
+ P<0.10; * P<0.05; ** P<0.01.
4. Discussion

In this study we assessed the relations between blood and brain serotonin (5-HT) measures, and the correlations between these 5-HT measures and the behavioural responses of pigs in a novelty test. Platelet 5-HT levels, measured at 13 weeks of age were related to hippocampal 5-HT activity at 19 weeks of age. Moreover, both platelet 5-HT level and hippocampal 5-HT activity were related to the time spent on explorative behaviours after introduction of a novel object in a novel environment (NOe) performed at 11 weeks of age. Hippocampal 5-HT activity was also related to the times spent standing alert and locomotion during NOe.

4.1. Relationships between peripheral and brain 5-HT measures

The first aim of this study was to investigate whether blood (platelet) 5-HT measures are correlated with brain 5-HT measures in pigs. Platelet 5-HT level, but not whole blood 5-HT, was related to 5-HT turnover and 5-HT concentration in the left and right hippocampus. Since 5-HT in platelets represents > 95% of blood 5-HT, whole blood 5-HT level is largely based on platelet 5-HT level (Celada et al., 1994; Da Prada and Picotti, 1979; Pussard et al., 1996), and indeed, platelet 5-HT level and whole blood 5-HT of the pigs were correlated, just as in humans (Jernej et al., 2000). The fact that correlations between hippocampal 5-HT measures and whole blood 5-HT level were less strong than those between hippocampal 5-HT measures and platelet 5-HT level, probably relies upon the number of platelets found in blood (Pussard et al., 1996), albeit intra-individual platelet numbers are fairly stable over time (Buckley et al., 2000). Also in other species, relations between diverse peripheral 5-HT measures and 5-HT measures in one or more brain regions have been reported (chickens: Uitdehaag et al., 2011; rats: Celada et al., 1994; Malyszko et al., 1993; Pietraszek et al., 1992b; mice: Pietraszek et al., 1992a; humans: Rausch et al., 2005). One of these studies, however, failed to find a correlation between the 5-HT concentrations in blood and brain, which was likely due to homogenizing the brain as a whole, but they did report correlations between blood and brain 5-HIAA (mice: Pietraszek et al., 1992a). In untreated rats, generally negative correlations were found between blood 5-HT and 5-HT concentrations in different brain areas, including the hippocampus and hypothalamus, with one exception, as the 5-HT concentration in the rat midbrain...
was positively correlated with blood 5-HT concentration (Pietraszek et al., 1992b). The authors suggested that this contrast may stem from the importance of the midbrain with respect to synthesis and release of 5-HT to other brain areas, and similarities between blood and brain daily rhythmicity was solely restricted to the midbrain (Pietraszek et al., 1992b). The positive correlations between blood and brain 5-HT found in other rat studies were likely due to providing drugs that reduce the activity of monoamine oxidase and thereby decrease 5-HT degradation into 5-HIAA (Malyszko et al., 1993) or drugs that increase the release of 5-HT and inhibits the 5-HT uptake (Celada et al., 1994). Others also report different drug effects (Chou et al., 1983) and 5-HT binding potentials (Yatham et al., 2000) in blood and brain, thus indicating that peripheral 5-HT measures are not the same as brain 5-HT measures. Nonetheless, peripheral 5-HT measures may partially reflect brain 5-HT measures since some blood and brain correlations were found in several species (Celada et al., 1994; Malyszko et al., 1993; Pietraszek et al., 1992a, b; Rausch et al., 2005; Uitdehaag et al., 2011), and both genetic (Lesch et al., 1993) and kinetic properties (Rausch et al., 2005) of blood and brain 5-HT mechanisms are related. Moreover, in long-term studies in humans (ranging in duration from weeks to years), intra-individual whole blood (Badcock et al., 1987; Ritvo et al., 1971; Yuwiler et al., 1981) and platelet (Jernej et al., 2000; Jernej and Cicin-Šain, 1990) serotonin levels were reported to be rather stable over time. Consequently, blood (platelet) 5-HT concentration has been identified as a stable individual trait in humans and rodents (Jernej and Cicin-Šain, 1990; Yuwiler et al., 1981). Similarly, the significant relationships of platelet 5-HT with both hippocampal 5-HT activity and behaviour during NOe supports the idea that blood 5-HT might reflect part of an individual pig’s consistent response pattern during challenging events. It should be noted, however, that only 5-HT measures in the hippocampus, and not those in the hypothalamus or frontal cortex were significantly correlated with platelet 5-HT level. This is in contrast with rat studies in which peripheral 5-HT levels correlated with not only hippocampal 5-HT measures (Pietraszek et al., 1992a, b), but also with 5-HT measures in the (frontal) cortex and hypothalamus (Celada et al., 1994; Malyszko et al., 1993; Pietraszek et al., 1992b). Several explanations for this discrepancy may be considered: species specificity (Pazos et al., 1985), a difference in monoamine oxidase activity in the different brain areas (see for a review Wang et al., 2013), a difference in methodology, where we took blood and brain samples six weeks apart and in other studies samples were obtained simultaneously (Celada et al., 1994; Malyszko et al., 1993; Pietraszek et al.,
Blood and brain serotonin, and behaviour in a novelty test

1992b), and the relative importance of the hippocampus in 5-HT functioning with regard to 5-HT receptor subtypes expression (Berumen et al., 2012). Platelet 5-HT uptake velocity was correlated with both whole blood and platelet 5-HT level, but it was not related to brain 5-HT measures, except for a correlation between 5-HIAA in the left frontal cortex. Most studies focused on the relation between uptake in platelets and synaptosomes (e.g. Chou et al., 1983; Smith et al., 1978, 2001), but in laying hens, the relation between platelet 5-HT uptake velocity and blood and brain 5-HT activity was studied and found to be present in one of two genetic chicken lines, suggesting that these relationships may be breed-specific (Uitdehaag et al., 2011).

4.2. Novelty test versus 5-HT measures

The second aim of this study was to assess the relationship between measures of brain and blood 5-HT activity and the behavioural responses of pigs in a challenging situation, the novelty test. Serotonin measures of our pigs were related to behaviours during the novelty test, particularly after the introduction of the novel object (NOe). Before the novel object was introduced - during novel environment exposure (NEe) - only platelet 5-HT uptake velocity and 5-HIAA concentrations in the right frontal cortex and left hippocampus were correlated to the frequency of vocalizations, frequency of excretions and the time spent on explorative behaviours, respectively. Although the relations between blood and brain 5-HT measures and vocalizations (Sánchez, 2003), excretions (Song et al., 2005; Thor, 2004) and explorative behaviours (Andersen et al., 2000a; Varty et al., 2002) might be related to (a lack of) fear or anxiety, the weak relations between blood or brain 5-HT measures and behavioural responses of pigs to NEe suggest that NEe was experienced differently by the pigs than NOe. One explanation may be that the pigs were more fearful during NOe compared to the NEe, which is supported by the changes in behaviour. During NOe, pigs vocalized and excreted more frequently, spent more time on standing alert and less time on locomotion and exploring the test arena. This suggests that NEe alone was not particularly fear-provoking for the pigs, which is in line with a previous study where an anxiolytic drug affected pig behaviour only in an elevated plus maze but not in a novel environment test (Andersen et al., 2000b). We, therefore, hypothesize that pigs demonstrate their level of fearfulness more evident in a more challenging situation such as NOe.
During NOe, whole blood and platelet 5-HT level, as well as hippocampal 5-HT measures were all related to time spent on exploration, and hippocampal 5-HT measures were also related to time spent on standing alert and locomotion. Introducing a novel object may induce curiosity (Cavigelli et al., 2011; Lauber et al., 2009) but is also likely to startle animals (Désiré et al., 2004; Lucki, 1998) and induce fear or anxiety (Forkman et al., 2007). Both whole blood and platelet 5-HT level of our pigs, and also right hippocampal 5-HT concentration and turnover, were related to time spent on exploring the test arena after introduction of the novel object. Generally, explorative behaviours are thought to reflect a low level of fear or anxiety (Korte and De Boer, 2003). Furthermore, in mice, explorative behaviours were related to 5-HT activity in the hippocampus (Brennan et al., 1981), and in rats exploration was affected by providing a 5-HT2 receptor agonist (Campbell and Merchant, 2003). Left and right hippocampal 5-HT turnover and right hippocampal 5-HT concentrations of our pigs were also related to time spent on standing alert (or ‘freezing’). Standing alert occurs most often during aversive conditions (Reimert et al., 2013), it has been associated to fear or anxiety (Forkman et al., 2007; Hashimoto et al., 1999; Vicens-Costa et al., 2011) and, in rats, to 5-HT concentrations in the prefrontal cortex (Hashimoto et al., 1999). Right hippocampal 5-HT turnover and 5-HIAA concentrations in the hypothalamus of our pigs were, furthermore, related to the level of locomotion the pigs expressed. Collectively, these results suggest that a higher level of locomotion is associated with a lower level of fear. In other animal species, locomotion was also suggested to be an indicator of the level of fear or anxiety an animal experiences ( Antoniadis and McDonald, 2000; Benetti et al., 2007; Campler et al., 2009), and locomotion has also been associated with the 5-HT system functioning (Lucki, 1998; Vanderwolf, 1989). However, some authors suggest that both standing alert (Vicens-Costa et al., 2011) and locomotion (Hessing et al., 1993; Van Reenen et al., 2004) might be measures of coping strategy rather than level of fear. Serotonin has been implicated in mechanisms involved in personality in humans (Hansenne et al., 2002) and from our results this might be true for pigs as well: here we hypothesize that serotonin could be related to either coping strategy or fearfulness, i.e. the “propensity to experience fear or anxiety” (Forkman et al., 2007). The coherent relation between 5-HT measures in brain and blood, and the behaviours exploration, standing alert and locomotion, does support a possible role for serotonin particularly in the pig’s fearfulness. Fearfulness is suggested to be a heritable trait that partially explains the individual behavioural differences.
observed in animals (Boissy, 1995). We observed behavioural responses during NOe at 11 weeks of age, determined blood 5-HT measures at 13 weeks of age, and brain 5-HT measures at 19 weeks of age. To obtain basal 5-HT values, blood and brain samples were collected apart from the novelty test. Sampling intervals of 5-HT measures were chosen to prevent stressful effects of blood collection on brain 5-HT measures. Therefore, we suggest that the relations we found between behaviour and measures of 5-HT in blood and brain indicate an underlying (personality) trait rather than states that vary in time. The existence of one or more stable underlying trait(s) was also proposed in laying hens where consistent line differences in fear behaviour and 5-HT measures due to selection for either high or low mortality were found (Bolhuis et al., 2009). Most animal studies focusing on behavioural responses in an experimental test, measure 5-HT during or directly after the behavioural test, implying that they measure a state the animal was in at that particular moment. This might explain the lack of relations we found between 5-HT measures in both the frontal cortex and hypothalamus, and behaviours observed during NOe - except for 5-HIAA in the hypothalamus and locomotion - while others did find relations between behaviour and 5-HT activity in (parts of) the cortex (Hashimoto et al., 1999; Vanderwolf, 1989). Both the frontal cortex and hypothalamus in humans and other animals serve many biological functions and are involved in fear behaviours (e.g. Colpaert, 1975; Hashimoto et al., 1999; Stanley and Jaynes, 1949). One of the roles of the frontal cortex is regulating attention (Olton et al., 1988), and the hypothalamus is involved in regulating the release of stress hormones (Kruk et al., 1998). Both brain structures may thus be of major importance with respect to 5-HT and behaviour at the time of a challenging event - a state of excitement or fear - and we cannot exclude the possibility that frontal cortex and hypothalamus 5-HT activity may have been related to the behaviours observed during the novelty test if the pigs would have been euthanized directly after ending the test. In sea snails, for example, serotonin is suddenly released after a startle, and is involved in the ‘Aplysia gill and siphon withdrawal reflex’ and sensitization of the reflex (see for a review Dayan and Huys, 2009). However, hippocampal 5-HT activity measured at 19 weeks of age in euthanized animals - a situation which probably reflected a non-fearful state - was related to behaviours observed during the novelty test at 11 weeks of age. This suggests that the hippocampus, and not the frontal cortex and hypothalamus, might be involved in a putative personality measure in pigs related to the trait fearfulness. The hippocampus has previously been associated
with fear or anxiety (Andrade and Beck, 2010; Antoniadis and McDonald, 2000, 2001; Dos Santos et al., 2010; Van der Staay et al., 2009) and emotional learning (Antoniadis and McDonald, 2000, 2001), and 5-HT has been associated with reflexes executed through learning (Dayan and Huys, 2009). Since, the right hippocampus of our pigs was the predominant brain structure involved in the behavioural responses of our pigs, hemisphere lateralization may play a role as well (Carlson and Glick, 1989; Delrue et al., 1994; Fitzgerald, 2012; Hecht, 2010; Rogers, 2010).

Our results may implicate that if tail biting pigs differ indeed in fearfulness from victims of being tail bitten or neutral pigs (i.e. not performing and receiving tail biting) as suggested by other authors (Zupan et al., 2012), they might also differ in 5-HT blood platelet storage and 5-HT system activity. If so, it should then be possible, by using behaviour during challenging events or by taking blood samples, to distinguish pigs on farm that may be predisposed to develop tail biting. However, first a clear relationship between 5-HT and tail biting, as well as other types of maladaptive responses, has to be determined before implementation in practice may be possible.

In conclusion, several behavioural responses observed during a novelty test - especially after exposure to a novel object - and blood and brain serotonin measures in pigs were correlated. Notably, blood platelet serotonin and serotonin activity in the right hippocampus both correlated significantly with time spent on exploring the test arena after the novel object was dropped. Our results suggest a role of serotonin in biological traits - such as fearfulness - underlying the behavioural responses of pigs during a challenging situation. These findings may help future research in understanding the development of maladaptive behavioural or physiological responses in pigs.

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References


Blood and brain serotonin, and behaviour in a novelty test


Blood and brain serotonin, and behaviour in a novelty test


Tail biting in pigs: blood serotonin and fearfulness as pieces of the puzzle?

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Abstract

Tail biting in pigs is a widespread problem in intensive pig farming. The tendency to develop this damaging behaviour has been suggested to relate to serotonergic functioning and personality characteristics of pigs. We investigated whether tail biting in pigs can be associated with blood serotonin and with their behavioural and physiological responses to novelty.

Pigs (n=480) were born in conventional farrowing pens and after weaning at four weeks of age they were either housed barren (B) or in straw-enriched (E) pens. Individual pigs were exposed to a back test and novel environment test before weaning, and after weaning to a novel object (i.e. bucket) test in an unfamiliar arena. A Principal Component Analysis on behaviours during the tests and salivary cortisol (novel object test only) revealed five factors for both housing systems, labeled ‘Early life exploration’, ‘Near bucket’, ‘Cortisol’, ‘Vocalizations & standing alert’, and ‘Back test activity’. Blood samples were taken at 8, 9 and 22 weeks of age to determine blood platelet serotonin. In different phases of life, pigs were classified as tail biter/non-tail biter based on tail biting behaviour, and as victim/non-victim based on tail wounds. A combination of both classifications resulted in four pig types: biters, victims, biter/victims, and neutrals.

Generally, only in phases of life during which pigs were classified as tail biters, they seemed to have lower blood platelet serotonin storage and higher blood platelet uptake velocities. Victims also seemed to have lower blood serotonin storage. Additionally, in B housing, tail biters seemed to consistently have lower scores of the factor ‘Near bucket’, possibly indicating a higher fearfulness in tail biters. Further research is needed to elucidate the nature of the relationship between peripheral 5-HT, fearfulness and tail biting, and to develop successful strategies and interventions to prevent and reduce tail biting.

Keywords: pigs, tail biting, blood serotonin, back test, novelty tests, enrichment
1. Introduction

Aberrant behaviours such as tail biting, i.e. the harmful or oral manipulations of the group mates’ tails, can both reflect and contribute to health and welfare problems in pigs (EFSA, 2007b). Tail biting is caused by many factors (Taylor et al., 2010), but the lack of exploration possibilities in the home pen is likely the main risk factor for its development (Zonderland et al., 2008). Accordingly, tail biting is generally seen as redirected explorative behaviour (Studnitz et al., 2007). Indeed, pigs kept in pens enriched with materials suitable for chewing and rooting, perform much less tail biting compared to pigs kept in rather barren pens (Van de Weerd et al., 2005; Zonderland et al., 2008). However, in barren housing systems not all pigs develop tail biting behaviour, and, conversely, in enriched housing systems still some pigs perform the behaviour (Ursinus et al., 2014). Previously, the existence of different types of tail biters was suggested (Taylor et al., 2010) and there may be different underlying motivations to display the damaging behaviour by pigs kept in diverging housing systems. Individual pigs may therefore be predisposed to develop tail biting behaviour. Tail biting behaviour has comparable characteristics with for instance feather pecking in laying hens (Brunberg et al., 2013b) and feather picking in parrots (Van Zeeland et al., 2009). The behaviours involved may be perceived as maladaptive as they seem an inadequate response given the living circumstances (Crespi, 2000; Mills, 2003). However, an important commonality of the problematic behaviours may be a malfunctional neurotransmitter system (Kops et al., 2013; Korte et al., 2009) which would imply that in some individuals the behaviours may be malfunctional rather than maladaptive only (Mills, 2003). Furthermore, it has been suggested that the propensity to develop aberrant behaviour is related to personality traits, such as the strategy (or ‘coping style’; Koolhaas et al., 1999) an animal adopts in challenging situations (Korte et al., 1998, 2009). Animals may be classified in either proactive or reactive individuals (Koolhaas et al., 1999). Briefly, proactive animals cope more actively with acute mild stress, develop more easily routines and seem more rigid in their responses to changes in their environment, whereas reactive animals respond more passively to acute stressors and seem more flexible in their behavioural responses (Bolhuis et al., 2004; Koolhaas et al., 1997). These coping styles may also relate to the functioning of the brain neurotransmitter systems (Bolhuis et al., 2000; Koolhaas et al., 2010) and it was previously suggested that these proactive animals may be more vulnerable to
develop compulsive disorders, which show similarities with feather pecking in laying hens and tail biting in pigs (Korte et al., 2009). Another personality trait that may contribute to the tendency of animals to develop aberrant behaviours is fearfulness or anxiety (Rodenburg et al., 2010; Zupan et al., 2012) which may be reflected in the response to novel situations (Forkman et al., 2007; Ursinus et al., 2013). Additionally, in pigs the behaviour in novelty tests has been associated with coping styles (Jansen et al., 2009), tail biting (Zupan et al., 2012), and serotonergic blood and brain parameters (Ursinus et al., 2013). Therefore, the main aim of our study was to explore whether tail biting in pigs, in a longitudinal study, is associated with behavioural and physiological responses to challenges, and also with blood serotonin. Blood serotonin is relatively easy to measure compared to brain serotonin and behaviours and may, thus, be valuable in understanding the mechanisms of tail biting. Pigs were subjected to a back test as the behaviour performed during this test may reflect a pig's coping style (Bolhuis et al., 2004; Reimert et al., 2013b), and they were exposed to two novelty tests, one before and one after weaning. After weaning, salivary cortisol measured around the novelty test was used to assess the pig's adrenal response to a challenging situation (Dickerson and Kemeny, 2004). Tail biting behaviour, tail damage, and blood serotonin were measured at different time points in life as we recently found that tail biting in pigs is not always consistent over different life stages (Ursinus et al., 2014). Previously, in laying hens (Brunberg et al., 2011) and pigs (Brunberg et al., 2013a) differences were found in gene expression profiles of the animals that performed damaging behaviours (feather peckers/tail biters), animals that received the damaging behaviours (victims of feather pecking/tail biting), and animals that were not involved in performing or receiving the damaging behaviours (neutral animals). Apart from focussing on pigs displaying tail biting behaviour, it seems, therefore, highly relevant to explore other types of pigs as well. Accordingly, our pigs were classified in tail biters/non tail biters and victims/non-victims of tail biting. Combining both classifications resulted in biters, victims, a combination of both (biter/victims), and pigs that never engaged in tail biting or receiving the behaviour (neutrals). We chose to use both barren and enriched pens as environmental enrichment strongly affects the prevalence of tail biting behaviour (e.g. Beattie et al., 1996; Ursinus et al., 2014), and may reveal different types of tail biters (Taylor et al., 2010).
2. Materials and methods

2.1. Ethics Statement

The experimental protocol followed during this study was approved by the Animal Care and Use Committee of Wageningen University (no. 2010055f) and then also adopted by the Animal Care and Use Committee of the University of Groningen, The Netherlands. Blood samples were taken near the home pens of the pigs and as quickly as possible to minimize stress. Pigs with severe tail wounds (i.e. tip of tail missing) were removed from the experiment and all pens (barren and enriched) received a jute sack from 8 weeks of age onward to keep tail biting in barren housing at an acceptable level.

2.2. Animals and housing

Pigs (n = 480) were born in 5 rounds at the experimental farm of TOPIGS Research Center IPG in Beilen (The Netherlands). Briefly, piglets were housed in a conventional (barren) farrowing pen with a sow crate. Tails and teeth were kept intact, but males were castrated. At four weeks of age piglets were transported to the experimental farm “De Haar” in Wageningen (The Netherlands). After weaning pigs were kept either barren (B) or enriched (E). The difference between E and B housing was the provision of wood shavings (12 kg at start, 3 kg added daily) and straw (1.5 kg daily) in E housing. B housed pigs received two handfuls of wood shavings daily, from six weeks onward. Additionally, from week 8 onward both B and E pens received a jute sack, to keep tail biting at an acceptable level in B pens. More details on housing of the pigs both pre- and post-weaning have previously been described (see Camerlink et al., 2013; Reimert et al., 2013b; Ursinus et al., 2014). Groups of pigs in a pen also differed in Indirect Genetic Effects (IGE) for growth; IGE results are presented elsewhere (see Camerlink et al., accepted; Reimert et al., 2014). Each pen consisted of six unrelated pigs with a 1:1 sex ratio, and at least two pigs of each back test (see below) classification (LR:HR ratio; Reimert et al., 2013b).
Chapter 4

2.3. Tail biting behaviour and tail damage

Four life phases were distinguished: one pre-weaning, and three post-weaning (1-3). These phases were, roughly, according to general production stages: piglet (0-4 weeks), weaner (4-8 weeks), grower (8-16 weeks), finisher (16-23 weeks). Pigs were, per phase, identified as tail biters and victims of being bitten based on home pen observations and tail damage scores, respectively (Ursinus et al., 2014). Tail biters were pigs involved in more than one tail biting incidence (i.e. nibbling, sucking or chewing at the tail of a pen mate) during a phase post-weaning. Pre-weaning, tail biters could not be identified properly and were, therefore, not considered in this study. Tail biting behaviour was observed during instantaneous scan samples with an interval of two min (30 samples/h) while using a Psion Workabout with Observer software (Noldus Information Technology, Wageningen, The Netherlands). In total six observation days (at 4, 5, 8, 11, 16, and 21 weeks of age) of six hours each (in total 1080 samples per pig) were considered and per phase two observation days (i.e. 360 samples per pig) were used. Victims of tail biting were pigs with a tail wound at time of weaning, or at least one time during a phase post-weaning (weeks 5-7, 8-15, and 16-23 for phase 1, 2 and 3, respectively) (Ursinus et al., 2014).

2.4. Behavioural tests

2.4.1. Pre-weaning back test

Piglets (n = 480) were subjected to a back test at approximately 14 days of age (Figure 1A) (see Reimert et al., 2013b). The test was carried out on two consecutive days, except for the final round where all 96 piglets were tested in one day. From each litter, individual piglets were placed in supine position for one min to observe their behavioural response which may range from vigorous struggling and screaming to immobility (see for more details Bolhuis et al., 2003; Melotti et al., 2011). Two observers conducted the test, one observer held the piglet and counted the number of struggles, and the other observer counted the number of vocalizations and registered latency times to first struggle and vocalization. Latency to first struggle was strongly correlated with number of struggles ($r = -0.84$, $P < 0.001$) and latency to first vocalization was strongly correlated with number of vocalizations ($r = -0.76$, $P < 0.001$) (analysed with Spearman’s rank correlation on residuals from a GLM with round as fixed effect, $n = 480$). Therefore, further analysis was restricted to numbers of struggles and vocalizations.
2.4.2. Pre-weaning novel environment exposure

Pre-weaning, individual piglets (n = 470) were, by litter, exposed to a novel environment (pNEe) at the age of 3.5 weeks (see also Reimert et al., 2013b). Testing was, per round, carried out on two consecutive days. The novel environment consisted of a 1.25 m × 1.25 m arena with dark wooden walls of 62.5 cm height, and concrete flooring (Figure 1B). A heating lamp with yellow lighting was placed above the arena. The test lasted for 2.5 min. Behaviours included in our study were percentage of time spent on walking (all forms of moving), standing alert (standing with head upward and ears pricked), exploring (walking or standing while nosing, licking or rooting floor or walls), and total number of vocalizations (grunts, grunt-squeals, squeals, and screams). Behaviours were recorded by two observers (one for continuous behaviours and one for behavioural events) who had each a Psion Workabout with Observer software (Noldus Information Technology, Wageningen, The Netherlands).

2.4.3. Post-weaning novel object exposure

Post-weaning, individual pigs (n = B: 224, E: 227) were subjected to a novelty test at 13 weeks of age (see also Reimert et al., 2014). The order of testing was
balanced for housing condition and sex. Testing was, per round, carried out on five consecutive days. The size of the test arena was 5 × 5 m, made of wooden walls (approximately 1.5 m high) and concrete flooring (Figure 1C). The arena (walls + floor) was darkened with grey painting. The first 5 min, pigs were exposed to the test arena (i.e. novel environment exposure or NEe), then a metal bucket was dropped from the ceiling (i.e. novel object exposure or NOe) and pigs were left in the arena for another 5 min. Total duration of the test was 10 min. In a previous study large behavioural differences were found between NEe and NOe when pigs were 11 weeks of age, pointing in the direction of NOe being more fearful for pigs post-weaning compared to NEe (Ursinus et al., 2013). Since we were especially interested in behavioural responses to potentially stressful situations, only the behaviours recorded during the NOe were used for further analysis. Behaviours considered were percentage of time spent on standing alert (standing with head upward and ears pricked), exploring the arena (nosing and rooting floor, nosing and rooting walls, and chewing), exploring the novel object (nosing, sniffing, rooting, licking or chewing the metal bucket), and total number of vocalizations (barks, grunts, grunt-squeals, squeals, and screams). Two observers with a Psion hand-held computer recorded continuous behaviours or behavioural events. Additionally, total distance covered (i.e. locomotion), and percentage of time spent near the door (~1.25 × 2.25 m in front of the door, with the door centred in the ~2.25 m, see also Reimert et al., 2014) and directly adjacent to the walls (excluding the door zone) were recorded by video tracking using EthoVision XT 8.5 (Noldus Information Technology, Wageningen, The Netherlands). Housing (B/E) effects on behavioural parameters of the NOe were not present which was previously described elsewhere (Reimert et al., 2014), and are therefore not further considered in our study.

2.5. Physiological measures

2.5.1. Serotonin measures

Blood samples were taken when the pigs were approximately 8 (T1), 9 (T2), and 22 (T3) weeks of age. The first (n = B: 237, E: 235 pigs) and last blood samples (n = B: 213, E: 221 pigs) were considered as basal samples. The second sample (n = B: 233, E: 230 pigs) was taken three days after a regrouping test, where pairs of pigs were, for another study, mixed with unfamiliar pigs during 24 h and then returned to their original home pen (see for more details Camerlink et al.,
2013), but no short-term effects on blood serotonergic measures were expected as they are thought to be rather stable over time (Jernej and Cicin-Šain, 1990; Yuwiler, et al., 1981). Samples were taken by venipuncture while the pigs were either placed on their backs (T1 and T2) or when held in a nose sling (T3). Order of blood collection was always balanced for housing. Blood was collected in EDTA containing tubes (9 ml) and stored on ice until transfer to the lab for further processing. Platelets (expressed in $10^9$ cells/l) were counted using a Sysmex F-820 Counter (Sysmex Corporation, Kobe, Japan).

2.5.1.1. Blood platelet serotonin level

Platelet serotonin (5-hydroxytryptamine or 5-HT) level was determined using a fluorimetric assay (Curzon and Green, 1970; Kluge et al., 1999) as previously described in Ursinus et al. (2013). Platelet 5-HT level was expressed in nmol/$10^9$ platelets.

2.5.1.2. Whole blood serotonin level

Serotonin in platelets represents > 95 % of 5-HT found in blood (Celada et al., 1994; Da Prada and Picotti, 1979). Therefore, we multiplied platelet 5-HT level by the number of platelets counted in whole blood ($10^9$ cells/l) to obtain whole blood 5-HT. Whole blood 5-HT was expressed in nmol/ml blood.

2.5.1.3. Blood platelet serotonin uptake velocity

Serotonin uptake velocity in blood platelets was measured by a radioactive uptake assay (Butler et al., 1992; Thies-Flechtner et al., 1994) as previously described in Ursinus et al. (2013). Platelet 5-HT uptake velocity was expressed as pmol/$10^9$ platelets/min.

2.5.2. Salivary cortisol measures

Before ($t = 0$ min) and after ($t = 15, 30,$ and $60$ min) the start of the novelty test at 13 weeks of age, pigs (n = B: 218, E: 218) were allowed to chew on two cotton buds to obtain saliva samples to measure cortisol concentrations by a radioimmunoassay kit (Reimert et al., 2014; Ruis et al., 1997). Pigs were previously habituated to chewing on the cotton buds. For each pig the deltas between the first and second sample ($\Delta t = 15-0$) (i.e. cortisol rise from the first sample to the highest observed peak), and between the second and final sample ($\Delta t = 15-60$) (i.e. cortisol recovery from the highest observed peak to the final sample) were...
calculated. The area under the curve (AUC) from time 0 to 60 was determined per pig using the linear trapezoidal rule. Housing (B/E) effects on salivary cortisol parameters were previously described elsewhere (Reimert et al., 2014) and therefore not further considered in our study. Shortly, B housed pigs had consistently higher values compared to E housed pigs, but cortisol responses to novelty did not differ between the two housing types.

2.6. Statistical Analysis

SAS version 9.2 (Statistical Analysis System Institute and Inc., 2002-2008) was used for all statistical analyses. The effect of type of pig (see below) was considered per phase as tail biting behaviour observed in individual pigs was inconsistent throughout life resulting in different classifications per pig per phase (Ursinus et al., 2014). Additionally, an ‘overall’ score per pig (ever i.e. in one of the three phases) a tail biter, and ever a victim or not) was used to assess the overall effect of type of pig on variables considered per phase.

2.6.1. The effect of housing and of type of pig on serotonin measures

The effect of housing (B/E) on serotonin measures was tested in a mixed model with a fixed effect of round (1-5) and random effect of pen (nested within round). Subsequently, the effect of type of pig with respect to tail biting (per phase and over all phases) on serotonin measures was tested separately for both types of housing (B/E). At time of weaning piglets were classified as either a victim (tail damage score = 4, tail wound; n = B: 14, E: 30) or non-victim of tail biting (tail damage score < 4, no tail wound; n = B: 226, E: 210). This classification in non-victim/victim (0/1) was tested as fixed effect in a mixed model, together with round (1-5) and the random effect of pen (nested within round). In each phase (1-3) post-weaning, pigs were classified as either a tail biter (at least twice involved in biting incident) (n = B: 29, 40, and 38, E: 0, 6, and 8 in phase 1-3, respectively) or a non-tail biter (n = B: 211, 199, and 183, E: 240, 232, and 223 in phase 1-3, respectively). In addition, all pigs were classified in each phase as either a victim (at least once a tail wound) (n = B: 76, 132, and 154, E: 26, 29, and 58 in phase 1-3, respectively) or a non-victim (no tail wounds) (n = B: 164, 107 and 68, E: 210, 214, 209 and 173 in phase 1-3, respectively). Both 0/1 classifications were treated as fixed effects with two levels, i.e. the effects of ‘biter’ (non-tail biter = 0 versus tail biter = 1) and of ‘victim’ (non-victim = 0 versus victim = 1), respectively. These
two fixed effects and their interaction were included in a mixed model, together with the fixed effect of round (1-5) and the random effect of pen (nested within round). Including the interaction between the effects of ‘biter’ and of ‘victim’ in the model made it possible to distinguish between the following four types of pigs: biter (‘biter’ = 1, ‘victim’ = 0) (n = B: 23, 22, and 11, E: 0, 5, and 2 for phase 1-3, respectively), victim (‘biter’ = 0 and ‘victim’ = 1) (n = B: 70, 114, and 126, E: 26, 28, and 52 for phase 1-3, respectively), a combination of both, here referred to as biter/victim (‘biter’ = 1 and ‘victim’ = 1) (n = B: 6, 18, and 27, E: 0, 1, and 6 for phase 1-3, respectively) and pigs not involved in tail biting or being tail bitten, here referred to as neutral pigs (‘biter’ = 0 and ‘victim’ = 0) (n = B: 141, 85, and 57, E: 214, 204, and 171 for phase 1-3, respectively). A similar approach was used to test the effect of an overall classification of each pig. Pigs were classified as tail biter if they were at least once a tail biter in any of the phases (n = B: 88, E: 14). Similarly, pigs were classified in victims if they were at least once a victim in any of the phase (n = B: 200, E: 109). Including the interaction of the effects of ‘biter’ and ‘victim’ resulted in biters (n = B: 15, E: 4), victims (n = B: 127, E: 99), biter/victims (n = B: 73, E: 10) and neutrals (n = B: 25, E: 127). Serotonin measures were logarithmically transformed if necessary to approach normal distribution of residuals.

2.6.2. Principal Component Analysis

A Principal Component Analysis (PCA) (Jolliffe, 1986) was conducted by type of housing (B/E) on variables from the back test, pre-weaning novel environment test (pNEe), and novel object test (NOe) including salivary cortisol measures, to examine whether variation in behavioural and physiological responses of the pigs (17 in total) could be summarized in a limited number of different factors (Van Reenen et al., 2004). Prior to PCA, variables were, if necessary, square root (number of vocalizations), arcsine square root (proportion of time spent exploring the bucket, and proportion of time spent near the door zone during NOe) or logarithmically (cortisol measures) transformed, and all variables were subjected to a general linear model with round (1-5) as fixed effect to obtain residuals used for the PCA. After extraction, principal components were scaled by their standard deviations (square roots of associated Eigenvalues or EV) and subjected to orthogonal rotation (varimax) to obtain independent factors.
2.6.3. The effect of type of pig on principal component factors

A mixed model analysis was performed to test whether type of pig (over all phases and per phase) with respect to tail biting, had an effect on the factors that were retained from the PCA. Similar to the analysis of serotonin measures, 0/1 classifications of pigs as ‘biter’ (non-tail biter = 0 versus tail biter = 1) and ‘victim’ (non-victim = 0 versus victim = 1) were included in the model as fixed effects, and their interaction was considered as well (resulting in the four pig types: biter, victim, biter/victim, neutral). Furthermore, the random effect of pen (nested within round) was included in the model. Round (1-5) was not included in the model, as variables were already corrected for the effect of round prior to PCA.

2.6.4. Correlations between serotonin measures and principal component factors

To assess consistency over time (T1, T2, and T3) with respect to blood serotonin storage (both expressed in whole blood and in blood platelets), a general linear model per type of housing (B/E) with round (1-5) as fixed effect was run on raw data of serotonin variables to obtain residuals. Thereafter, Spearman’s rank correlation coefficients (as not all variables approached normality) were calculated between residuals of serotonin measures. A similar procedure was used to determine possible relationships between serotonin measures and factors obtained by the PCA. Spearman’s rank correlation coefficients were calculated between residuals of serotonin measures and factors.

3. Results

3.1. The effect of housing and of type of pig on serotonin measures

Housing significantly affected platelet 5-HT uptake velocity (determined at T3 only), where B housed pigs had higher velocities compared to E housed pigs (P < 0.05) (Table 1). No other significant effects of housing were found with respect to 5-HT measures, except that B housed pigs tended to have higher platelet 5-HT levels at T1 (P < 0.10).

Whole blood 5-HT measured at T1 was significantly (P < 0.001) positively correlated with whole blood 5-HT measured at T2 (B: r = 0.42, E: r = 0.44) and T3 (B: r = 0.40, E: r = 0.36), and T2 and T3 were also significantly (P < 0.001) correlated (B: r = 0.43, E: r = 0.38). Corresponding results were found for blood platelet 5-HT measures (all P < 0.001), where T1 was correlated with T2 (B: r = 0.43, E: r = 0.41) and T3 (B: r = 0.37, E: r = 0.38), and T2 was also correlated with T3 (B: r = 0.37, E: r = 0.29).
3.1.1. Barren housing

Considering a pig’s life as a whole, no significant main effects of ‘biter’ (i.e. non-tail biters versus tail biters) or ‘victim’ (i.e. non-victims versus victims), and no significant interaction between these effects (i.e. allowing for the distinction between tail biters, victims, biter/victims, and neutral pigs) were found with respect to the blood 5-HT measures (data not shown). However, considering each phase of life separately did reveal relationships between type of pigs and serotonin measures. Victims at time of weaning had, when B housed post-weaning, lower whole blood and platelet 5-HT levels at T1 and T3 compared to non-victims (Table 2). Tail biters classified during phase 1 post-weaning tended to have a lower platelet 5-HT level at T1 compared to non-tail biters (Table 3). Tail biters of phase 2 had a significantly lower platelet 5-HT level at T2 and they tended to have a lower whole blood 5-HT level at T2 compared to non-tail biters. Furthermore, phase 3 tail biters had significantly lower platelet 5-HT levels at T2 compared to non-tail biters. Victims of phase 3 had significantly lower platelet 5-HT uptake velocities, and tended to have higher platelet 5-HT levels at T2 compared to non-victims. Finally, a significant interaction between ‘biter’ and ‘victim’ revealed that tail biters of phase 3 had lower whole blood 5-HT at T2, and tended to have higher platelet 5-HT uptake velocities at T3 compared to victims, biter/victims, and neutral pigs.

Table 1. Blood 5-HT measures at 8 (T1), 9 (T2), and 22 (T3) weeks of age in barren or enriched housed pigs.

<table>
<thead>
<tr>
<th>Blood measures</th>
<th>Barren</th>
<th>Enriched</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood 5-HT T1</td>
<td>12.6 ± 0.52</td>
<td>11.4 ± 0.52</td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T2</td>
<td>12.0 ± 0.60</td>
<td>11.9 ± 0.60</td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T3</td>
<td>7.3 ± 0.48</td>
<td>7.6 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT uptake T3</td>
<td>45.4 ± 1.43</td>
<td>40.1 ± 1.41</td>
<td>*</td>
</tr>
<tr>
<td>Platelet 5-HT level T1</td>
<td>22.9 ± 0.80</td>
<td>21.3 ± 0.80</td>
<td>+</td>
</tr>
<tr>
<td>Platelet 5-HT level T2</td>
<td>20.2 ± 1.00</td>
<td>20.7 ± 1.01</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT level T3</td>
<td>18.4 ± 0.91</td>
<td>19.5 ± 0.90</td>
<td></td>
</tr>
</tbody>
</table>

* Untransformed LSmeans ± SEM.

* Whole blood 5-HT level in nmol/ml; Platelet 5-HT level in nmol/platelet 10⁹; Platelet 5-HT uptake velocity in pmol/platelet 10⁹/min.

+ P < 0.10; * P < 0.05.
### Table 2. Blood 5-HT measures at 8 (T1), 9 (T2), and 22 (T3) weeks of age in barren or enriched housed victims and non-victims of tail biting at time of weaning.

<table>
<thead>
<tr>
<th>Blood measures&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Victim</th>
<th>Non-victim</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Barren</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T1</td>
<td>8.1 ± 1.75</td>
<td>12.8 ± 0.59</td>
<td>**</td>
</tr>
<tr>
<td>Whole blood 5-HT T2</td>
<td>10.0 ± 1.94</td>
<td>12.1 ± 0.61</td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T3</td>
<td>2.1 ± 1.30</td>
<td>7.6 ± 0.49</td>
<td>***</td>
</tr>
<tr>
<td>Platelet 5-HT uptake T3</td>
<td>45.6 ± 5.96</td>
<td>45.3 ± 1.48</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT level T1</td>
<td>17.7 ± 2.76</td>
<td>23.1 ± 0.91</td>
<td>*</td>
</tr>
<tr>
<td>Platelet 5-HT level T2</td>
<td>19.4 ± 3.02</td>
<td>20.3 ± 0.99</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT level T3</td>
<td>11.2 ± 2.73</td>
<td>18.9 ± 0.73</td>
<td>**</td>
</tr>
<tr>
<td><strong>Enriched</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T1</td>
<td>11.3 ± 1.13</td>
<td>11.4 ± 0.46</td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T2</td>
<td>13.2 ± 1.35</td>
<td>11.7 ± 0.57</td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T3</td>
<td>8.1 ± 1.12</td>
<td>7.5 ± 0.51</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT uptake T3</td>
<td>45.5 ± 3.48</td>
<td>39.4 ± 1.52</td>
<td>+</td>
</tr>
<tr>
<td>Platelet 5-HT level T1</td>
<td>21.4 ± 1.97</td>
<td>21.3 ± 0.77</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT level T2</td>
<td>25.8 ± 2.35</td>
<td>20.0 ± 0.95</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT level T3</td>
<td>19.8 ± 2.62</td>
<td>19.5 ± 1.09</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Untransformed LSmeans ± SEM.

<sup>b</sup> Whole blood 5-HT level in nmol/ml; Platelet 5-HT level in nmol/platelet 10<sup>9</sup>; Platelet 5-HT uptake velocity in pmol/platelet 10<sup>9</sup>/min.

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001.
Table 3. Blood 5-HT measures at 8 (T1), 9 (T2) and 22 (T3) weeks of life and given per type of pig (with respect to tail biting) in barren housing over three phases of life (weaner, grower, finisher) post-weaning.

<table>
<thead>
<tr>
<th>Blood measuresa</th>
<th>Biter</th>
<th>Victim</th>
<th>Biter/Victim</th>
<th>Neutral</th>
<th>P-valuesb</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T1</td>
<td>11.3 ± 1.36</td>
<td>14.6 ± 0.91</td>
<td>11.1 ± 2.57</td>
<td>11.9 ± 0.70</td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T2</td>
<td>12.8 ± 1.58</td>
<td>12.7 ± 0.98</td>
<td>10.9 ± 2.87</td>
<td>11.5 ± 0.73</td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T3</td>
<td>7.0 ± 1.05</td>
<td>7.4 ± 0.73</td>
<td>6.5 ± 2.06</td>
<td>7.3 ± 0.57</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT uptake T3</td>
<td>42.9 ± 4.31</td>
<td>49.4 ± 2.81</td>
<td>43.4 ± 11.67</td>
<td>43.7 ± 1.93</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT level T1</td>
<td>19.1 ± 2.15</td>
<td>23.9 ± 1.43</td>
<td>18.1 ± 4.06</td>
<td>23.2 ± 1.09</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT level T2</td>
<td>22.4 ± 2.45</td>
<td>21.3 ± 1.55</td>
<td>18.6 ± 4.44</td>
<td>19.4 ± 1.17</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT level T3</td>
<td>16.6 ± 2.04</td>
<td>18.2 ± 1.32</td>
<td>14.6 ± 4.25</td>
<td>19.0 ± 0.90</td>
<td></td>
</tr>
<tr>
<td><strong>Phase 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T1</td>
<td>10.3 ± 1.42</td>
<td>12.7 ± 0.73</td>
<td>14.0 ± 1.53</td>
<td>12.6 ± 0.83</td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T2</td>
<td>10.5 ± 1.60</td>
<td>12.0 ± 0.78</td>
<td>10.9 ± 1.72</td>
<td>12.5 ± 0.88</td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T3</td>
<td>6.9 ± 1.11</td>
<td>7.2 ± 0.59</td>
<td>6.4 ± 1.21</td>
<td>7.7 ± 0.66</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT uptake T3</td>
<td>49.2 ± 4.80</td>
<td>45.1 ± 2.09</td>
<td>46.2 ± 5.18</td>
<td>44.5 ± 2.52</td>
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<tr>
<td>Platelet 5-HT level T1</td>
<td>20.2 ± 2.23</td>
<td>22.3 ± 1.13</td>
<td>22.4 ± 2.39</td>
<td>24.3 ± 1.27</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT level T2</td>
<td>17.9 ± 2.48</td>
<td>20.3 ± 1.24</td>
<td>18.1 ± 2.67</td>
<td>21.0 ± 1.40</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT level T3</td>
<td>19.6 ± 2.24</td>
<td>18.8 ± 1.00</td>
<td>15.8 ± 2.42</td>
<td>18.2 ± 1.14</td>
<td></td>
</tr>
<tr>
<td><strong>Phase 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T1</td>
<td>11.7 ± 1.96</td>
<td>12.7 ± 0.73</td>
<td>11.6 ± 1.28</td>
<td>12.4 ± 0.97</td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T2</td>
<td>7.0 ± 1.15</td>
<td>11.7 ± 0.68b</td>
<td>12.3 ± 1.32b</td>
<td>11.5 ± 0.96b</td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T3</td>
<td>5.7 ± 1.42</td>
<td>6.8 ± 0.47</td>
<td>6.6 ± 0.90</td>
<td>7.5 ± 0.65</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT uptake T3</td>
<td>63.2 ± 7.66</td>
<td>44.1 ± 1.90c</td>
<td>48.6 ± 4.28c</td>
<td>44.6 ± 2.85c</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT level T1</td>
<td>25.7 ± 3.12</td>
<td>22.2 ± 1.14</td>
<td>25.4 ± 2.03</td>
<td>22.6 ± 1.53</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT level T2</td>
<td>15.9 ± 3.42</td>
<td>19.8 ± 1.10</td>
<td>21.7 ± 2.10</td>
<td>18.8 ± 1.54</td>
<td></td>
</tr>
<tr>
<td>Platelet 5-HT level T3</td>
<td>21.3 ± 2.89</td>
<td>17.8 ± 0.85</td>
<td>16.8 ± 1.84</td>
<td>18.5 ± 1.23</td>
<td></td>
</tr>
</tbody>
</table>

- a Untransformed LSmeans ± SEM. LSmeans lacking a common letter differ by P < 0.05 (a/b) or P < 0.10 (y/z).
- b Whole blood 5-HT level in nmol/ml; Platelet 5-HT level in nmol/platelet 10⁹; Platelet 5-HT uptake velocity in pmol/platelet 10⁹/min.
- B = Main effect of tail biter; V = Main effect of victim; B×V = Interaction between main effects resulting in Biter, Victim, Biter/Victim and Neutral.
- P < 0.10; * P < 0.05; ** P < 0.01.
Table 4. Blood 5-HT measures at 8 (T1), 9 (T2) and 22 (T3) weeks of life and given per type of pig (with respect to tail biting) in enriched housing over three phases of life (weaner, grower, finisher) post-weaning.

<table>
<thead>
<tr>
<th>Blood measures(^b)</th>
<th>Biter</th>
<th>Victim</th>
<th>Biter/victim</th>
<th>Neutral</th>
<th>P-value(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T1</td>
<td>-</td>
<td>11.7 ± 1.26</td>
<td>-</td>
<td>11.3 ± 0.46</td>
<td>-</td>
</tr>
<tr>
<td>Whole blood 5-HT T2</td>
<td>-</td>
<td>12.7 ± 1.51</td>
<td>-</td>
<td>11.8 ± 0.57</td>
<td>-</td>
</tr>
<tr>
<td>Whole blood 5-HT T3</td>
<td>-</td>
<td>10.1 ± 1.28</td>
<td>-</td>
<td>7.3 ± 0.49</td>
<td>-</td>
</tr>
<tr>
<td>Platelet 5-HT uptake T3</td>
<td>-</td>
<td>41.6 ± 3.87</td>
<td>-</td>
<td>40.0 ± 1.51</td>
<td>-</td>
</tr>
<tr>
<td>Platelet 5-HT level T1</td>
<td>-</td>
<td>20.8 ± 2.19</td>
<td>-</td>
<td>21.4 ± 0.77</td>
<td>-</td>
</tr>
<tr>
<td>Platelet 5-HT level T2</td>
<td>-</td>
<td>21.9 ± 2.64</td>
<td>-</td>
<td>20.6 ± 0.95</td>
<td>-</td>
</tr>
<tr>
<td>Platelet 5-HT level T3</td>
<td>-</td>
<td>27.5 ± 2.95</td>
<td>-</td>
<td>18.6 ± 1.05</td>
<td>-</td>
</tr>
<tr>
<td><strong>Phase 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T1</td>
<td>11.9 ± 2.60</td>
<td>12.0 ± 1.16</td>
<td>12.9 ± 5.83</td>
<td>11.3 ± 0.46</td>
<td>***</td>
</tr>
<tr>
<td>Whole blood 5-HT T2</td>
<td>15.9 ± 3.07</td>
<td>12.9 ± 1.40</td>
<td>14.3 ± 6.86</td>
<td>11.7 ± 0.59</td>
<td>-</td>
</tr>
<tr>
<td>Whole blood 5-HT T3</td>
<td>7.1 ± 2.73</td>
<td>4.3 ± 1.11</td>
<td>-</td>
<td>8.1 ± 0.47</td>
<td>***</td>
</tr>
<tr>
<td>Platelet 5-HT uptake T3</td>
<td>59.1 ± 7.98</td>
<td>35.7 ± 3.35</td>
<td>-</td>
<td>40.3 ± 1.53</td>
<td>*</td>
</tr>
<tr>
<td>Platelet 5-HT level T1</td>
<td>20.2 ± 4.54</td>
<td>21.8 ± 2.01</td>
<td>15.6 ± 10.18</td>
<td>21.3 ± 0.77</td>
<td>-</td>
</tr>
<tr>
<td>Platelet 5-HT level T2</td>
<td>28.0 ± 5.42</td>
<td>23.7 ± 2.42</td>
<td>22.2 ± 12.15</td>
<td>20.1 ± 0.98</td>
<td>-</td>
</tr>
<tr>
<td>Platelet 5-HT level T3</td>
<td>18.8 ± 6.56</td>
<td>14.4 ± 2.61</td>
<td>-</td>
<td>20.2 ± 1.03</td>
<td>*</td>
</tr>
<tr>
<td><strong>Phase 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood 5-HT T1</td>
<td>8.1 ± 4.01</td>
<td>11.5 ± 0.87</td>
<td>14.6 ± 2.45</td>
<td>11.1 ± 0.51</td>
<td>-</td>
</tr>
<tr>
<td>Whole blood 5-HT T2</td>
<td>9.6 ± 4.89</td>
<td>11.3 ± 1.04</td>
<td>13.9 ± 2.98</td>
<td>12.2 ± 0.62</td>
<td>-</td>
</tr>
<tr>
<td>Whole blood 5-HT T3</td>
<td>4.0 ± 3.87(^a)</td>
<td>6.3 ± 0.88(^a)</td>
<td>11.0 ± 2.42(^a)</td>
<td>7.9 ± 0.54(^a)</td>
<td>*</td>
</tr>
<tr>
<td>Platelet 5-HT uptake T3</td>
<td>42.4 ± 16.20</td>
<td>38.9 ± 2.71</td>
<td>32.3 ± 7.13</td>
<td>40.8 ± 1.59</td>
<td>-</td>
</tr>
<tr>
<td>Platelet 5-HT level T1</td>
<td>16.8 ± 5.94</td>
<td>21.2 ± 1.26</td>
<td>25.1 ± 3.61</td>
<td>20.9 ± 0.72</td>
<td>-</td>
</tr>
<tr>
<td>Platelet 5-HT level T2</td>
<td>18.0 ± 8.70</td>
<td>19.5 ± 1.78</td>
<td>24.9 ± 5.22</td>
<td>21.2 ± 1.01</td>
<td>-</td>
</tr>
</tbody>
</table>
| Platelet 5-HT level T3 | 10.6 ± 9.17\(^a\) | 15.9 ± 2.00\(^b\) | 29.2 ± 5.63\(^b\) | 20.3 ± 1.16\(^b\) | * | **

\(^a\) Untransformed LSmeans. LSmeans lacking a common letter differ by P < 0.05 (a/b) or P < 0.10 (y/z).
\(^b\) Whole blood 5-HT level in nmol/ml; Platelet 5-HT level in nmol/platelet 10^9; Platelet 5-HT uptake velocity in pmol/platelet 10^9/min.
\(^c\) B = Main effect of tail biter; V = Main effect of victim; B×V = Interaction between main effects resulting in Biter, Victim, Biter/Victim and Neutral.

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001.
3.1.2. Enriched housing

Also when considering E housed pigs throughout life, no relationships were found between type of pigs (in terms of either significant fixed effects of ‘biter’ and ‘victim’ or their interaction) and 5-HT measures, although a tendency for an interaction between tail biters and victims was found with respect to platelet 5-HT measured at T3 (P < 0.10, but post-hoc analysis revealed no pairwise differences; untransformed LSmeans ± SEM: biters: 14.4 ± 6.62, victims: 19.3 ± 1.55, biter/victims: 26.7 ± 4.85, neutrals: 19.3 ± 1.38) (non-significant findings not shown). Victims at time of weaning tended to have a higher platelet 5-HT uptake velocity at T3 compared to non-victims when E housed post-weaning (Table 2). Pigs identified as victim of tail biting during phase 2 post-weaning had lower whole blood 5-HT levels and platelet 5-HT levels at T3 compared to non-victims (Table 4). Tail biters identified during phase 2 had higher platelet 5-HT uptake velocities at T3 compared to non-tail biters. In phase 3, a significant interaction was observed between ‘biter’ and ‘victim’, where tail biters tended to have lower whole blood 5-HT levels at T3 compared to biter/victims and neutral pigs. A significant interaction between ‘biter’ and ‘victim’ was also found for platelet 5-HT levels at T3 (P < 0.01), where tail biters had significantly lower levels compared to biter/victims and neutral pigs, and tended to have lower levels compared to victims of tail biting.

3.2. Performance in behavioural tests by different types of pigs

3.2.1. Principal component analysis

In B housing, six factors were retained from the PCA (EV > 1). However, only five factors were retained from the PCA to maintain an equal number of factors for both B and E housing as in E housing five factors had an EV > 1 (Table 5 and 6). The factors of B housing together explained 87 % of the total variance and of E housing this was 93 %. Factors retained in B and E housing were largely similar. Each factor was labelled according to the importance of measures defined by loadings. The factor ‘Early life exploration’ had high positive loadings for the proportion of time exploring the novel arena and walking, and a high negative loading for the proportion of time spent standing alert during the pNEe. The proportion of time spent exploring the bucket presented during NOe loaded positively, and proportions of time spent near the wall zone and standing alert loaded negatively (although this was for the latter variable less prominent in E
housing), on the factor ‘Near bucket’. The factor ‘Cortisol’ summarized mainly basal cortisol and the area under the curve, and to a lesser extent also changes (i.e. recovery) in cortisol determined around the novelty test post-weaning. In B housed pigs, high scores on the factor ‘Vocalizations & standing alert’ were associated with high frequencies of vocalizing during pNEe and NOe and with a low proportion of time spent exploring the arena during NOe. The loading pattern of this factor was slightly different in E housed pigs: here the factor ‘Vocalizations and standing alert’ was less clearly determined by frequencies of vocalizations during pNEe and NOe (with moderate loadings of 0.20 and 0.41, respectively), but had a high positive loading of the proportion of time spent standing alert, and a high negative loading of the proportion of time spent exploring the arena during NOe. Variables recorded during the back test, i.e. numbers of struggles and vocalizations, exclusively loaded on the factor labelled ‘Back test activity’.

3.2.1.1. Barren housing

Overall, B housed pigs classified as tail biters had significantly lower ‘Near bucket’ scores compared to non-tail biters (tail biters: -0.24 ± 0.14, non-tail biters: 0.12 ± 0.11, P < 0.05). Furthermore, tail biters tended to have a higher ‘Back test Activity’ compared to non-tail biters (tail biters: 0.21 ± 0.13, non-tail biters: -0.09 ± 0.11, P < 0.10) and more specifically it tended to be so compared to victim and neutral pigs (biter: 0.42 ± 0.24, victim: -0.02 ± 0.09, neutral: -0.17 ± 0.19, P < 0.10). No other significant (or tendencies to) relationships between the overall classification of pigs and PCA factors were found (data not shown). When considering phases of life separately, piglets with a tail wound at weaning had lower ‘Vocalizations & standing alert’ scores (P < 0.05) and tended to have lower ‘Cortisol’ scores (P < 0.10) (Figure 2A). Tail biters identified during phase 1 tended to have lower ‘Near bucket’ scores (P < 0.10), and tail biters of phase 3 had significantly (P < 0.05) lower ‘Near bucket’ scores compared to non-tail biters (Figure 3A and 3C). During phase 2 post-weaning, an interaction between ‘biter’ and ‘victim’ was found with respect to ‘Early life exploration’, where victims had significantly lower scores of this factor compared to neutral pigs (P < 0.05), but not compared to tail biters and biter/victims (Figure 3B). Furthermore, victims identified during phase 2 tended to have lower ‘Vocalizations & standing alert’ scores compared to non-victims (P < 0.10), but an interaction between ‘biter’ and ‘victim’ tended to be present as well and revealed that tail biters seemed to have higher scores compared to victims, biter/victims and neutrals (all P < 0.10) (Figure 3B).
Table 5. Loadings\(^*\) on the first five factors extracted by principal component analysis, after orthogonal rotation, of variables recorded in barren housed pigs during a back test at 2 weeks of age, novel environment test at 3 weeks of age and a novel object test at 13 weeks of age. Proportions of total variation explained by each factor are given.

<table>
<thead>
<tr>
<th>Measures</th>
<th>Early life exploration</th>
<th>Near bucket</th>
<th>Cortisol</th>
<th>Vocalizations &amp; standing alert</th>
<th>Back test activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Back test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of struggles</td>
<td>-0.02</td>
<td>-0.05</td>
<td>-0.02</td>
<td>0.14</td>
<td>0.72</td>
</tr>
<tr>
<td>No. of vocalizations</td>
<td>-0.05</td>
<td>-0.04</td>
<td>0.03</td>
<td>0.34</td>
<td>0.71</td>
</tr>
<tr>
<td><strong>Pre-weaning novel environment exposure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exploration (%)</td>
<td>0.76</td>
<td>0.01</td>
<td>-0.05</td>
<td>-0.14</td>
<td>0.01</td>
</tr>
<tr>
<td>Walking (%)</td>
<td>0.77</td>
<td>0.03</td>
<td>0.01</td>
<td>0.18</td>
<td>0.04</td>
</tr>
<tr>
<td>Standing alert (%)</td>
<td>-0.83</td>
<td>-0.03</td>
<td>0.05</td>
<td>-0.19</td>
<td>-0.04</td>
</tr>
<tr>
<td>No. of vocalizations</td>
<td>0.22</td>
<td>0.03</td>
<td>-0.03</td>
<td>0.65</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Novel object exposure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of vocalizations</td>
<td>-0.07</td>
<td>0.02</td>
<td>-0.04</td>
<td>0.63</td>
<td>0.11</td>
</tr>
<tr>
<td>Standing alert (%)</td>
<td>-0.23</td>
<td>-0.65</td>
<td>0.02</td>
<td>0.20</td>
<td>-0.45</td>
</tr>
<tr>
<td>Exploration (%)</td>
<td>0.25</td>
<td>0.10</td>
<td>-0.08</td>
<td>-0.50</td>
<td>0.49</td>
</tr>
<tr>
<td>Exploring bucket (%)</td>
<td>-0.02</td>
<td>0.81</td>
<td>0.04</td>
<td>0.27</td>
<td>-0.10</td>
</tr>
<tr>
<td>Distance covered (m)</td>
<td>0.23</td>
<td>0.18</td>
<td>0.01</td>
<td>0.47</td>
<td>0.11</td>
</tr>
<tr>
<td>At door zone (%)</td>
<td>-0.04</td>
<td>-0.26</td>
<td>-0.04</td>
<td>0.07</td>
<td>0.03</td>
</tr>
<tr>
<td>At wall zone (%)</td>
<td>0.10</td>
<td>-0.74</td>
<td>-0.01</td>
<td>-0.32</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Salivary cortisol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal cortisol</td>
<td>-0.10</td>
<td>0.17</td>
<td>0.83</td>
<td>0.01</td>
<td>-0.13</td>
</tr>
<tr>
<td>Cortisol change (t=15 - t=0)</td>
<td>0.08</td>
<td>-0.10</td>
<td>0.18</td>
<td>-0.06</td>
<td>0.23</td>
</tr>
<tr>
<td>Cortisol change (t=15 - t=60)</td>
<td>0.04</td>
<td>-0.06</td>
<td>0.52</td>
<td>-0.03</td>
<td>0.17</td>
</tr>
<tr>
<td>Cortisol area under the curve</td>
<td>-0.09</td>
<td>0.11</td>
<td>0.85</td>
<td>0.01</td>
<td>-0.05</td>
</tr>
<tr>
<td><strong>Variance explained (%)</strong></td>
<td>24.20</td>
<td>20.59</td>
<td>15.36</td>
<td>14.15</td>
<td>12.84</td>
</tr>
</tbody>
</table>

* Loadings > 0.30 or < -0.30 are indicated in italics, and loadings > 0.50 or < -0.50 are also indicated in bold.
Table 6. Loadings on the first five factors extracted by principal component analysis, after orthogonal rotation, of variables recorded in enriched housed pigs during a back test at 2 weeks of age, a novel environment test at 3 weeks of age and a novel object test at 13 weeks of age. Proportions of total variation explained by each factor are given.

<table>
<thead>
<tr>
<th>Measures</th>
<th>Early life exploration</th>
<th>Near bucket</th>
<th>Cortisol</th>
<th>Vocalizations &amp; standing alert</th>
<th>Back test activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Back test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of struggles</td>
<td>-0.09</td>
<td>-0.07</td>
<td>0.08</td>
<td>-0.05</td>
<td><strong>0.72</strong></td>
</tr>
<tr>
<td>No. of vocalizations</td>
<td>-0.01</td>
<td>-0.04</td>
<td>0.12</td>
<td>-0.03</td>
<td><strong>0.80</strong></td>
</tr>
<tr>
<td><strong>Pre-weaning novel environment exposure</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exploration (%)</td>
<td><strong>0.75</strong></td>
<td>0.01</td>
<td>0.02</td>
<td>-0.08</td>
<td>-0.16</td>
</tr>
<tr>
<td>Walking (%)</td>
<td><strong>0.78</strong></td>
<td>0.02</td>
<td>0.03</td>
<td>-0.03</td>
<td>0.23</td>
</tr>
<tr>
<td>Standing alert (%)</td>
<td><strong>-0.78</strong></td>
<td>-0.03</td>
<td>-0.05</td>
<td>-0.01</td>
<td>-0.08</td>
</tr>
<tr>
<td>No. of vocalizations</td>
<td>0.24</td>
<td>0.05</td>
<td>-0.03</td>
<td>0.20</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>Novel object exposure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of vocalizations</td>
<td>0.08</td>
<td>0.14</td>
<td>-0.16</td>
<td>0.41</td>
<td>0.32</td>
</tr>
<tr>
<td>Standing alert (%)</td>
<td>-0.19</td>
<td>-0.33</td>
<td>-0.01</td>
<td><strong>0.82</strong></td>
<td>-0.12</td>
</tr>
<tr>
<td>Exploration (%)</td>
<td>0.02</td>
<td>-0.36</td>
<td>0.05</td>
<td><strong>-0.82</strong></td>
<td>0.00</td>
</tr>
<tr>
<td>Exploring bucket (%)</td>
<td>0.07</td>
<td><strong>0.93</strong></td>
<td>-0.04</td>
<td>0.01</td>
<td>-0.02</td>
</tr>
<tr>
<td>Distance covered (m)</td>
<td>0.16</td>
<td>0.36</td>
<td>-0.19</td>
<td>-0.12</td>
<td>0.41</td>
</tr>
<tr>
<td>At door zone (%)</td>
<td>-0.01</td>
<td>-0.33</td>
<td>-0.01</td>
<td>-0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>At wall zone (%)</td>
<td>-0.01</td>
<td><strong>-0.82</strong></td>
<td>0.09</td>
<td>-0.01</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Salivary cortisol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal cortisol</td>
<td>-0.01</td>
<td>-0.07</td>
<td><strong>0.85</strong></td>
<td>0.00</td>
<td>-0.02</td>
</tr>
<tr>
<td>Cortisol change (t=15 - t=0)</td>
<td>-0.14</td>
<td>0.19</td>
<td>0.07</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>Cortisol change (t=15 - t=60)</td>
<td>-0.06</td>
<td>0.02</td>
<td>-0.27</td>
<td>0.09</td>
<td>-0.05</td>
</tr>
<tr>
<td>Cortisol area under the curve</td>
<td>-0.04</td>
<td>0.06</td>
<td><strong>0.88</strong></td>
<td>0.04</td>
<td>-0.01</td>
</tr>
<tr>
<td><strong>Variance explained (%)</strong></td>
<td><strong>20.79</strong></td>
<td><strong>24.35</strong></td>
<td><strong>15.93</strong></td>
<td><strong>14.39</strong></td>
<td><strong>17.06</strong></td>
</tr>
</tbody>
</table>

* Loadings > 0.30 or < -0.30 are indicated in italics, and loadings > 0.50 or < -0.50 are also indicated in bold.
Figure 2. Factor scores of victims and non-victims of tail biting at time of weaning. Behavioural and physiological responses of pigs to novelty (pre- and post-weaning) were summarized in five factors using a PCA. Factor scores are presented per type of pig, i.e. victim (with tail wound) or non-victim (without tail wound) at time of weaning. A: Barren housing. B: Enriched housing. + P < 0.10, * P < 0.05.
Figure 3. Factor scores of barren housed pigs classified as biters, victims, biter/victims or neutrals with respect to tail biting post-weaning. Behavioural and physiological responses of pigs to novelty (pre- and post-weaning) were summarized in five factors using a PCA. Factor scores are presented per type of pig, i.e. tail biter, victim (with tail wound), both tail biter and victim (biter/victim), or pigs that were neither tail biter or victim (neutral). A: Phase 1 post-weaning. B: Phase 2 post-weaning. C: Phase 3 post-weaning. + P < 0.10, * P < 0.05. Pig types lacking a common letter differ significantly (a/b) or tend to do so (x/y/z).
Figure 4. Factor scores of enriched housed pigs classified as biters, victims, biter/victims or neutrals with respect to tail biting post-weaning. Behavioural and physiological responses of pigs to novelty (pre- and post-weaning) were summarized in five factors using a PCA. Factor scores are presented per type of pig, i.e. tail biter, victim (with tail wound), both tail biter and victim (biter/victim), or pigs that were neither tail biter or victim (neutral). A: Phase 1 post-weaning. B: Phase 2 post-weaning. C: Phase 3 post-weaning.
3.2.1.2. Enriched housing

Classifying the E housed pigs once (‘overall’) with respect to tail biting and thereby combining all phases of life, did not reveal any relationships with the factors obtained from the PCA (‘Early life exploration’, ‘Cortisol’, ‘Near bucket’, ‘Vocalizations & standing alert’, and Back test activity’) (data not shown). Neither having a tail wound at the time of weaning (Figure 2B) nor type of pig post-weaning had an effect on the five factors (Figure 4).

3.3. Correlations between blood 5-HT and performance in behavioural tests

3.3.1. Barren housing

In B housed pigs, ‘Vocalizations & standing alert’ was significantly positively correlated with platelet 5-HT levels determined at T1 (r = 0.15, P < 0.05) and it tended to be so for T2 (r = 0.13, P < 0.10) and T3 (r = 0.12, P < 0.10). Furthermore, also ‘Early life exploration’ tended to be positively correlated with platelet 5-HT levels determined at T1 (r = 0.12, P < 0.10) and T2 (r = 0.12, P < 0.10). No other correlations were found between the factors retained from the PCA and 5-HT measures.

3.3.2. Enriched housing

In E housed pigs, ‘Back test activity’ was significantly positively correlated with whole blood 5-HT measured at T1 (r = 0.23, P < 0.001) and T2 (r = 0.20, P < 0.01), and platelet 5-HT levels of T1 (r = 0.20, P < 0.01) and T2 (r = 0.23, P < 0.001). Furthermore, ‘Cortisol’ tended to be negatively correlated with whole blood 5-HT levels at T2 (r = -0.13, P < 0.10) and with platelet 5-HT levels at T2 (r = -0.14, P < 0.10).
4. Discussion

The main aim of our study was to explore whether tail biting in pigs is associated with behavioural and physiological responses to challenges, and also with blood serotonin. To our knowledge, this experiment is the first to provide evidence suggesting that tail biting in pigs is possibly linked to both fearfulness and the blood 5-HT system.

4.1. The effect of housing

Platelet serotonin (5-HT) uptake velocities were significantly higher in barren (B) compared to enriched (E) housed pigs. Except for one tendency for higher 5-HT platelet storage in B housed pigs than in E housed pigs, no housing effects on blood 5-HT levels were found. In a previous study in pigs (Ursinus et al., 2013), housing affected blood platelet 5-HT measures also only marginally and not significantly. Blood 5-HT has been associated with, amongst others, stress physiology (Fichtner et al., 1995; Naesh et al., 2001), gut motility (Kemperman et al., 2007), and immune functioning (León-Ponte et al., 2007) which may be affected by type of housing. B housed pigs are likely to suffer from stress due to the inability to perform species specific behaviours (EFSA, 2007a). In humans, stress may reduce platelet 5-HT uptake sites (patients with PTSD; Fichtner et al., 1995) and velocity (after surgery; Naesh et al., 2001). This altered platelet 5-HT uptake may be related to changes in HPA-axis responses, although cause and effect remain undecided (Naesh et al., 2001). Environmental enrichment in pigs, as compared with barren housing, has been shown to affect HPA-axis (re)activity at different levels indeed (De Jong et al., 2000; Morrison et al., 2007) and also in our E and B pigs differences in cortisol levels (but not in response to novelty) were found (see Reimert et al., 2014). Furthermore, in E housed pigs a tendency for a negative correlation between salivary cortisol and 5-HT storage was observed, suggesting a link between HPA-axis functioning and platelet 5-HT. In addition, E housed pigs likely have an increased gut motility (Pond, 1987) due to the availability of highly fibrous straw. In human babies, increased gut motility was related to increased platelet 5-HT levels (Kemperman et al., 2007), and in chickens the availability of fibrous litter for foraging, which likely affects gut motility, also affected levels of blood 5-HT (De Haas et al., 2014). Also in pigs, provision of dietary fibres has been shown to affect whole blood 5-HT (Souza da
Silva et al., 2014). Hence, also gut motility may relate to blood 5-HT (uptake) in different ways. Collectively, these findings suggest that the effect of housing on platelet 5-HT uptake velocity in our pigs may be related to both stress (B pigs) and the presence of fibrous foraging material, i.e. straw (E pigs). However, the exact underlying mechanisms remain unclear and the existence of other aspects involved cannot be excluded as peripheral 5-HT also serves other functions and plays, for instance, a role in immune responses (León-Ponte et al., 2007).

Relationships between behaviours may be affected by the environment (e.g. Bell and Sih, 2007; Pearish et al., 2013), which emphasizes for our study the importance of studying relationships between tail biting and other pig characteristics in different housing systems. Nevertheless, in E housing far less tail biting and tail damage was observed and only few E housed tail biters were identified (Ursinus et al., 2014). Consequently, in E housed pigs, relations between tail biting and serotonergic, behavioural and cortisol measures were explored, but results should, given these low numbers of tail biters, be considered with caution.

4.2. Tail biting and its relation with blood serotonin

Classifications of pigs according to performing and/or being the victim of tail biting at any phase of life, i.e. over the whole observation period, did not relate to peripheral serotonergic measures. Tail biting behaviour observed in individual pigs was, however, inconsistent throughout life (Ursinus et al., 2014), which may explain the lack of relationships between this ‘overall’ characterization with respect to being the actor and recipient of tail biting (combination of all phases in life) and 5-HT measures. However, our results also show that relationships between tail biting and 5-HT seemed to be present in pigs of both housing systems when specific phases in a pig’s life were considered. Generally, in several phases of life (although not all), tail biters had lower whole blood and platelet 5-HT levels and higher platelet 5-HT uptake velocities compared to either non-tail biters or other types of pigs (victims, biter/victims, neutrals). This seems in line with lower 5-HT values in whole blood or platelets found in humans with mental disorders such as obsessive compulsive disorders (OCD) (Delorme et al., 2005) and depression (Cleare, 1997; Coppen et al., 1976), and in laying hens that perform feather pecking behaviour (Bolhuis et al., 2009; Rodenburg et al., 2009). Additionally, in laying hens selected for a low mortality due to severe feather
Tail biting, blood serotonin and fear

pecking and other injurious behaviours, lower platelet 5-HT uptake velocities were found (Bolhuis et al., 2009; Uitdehaag et al., 2011). Therefore, tail biters may suffer from a (temporary) change in the blood 5-HT system. Remarkably though, victims of tail biting also seemed to have lower whole blood and platelet 5-HT levels, but had a significantly lower or tendency for a higher platelet 5-HT uptake velocity, compared to non-victims. It is possible, that victims of tail biting may also suffer from depressive-like symptoms (e.g. due to B housing or to being tail bitten) and also develop a malfunctional blood 5-HT system, although not entirely identical to that observed in tail biters. In pigs, especially the neutral animals not involved in the damaging behaviour at all, were found to differ in gene expression profiles (some of the genes were associated with production, sociality, and novelty seeking) compared to animals that either displayed or received the damaging behaviour (Brunberg et al., 2013a). Although in our study both tail biters and victims were found to differ from non-tail biters or non-victims, or other pig types, a clear and consistent difference between neutral pigs and both tail biters and victims was not found. This difference in findings may be explained by the type of pigs used. In the current study, we used all available animals including a group of pigs that were both tail biter and tail bite victim, instead of a selected set of (possibly the most extreme) animals (biter, victim, neutral) as was done by others (Brunberg et al., 2013a).

In B housed pigs, whole blood and platelet 5-HT measured at T2 (i.e. week 9) were related to tail biting in phases 2 and 3 post-weaning. In E housing, associations between type of pig, with respect to tail biting and 5-HT measures were found at T3 (i.e. week 22) only. Additionally, 5-HT measures determined at T1 (i.e. week 8) were not significantly related to any type of pig in any phase, except for pigs that were victims of tail biting at the time of weaning and subsequently B housed. It seems, therefore, plausible that, rather than suffering from a malfunctional blood 5-HT system from early life onward, pigs of both housing systems develop changes in their blood 5-HT system during life. Average blood 5-HT values determined at T1 and T2 were similar, most likely because the time span between the two samples was very short and decreases in 5-HT levels due to aging, as found in humans (Jernej et al., 2000), were not yet present. However, significant associations between tail biting and blood 5-HT were present in B housed pigs at T2. This suggests that temporarily mixing unfamiliar pigs can trigger relationships between tail biting and blood 5-HT when also unfavourably housed. As our blood samples were taken 3 days after mixing the pigs and the
half life time of 5-HT in blood is approximately 3-4 days (Kemperman et al., 2007; Koe and Weissman, 1966), it is not impossible that some changes in the blood 5-HT system of individual pigs developed after mixing. Especially when mixing resulted in a lower feed intake (Hyun et al., 1998) and consequently limited dietary tryptophan intake that is needed to synthesize 5-HT (Azmitia, 2010). Dietary tryptophan levels (Martínez-Trejo et al., 2009) and blood tryptophan measures have been associated with tail biting behaviour in pigs (Valros et al., 2013). Additionally, correlations between blood 5-HT values determined at different time points were not extremely high (although clearly and significantly present), suggesting fluctuations in blood 5-HT levels of individual pigs. Remarkably, in E housing also some relations were found between type of pig and 5-HT measures in later life. Here, changes in the 5-HT system may have developed due to a change in tryptophan availability or demand caused by a depressed feed intake, stress (Keszthelyi et al., 2012) (e.g. due to restricted space (Morgan and Tromborg, 2007) caused by a pig’s growth) or gut (mal)functioning (Kemperman et al., 2007; Spiller, 2008), although this may also be true in B housed pigs.

According to our results, pigs involved in tail biting do not consistently exhibit changes in the blood 5-HT system that may have led to tail biting behaviour or becoming a victim of tail biting. However, in phases of life during which individual pigs display tail biting, a clear relationship with the 5-HT system seems to exist, suggesting that tail biting with its accompanying blood 5-HT levels (lower 5-HT storage and higher platelet uptake velocity in tail biters) fluctuate throughout time. The present findings seem to support the existence of a relationship between the blood 5-HT system and tail biting (Valros et al., 2013), similar to the relationship between 5-HT and feather pecking in laying hens (Bolhuis et al., 2009; Rodenburg et al., 2009), but in our pigs this relationship seems more state-like than trait-like. This does, however, not exclude the possible existence of trait-like differences in 5-HT system functioning in other types of tail biters (such as obsessive tail biters, see also Taylor et al., 2010) than observed in our study (likely two-stage tail biters, see Ursinus et al., 2014), which may predispose these animals to perform tail biting behaviour.

4.3. Tail biting and its relation with responsiveness in pigs and blood serotonin

A fairly consistent relationship was observed in B housed tail biters with the factor ‘Near bucket’ (retained from a PCA) during a novel object test post-
weaning, i.e. tail biting pigs spent or tended to spend less time near a metal bucket introduced during a novel environment test, and spent more time standing alert (a vigilance behaviour; Reimert et al., 2013a), and in the wall zones compared to non-tail biters. In E housing no such relation was found, probably due to the low level of tail biting observed. Avoiding a novel object (Van Reenen et al., 2009), standing alert (Hashimoto et al., 1999; Vicens-Costa et al., 2011), and seeking the walls of an arena (also called ‘Thigmotaxis’) (Gershenfeld and Paul, 1997; Kallai et al., 2007) altogether suggests the presence of a higher fearfulness in B housed tail biters at times of challenge in an unfamiliar environment and without other pigs present. Also in humans with behavioural disorders (Ashwani et al., 2011; Torres et al., 2006) or other animals displaying maladaptive behaviour (e.g. poultry: De Haas et al., 2013; Rodenburg et al., 2010; dogs: Seksel and Lindeman, 2001; cats: Ghaffari and Sabzevari, 2010) higher levels of fear or anxiety were suggested. Furthermore, the lower blood 5-HT levels in our tail biters in combination with a higher fearfulness seem to support previous findings in pigs where lower levels of exploring an unfamiliar arena (indicative of higher fearfulness; Korte and De Boer, 2003) was associated with lower whole blood and platelet 5-HT levels (Ursinus et al., 2013). However, in a different study, it was suggested that tail biting pigs were less fearful compared to victim pigs, which was based on the shorter latency time to touch a novel object in the home pen, the longer contact duration with the novel object and lower levels of locomotion (Zupan et al., 2012). Notably, however, in this particular study tail biters responded to exposure to the novel object test with a decrease of heart rate variability in comparison with victims; this indicates suppression of the parasympathetic nervous system which is also found in human patients with panic disorders (Friedman and Thayer, 1998). Moreover, different measures of heart rate variability were found to be significantly intercorrelated in tail biters but not in victims or control pigs (Zupan et al., 2012); similar intercorrelations were demonstrated in human panickers (Friedman and Thayer, 1998). The present finding in tail biting pigs, therefore, seem to agree with the notion that altered 5-HT system functioning, high fearfulness and high levels of impulsive behaviours are related (see for a review Carver and Miller, 2006).

Over all phases (but not in separate phases), being a tail biter in our study tended to be associated with a higher ‘Back test activity’ (indicative of a proactive coping strategy; Bolhuis et al., 2004; Reimert et al., 2013b), compared to a non-tail biter. This is in line with previous findings in parrots (Van Zeeland et al., 2013),
where feather pickers likely had a proactive coping strategy. In laying hens it was suggested that the initial (first order) feather peckers may be proactive copers (Kops, 2014), whereas the hens attracted to already damaged or ruffled feathers (second order peckers) may be reactive copers (Kops et al., 2013). Interestingly, in one life phase, tail biters also tended to have higher ‘Vocalizations & standing alert’ scores compared to victims, biter/victims and neutrals, whereas victims had lower scores of this factor compared to non-victims. Moreover, B housed pigs with higher ‘Vocalizations & standing alert’ scores had higher platelet 5-HT levels, and E housed pigs with a higher ‘Back test activity’ also had higher whole blood and platelet 5-HT levels. Altogether, this suggests that blood 5-HT measures in pigs may be related to more than one personality dimension, which was also proposed by others (Carver and Miller, 2006). Although the relationship between tail biting and coping strategy was not significantly nor consistently present, considering interactions between 5-HT, fearfulness and other personality dimensions may be relevant in understanding problematic behaviours such as tail biting in pigs.

**Conclusions**

Generally, within specific phases of life, tail biters and to a lesser extent also victims seemed to have lower levels of blood serotonin compared to non-performers/receivers. Tail biters also seemed to have higher blood platelet uptake velocities. Furthermore, our results show the importance of considering different phases in a pig’s life with respect to relationships between (problematic) behaviours and serotonergic measures as both may fluctuate in time. Additionally, barren housed tail biters seemed more fearful after a challenging event. Taken together, considering both behavioural responses to challenging events and blood serotonergic measures in pigs may help in characterizing and identifying individuals at risk for developing damaging behaviours such as tail biting. Further research is needed to elucidate the nature of the relationship between peripheral 5-HT and tail biting, and to develop successful preventive strategies and interventions.
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Tail biting, blood serotonin and fear


Indirect genetic effects for growth rate in domestic pigs alter aggressive and manipulative biting behaviour

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Abstract

Indirect genetic effects (IGEs) are heritable effects of an individual on trait values of another, and are a result of social interactions. The objective was to determine the consequences of selection for IGEs for growth (IGEg) on the behavioural repertoire of pigs in a G×E treatment design. One generation of selection was applied to create a high vs. low IGEg contrast in 480 pigs (4-23 weeks of age) housed in barren and straw-enriched pens (n = 80). High IGEg pigs showed 44% less aggressive biting (P = 0.03), 24% less ear biting (P = 0.004), and 34% less biting on enrichment material (P = 0.03). High IGEg pigs had a lower tail damage score (high 2.0; low 2.2; P = 0.004), and consumed 30% less jute sacks (P = 0.002). Straw-bedding reduced biting behaviours additive to the effects of IGEg (P < 0.01), resulting in no G × E interactions. In conclusion, selection on high IGEg reduced potentially harmful biting behaviours in pigs.

Key words: behaviour, indirect genetic effects, genotype-environment interaction, pig, tail biting, response to selection
1. Introduction

Social interactions among individuals may affect a variety of phenotypic traits (e.g. Frank, 2007). If these social effects on others are heritable they may affect response to selection, and thereby alter the outcome of both evolutionary processes in natural populations, and artificial selection programs in agriculture (e.g. Bijma and Wade, 2008; Griffing, 1967; McGlothlin et al., 2010). The impact of social interactions on response to selection can be studied within the framework of indirect genetic effects (IGEs). An indirect genetic effect (IGE), also known as an associative, social-, or competitive genetic effect, or a social breeding value, is a heritable effect of an individual on the trait values of its social partners (Griffing, 1967; Moore et al., 1997). For example, an individual may reduce the growth of its social partners because it carries genes making it highly competitive. IGEs are relevant both for the evolution of natural populations, and for response to artificial selection in domestic and agricultural populations, ranging from trees to laboratory animals and livestock (Bijma, 2011; Wolf et al., 1998). Theory predicts that IGEs affect the response to selection (Bijma et al., 2007; Griffing, 1967; Moore et al., 1997), and there is a growing body of evidence for the existence of IGEs (e.g. Alemu et al., 2014; Peeters et al., 2012). Studies indicate that competitive, aggressive, or injurious behaviours, but also cooperation, may underlie the observed IGEs (Agrawal et al., 2001; Alemu et al., 2014; Mutic and Wolf, 2007; Rodenburg et al., 2010; Wilson et al., 2009). The link between IGEs and behaviour is especially relevant to livestock populations, where behaviour is an important component of animal welfare. First selection experiments in poultry yielded promising results on production and behaviour (e.g. Muir, 1996; Muir et al., 2013; Rodenburg et al., 2010). Yet, we are only at the start of discovering mechanisms underlying IGEs, and there is an urge for more empirical research (Wilson, 2013).

In domestic pigs (*Sus scrofa*), IGEs affect growth rate (here denoted as IGEg), meaning that pigs differ in the heritable effect they express on the growth rate of their pen mates (e.g. Bergsma et al., 2013). Commercially kept pigs have been selected primarily for growth rate and are kept in barren environments, which both may have increased competitive and aberrant behaviour (Rodenburg and Turner, 2012). Aberrant behaviour, such as repeatedly chewing on tails or ears of group mates, may affect growth and health of the bitten animal and is considered a severe welfare problem in pig husbandry (e.g. Schröder-Petersen and Simonsen, 2001). Selection on IGEg might contribute to a solution to
Chapter 5

simultaneously improve both productivity and welfare (Rodenburg et al., 2010).

Consequences of selection for IGEg on the behavioural repertoire of pigs are largely unknown, as well as the potential dependency of IGEg on the environment. The genetic disposition for certain behaviours, for example aggression, may be expressed differently depending on the environment (e.g. Barr et al., 2003). It is therefore important to consider genotype-environment interactions (G × E) to assess whether changes due to selection for IGEs are consistent across environments (Danielson-François et al., 2009).

Here we studied whether selection for IGEs for growth (IGEg) alters the behaviour of pigs. Using a one generation selection experiment, we investigated which behaviours are targeted by selection for IGEg. In addition, we studied whether interactions exist between IGEg and the environment (G × E) in which the behaviour is expressed. Hereto, pigs were divergently selected for IGEg, and housed in contrasting conditions (barren versus straw-enriched) that were expected to yield differences in behaviour. This is one of the first selection experiments on IGEs in a large mammal. The results will provide insight in the mechanisms underlying IGEs for growth, and in the potential of selection on IGEs to improve social interactions between group living animals.

2. Materials and Methods

2.1. Genetic selection on IGE for growth (IGEg)

Background information on IGEs, and the estimation of IGEs for growth during the finishing phase (from 25 – 110 kg) for the current trial, here denoted as IGEg, has been given in detail in Camerlink et al. (2013). Briefly, sows (64 Topigs-20 sows: sow line of Great Yorkshire × Dutch Landrace) and boars (24 Tempo boars: commercial synthetic boar line with Great Yorkshire genetic background) were selected based on their estimated breeding value for IGEg. Sires and dams with the most extreme high and low IGEg of the available population were mated within their IGEg group (high vs. low), while the direct breeding value was kept equal between groups. This resulted in a contrast of 14 g ADG (Average Daily Gain) between high and low IGEg offspring (40 high IGEg litters and 40 low IGEg litters). Hence, high IGEg offspring would increase the growth of their pen mates, whereas low IGEg offspring would decrease the growth of their pen
mates. Offspring were studied over five batches of 96 pigs each ($n = 480$), between September 2010 and February 2012. The Animal Care and Use Committee of Wageningen University approved the experiment (Protocol Number: 2010055f).

### 2.2. Animals and housing

Piglets were born in conventional farrowing pens with farrowing crates (TOPIGS experimental farm, Beilen, The Netherlands). Tails and teeth were kept intact. Male piglets were castrated (at 3 d of age), because IGEg have currently been estimated on gilts and castrated males. Cross fostering was applied only if litter sizes exceeded 14 piglets, and always within the same IGEg group. At ~14 days of age, piglets were subjected to the back test to assess their coping style (Hessing et al., 1993). Classification of piglets based on their response in the back test, for which no relationship with IGEg was found (Reimert et al., 2013), was used to standardize group composition with regard to coping style. Piglets were weaned at 26 days of age, whereby maximum eight piglets per sow were selected. Selection was based on good health, sex, and back test response. Selected piglets ($n = 480$ in total) were transported to experimental farm De Haar (Wageningen, The Netherlands).

From weaning to slaughter (4 – 23 wk of age), a $2 \times 2$ experimental arrangement was applied with IGEg (low vs. high) and housing conditions (barren vs. enriched) as factors at the pen level. Pigs were housed with six per pen, leading to 80 pens in total. Group composition was balanced for sex (1:1) and back test classification (at least two of each classification). Half of the pigs from each IGEg group, and half of the selected piglets from each sow, were allocated to barren pens, and the other half to enriched pens.

Barren pens had a floor which was half solid concrete and half slatted. Enriched pens had a solid floor with a bedding of 12 kg of wood shavings and 1.5 kg of straw. Fresh wood shavings (3 kg / pen) and straw (0.25 – 1.5 kg / pen depending on age) were added to enriched pens daily. Pen dimensions were either $1.90 \times 3.20$ m or $2.25 \times 3.25$ m ($1 - 1.2$ m$^2$ / pig), depending on batch, and were within batch equal between barren and enriched pens. All pens had a metal chain with ball attached to the pen wall as toy. Dry pelleted commercial feed was offered *ad libitum* from a single space feeder. Feed was provided according to commercial practice, with a total of four feed changes whereby on the first day the old and new feed types were mixed to create a gradual transition between feed types. Water was continuously available from a single nipple drinker per pen.
Temperature was until 10 days after weaning set at a minimum of 25 °C, and was hereafter set at 22 °C for 3 weeks, followed by 20 °C until slaughter. Lights and a radio were on from 7:00 till 19:00 h. To reduce damaging tail biting behaviour, i.e. chewing on the tail of a conspecific which can lead to injury and in extreme cases even to mortality of the bitten animal, all pens received a handful of wood shavings per day from week six onwards and a jute sack was attached to the wall from week eight onwards. Pigs were housed in these pens from weaning until slaughter. Due to diverse health reasons including tail biting, 18 high IGEg and 11 low IGEg pigs were removed from the experiment.

2.3. Behavioural observations

Behaviours of individual pigs were recorded at 4, 5, 8, 12, 16, and 21 wk of age. Each pig was identified by a spray marked number on the back, which was refreshed before behavioural observations. Behaviour, as described in Table A1 (Appendix), was scored during live observations using 2-min instantaneous scan sampling for 6 h during the active period of the day, consisting of six 1 h blocks from 8:00 – 11:30 h and from 14:00 – 17:30 h with after each hour a 15 minute break. This procedure resulted in 180 observations per pig per observation day, with one observation day in each of the weeks mentioned. The Observer 5.0 software package (Noldus Information Technology B.V., Wageningen, The Netherlands) installed on a hand-held computer was used for behaviour recordings. Observations were carried out by observers who were unaware of the IGEg of the pigs.

2.4. Tail damage scores

Tail damage scores can serve as an indicator for the amount of tail biting behaviour in a pen. Scores were obtained using an adapted procedure from Zonderland et al. (2008). Scores ranged from 1 – 4, with score 1 being no visible tail damage; score 2 for hair removed from the tail; score 3 for bite marks; and score 4 for a clearly visible wound. Tail damage was scored each week on each individual pig, leading up to 20 observations per pig. When a pig had to be removed from the trial due to being bitten severely its score was set to 4 for the remaining period till slaughter. When a tail biter had to be removed from the pen it kept its last score before being removed from the pen. Scores were obtained by multiple observers who were trained to score in the same way, and who were unaware of the IGEg of the pigs.
2.5. Interventions to limit damage due to tail biting

Oral manipulation amongst pigs is the repeatedly biting on the tail, ear or paw of a group member, and may result in injury, impaired health or mortality of the bitten animal. Oral manipulation such as tail biting may start harmlessly, but when no measures are taken many animals may be severely damaged (Statham et al., 2009). During the trial, measures were taken to reduce tail biting to an acceptable level to prevent the loss of animals and to guarantee a certain level of animal welfare. Tail biting wounds became significant from six weeks of age. To reduce the amount of damaging tail biting behaviour, a handful of wood shavings was provided to each pen from week six onward and from week eight a jute sack was attached to the pen wall as material to chew on. The jute sack was a commercially available sack of approximately 60 × 105 cm, which was over the width attached to the pen wall and was replaced when there was less than 1/3 of the sack left (Figure 1). When the sack was replaced, the remainders were approximated in cm². The amount of jute sack that was 'consumed' was noted by pen. To reduce tail biting, the tails of bitten pigs were alternating between days covered with the aversive P.B.H. spray (Kommer Biopharm B.V.) or Stockholm tar (Rapide®). Pigs were removed from the pen when they had a reduction in tail length, irrespective of the amount of reduction. Six high IGEg pigs and three low IGE pigs, from 8 different pens in total, were removed from the trial due to reduced tail length. One tail biter (low IGEg) was removed to limit further tail damage of its five pen mates.

2.6. Data analyses

Statistical analyses were performed using SAS (SAS 9.2, Institute Inc.). Data were analysed and presented by production phase as applied in commercial pig farming to facilitate comparison between animal behaviour studies. The nursery phase is from 4 to 8 wk of age, whereas the finishing phase is generally from 8 wk of age till slaughter (here at 23 wk of age).

Behavioural scans were analysed on pen level (n = 80) and averaged over production phase (nursery phase: observations wk 4, 5, and 8; finishing phase: observations wk 11, 12, 16 and 21). Hereto the behaviours of pigs were averaged by pen (6 pigs / pen). Residuals of the response variables were checked for normality, and if needed, behaviours were arcsine square root transformed. Behaviours by pen and production phase were analysed in a General Linear Model (GLM
Procedure), and included IGEg group, housing condition, the interaction between IGEg group and housing condition, and batch as fixed class effects.

The weekly tail damage scores were averaged into two scores per pig, one for the nursery phase (wk 4 – 7) and one for the finishing phase (wk 8 – 23). Scores were analysed at individual animal level (n = 480) in a Generalized Linear Mixed Model (MIXED Procedure) with IGEg group, housing condition, the interaction between IGEg group and housing condition, sex, and batch as fixed class effects, and as random factor pen nested within IGEg group, housing condition and batch.

The total cm² of ‘consumed’ jute sacks per pen (from wk 8 – 23) was analysed at pen level (n = 80) in a General Linear Model (GLM Procedure) with IGE group, housing condition, the interaction between IGE group and housing condition, and batch as fixed class effects. To facilitate the interpretation of consumed bags in cm², results are presented in number of jute sacks consumed (total cm² / (60 × 105)). The amount of jute sacks per pen was correlated to the average tail damage scores per pen by Pearson correlation.

In the Results, average trait values for the treatments will be reported as (untransformed) LSmeans ± SEM.

Figure 1. Jute sack attached to pen wall as distraction material to limit tail biting. The sack was replaced when the sack was ‘consumed’ till the dashed line or further.
3. Results

3.1. Nursery phase

Over the observation moments between wk 4 and 8 of age, differences in behaviour between the IGEg groups were small, and did not show a systematic pattern. Pigs with high IGEg showed 20% less nosing contact with pen mates (nose-nose and nose-body contact), and tended to show 25% less aggressive biting (Table I). In addition, high IGEg pigs tended to spend less time lying inactive and defecate less than low IGEg pigs (Table I). There was no difference in overall activity (all activity minus lying inactive and sleeping) \( (P = 0.54) \), the sum of all explorative behaviours (see Appendix for behaviours) \( (P = 0.55) \), or the sum of all aggressive behaviours \( (P = 0.85) \). IGEg group interacted with housing condition for drinking and belly nosing, and tended to interact for rooting, nose contact, and head knocks (Table II). Other behaviours were not significantly affected by IGEg group, or its interaction with housing.

Table I. Behaviours during the nursery and finishing phase in percentage of behavioural scans for the difference between high and low IGEg pigs. Values are LSmeans of untransformed data with standard error (SEM).

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>High IGEg</th>
<th>Low IGEg</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nursery (4 – 7 wk)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggressive biting</td>
<td>0.09 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>Lying inactive</td>
<td>11.20 ± 0.30</td>
<td>11.90 ± 0.30</td>
<td>0.08</td>
</tr>
<tr>
<td>Nose contact</td>
<td>0.24 ± 0.02</td>
<td>0.30 ± 0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>Urinate/defecate</td>
<td>0.37 ± 0.20</td>
<td>0.43 ± 0.20</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Finishing (8 – 23 wk)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggressive biting</td>
<td>0.03 ± 0.005</td>
<td>0.05 ± 0.005</td>
<td>0.006</td>
</tr>
<tr>
<td>Comfort behaviour</td>
<td>0.10 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.005</td>
</tr>
<tr>
<td>Chewing toy</td>
<td>0.90 ± 0.13</td>
<td>1.50 ± 0.13</td>
<td>0.005</td>
</tr>
<tr>
<td>Ear biting</td>
<td>0.11 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Urinate/defecate</td>
<td>0.30 ± 0.02</td>
<td>0.34 ± 0.02</td>
<td>0.10</td>
</tr>
</tbody>
</table>

1 Only significant results are included in the table \( (P < 0.10) \).
3.2. Finishing phase

During the finishing phase, when pigs were observed at 12, 16 and 21 wk of age, high IGEg pigs showed systematically less biting behaviour than low IGEg pigs. Although the frequencies of the observed behaviours are low, of the observed time high IGEg pigs spent 40 % less on aggressive biting of pen mates, and 27 % less on oral manipulation in the form of biting the ears of pen mates than low IGEg pigs did (Table I). High IGEg pigs were not only biting their pen mates less, but also their environment. They were chewing 40 % less on the distraction materials provided, which were the chain with ball and jute sack (Table I). High IGEg pigs were 40 % more often observed to perform comfort behaviour, such as scratching the skin (Table I). Similar to the nursery phase, high IGEg pigs tended to urinate and defecate less than low IGEg pigs (Table I). There was no difference between the IGE groups in overall activity (P = 0.31), explorative behaviour (P = 0.46), or aggressive behaviour (P = 0.29). There was a significant interaction between IGEg group and housing condition for lying inactive and locomotion, and there tended to be G × E interactions for comfort behaviour, drinking, pen exploration, and nosing objects (Table III).

3.3. Effect of housing condition on behaviour

Enrichment with straw significantly influenced almost all behaviours during the nursery and finishing phase (Table II, Table III). Pigs in enriched pens were more active compared to pigs in barren pens, which was seen from less time spent on sleeping, lying inactive and standing. Pigs in enriched pens especially showed less tail biting, ear biting, and belly nosing, and instead spent more time on play, comfort behaviour, and nosing and rooting the pen than pigs in barren pens.
### Table II. Behaviours during the nursery phase (wk 4 – 7) in percentage of behavioural scans for each treatment group: high and low IGEg pigs both in barren (B) and enriched (E) pens (n = 80 in total), with P-values for the difference between IGEg groups (P-IGE), the difference between housing conditions (P-HC), and their interaction (IGE×HC). Values are LSmeans of untransformed data with standard error (SEM).

<table>
<thead>
<tr>
<th>Behav. nursery</th>
<th>High E</th>
<th>High B</th>
<th>Low E</th>
<th>Low B</th>
<th>SEM</th>
<th>P-IGE</th>
<th>P-HC</th>
<th>IGE×HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleeping</td>
<td>38.0</td>
<td>47.0</td>
<td>39.0</td>
<td>46.0</td>
<td>1.1</td>
<td>0.94</td>
<td>&lt;0.001</td>
<td>0.30</td>
</tr>
<tr>
<td>Lying inactive</td>
<td>9.6</td>
<td>12.8</td>
<td>10.6</td>
<td>13.2</td>
<td>0.39</td>
<td>0.08</td>
<td>&lt;0.001</td>
<td>0.44</td>
</tr>
<tr>
<td>Standing</td>
<td>2.2</td>
<td>2.9</td>
<td>2.0</td>
<td>2.7</td>
<td>0.2</td>
<td>0.31</td>
<td>&lt;0.001</td>
<td>0.91</td>
</tr>
<tr>
<td>Locomotion</td>
<td>2.5</td>
<td>2.5</td>
<td>2.6</td>
<td>2.7</td>
<td>0.13</td>
<td>0.24</td>
<td>0.89</td>
<td>0.72</td>
</tr>
<tr>
<td>Sitting</td>
<td>0.82</td>
<td>0.90</td>
<td>0.78</td>
<td>1.0</td>
<td>0.06</td>
<td>0.60</td>
<td>0.01</td>
<td>0.25</td>
</tr>
<tr>
<td>Comfort behav.</td>
<td>0.31</td>
<td>0.24</td>
<td>0.30</td>
<td>0.24</td>
<td>0.03</td>
<td>0.92</td>
<td>0.02</td>
<td>0.87</td>
</tr>
<tr>
<td>Eating</td>
<td>7.9</td>
<td>8.2</td>
<td>8.7</td>
<td>8.3</td>
<td>0.2</td>
<td>0.89</td>
<td>0.09</td>
<td>0.56</td>
</tr>
<tr>
<td>Drinking</td>
<td>1.5</td>
<td>1.8</td>
<td>1.6</td>
<td>1.5</td>
<td>0.09</td>
<td>0.26</td>
<td>0.22</td>
<td>0.02</td>
</tr>
<tr>
<td>Urinate/defecate</td>
<td>0.29</td>
<td>0.45</td>
<td>0.36</td>
<td>0.5</td>
<td>0.03</td>
<td>0.09</td>
<td>&lt;0.001</td>
<td>0.75</td>
</tr>
<tr>
<td>Playing</td>
<td>1.1</td>
<td>0.63</td>
<td>0.96</td>
<td>0.66</td>
<td>0.1</td>
<td>0.66</td>
<td>&lt;0.001</td>
<td>0.46</td>
</tr>
<tr>
<td>Exploration floor</td>
<td>16.0</td>
<td>11.0</td>
<td>14.0</td>
<td>11.0</td>
<td>0.5</td>
<td>0.14</td>
<td>&lt;0.001</td>
<td>0.43</td>
</tr>
<tr>
<td>Nosing object</td>
<td>1.8</td>
<td>2.5</td>
<td>1.9</td>
<td>2.9</td>
<td>0.14</td>
<td>0.13</td>
<td>&lt;0.001</td>
<td>0.37</td>
</tr>
<tr>
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<td>1.6</td>
<td>5.4</td>
<td>2.2</td>
<td>0.4</td>
<td>0.49</td>
<td>&lt;0.001</td>
<td>0.10</td>
</tr>
<tr>
<td>Rooting object</td>
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<td>0.26</td>
<td>0.09</td>
<td>0.31</td>
<td>0.03</td>
<td>0.23</td>
<td>&lt;0.001</td>
<td>0.59</td>
</tr>
<tr>
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<td>10.0</td>
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<td>0.5</td>
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<td>0.69</td>
</tr>
<tr>
<td>Chewing toy</td>
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<td>0.33</td>
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<td>0.37</td>
<td>0.04</td>
<td>0.26</td>
<td>0.0002</td>
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</tr>
<tr>
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<td>0.87</td>
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<td>0.09</td>
<td>0.20</td>
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<td>0.02</td>
</tr>
<tr>
<td>Mounting</td>
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<td>0.23</td>
<td>0.19</td>
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<td>0.53</td>
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<tr>
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<td>0.18</td>
<td>0.15</td>
<td>0.25</td>
<td>0.03</td>
<td>0.18</td>
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<td>0.15</td>
</tr>
<tr>
<td>Head knock</td>
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<td>0.09</td>
<td>0.13</td>
<td>0.02</td>
<td>0.17</td>
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<td>0.07</td>
</tr>
<tr>
<td>Biting</td>
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<td>0.10</td>
<td>0.09</td>
<td>0.14</td>
<td>0.01</td>
<td>0.06</td>
<td>0.005</td>
<td>0.23</td>
</tr>
<tr>
<td>Fighting at feeder</td>
<td>0.09</td>
<td>0.11</td>
<td>0.08</td>
<td>0.10</td>
<td>0.01</td>
<td>0.54</td>
<td>0.23</td>
<td>0.99</td>
</tr>
<tr>
<td>Tail biting</td>
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<td>0.15</td>
<td>0.02</td>
<td>0.17</td>
<td>0.02</td>
<td>0.80</td>
<td>&lt;0.001</td>
<td>0.46</td>
</tr>
<tr>
<td>Ear biting</td>
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<td>0.40</td>
<td>0.09</td>
<td>0.37</td>
<td>0.03</td>
<td>0.63</td>
<td>&lt;0.001</td>
<td>0.92</td>
</tr>
<tr>
<td>Manip. other</td>
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<td>0.50</td>
<td>0.15</td>
<td>0.50</td>
<td>0.04</td>
<td>0.40</td>
<td>&lt;0.001</td>
<td>0.30</td>
</tr>
</tbody>
</table>
Table III. Behaviours during the finishing phase (wk 8 – 23) in percentage of behavioural scans for each treatment group: high and low IGEg pigs both in barren (B) and enriched (E) pens, with P-values for the difference between IGEg groups (P-IGE), the difference between housing conditions (P-HC), and their interaction (IGE×HC). Values are LSmeans of untransformed data with standard error (SEM).

<table>
<thead>
<tr>
<th>Behav. finishing</th>
<th>High E</th>
<th>High B</th>
<th>Low E</th>
<th>Low B</th>
<th>SEM</th>
<th>P-IGE</th>
<th>P-HC</th>
<th>IGE×HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleeping</td>
<td>51</td>
<td>55</td>
<td>50</td>
<td>53</td>
<td>1</td>
<td>0.14</td>
<td>0.004</td>
<td>0.54</td>
</tr>
<tr>
<td>Lying inactive</td>
<td>14</td>
<td>17</td>
<td>16</td>
<td>17</td>
<td>0.4</td>
<td>0.12</td>
<td>0.002</td>
<td>0.15</td>
</tr>
<tr>
<td>Standing</td>
<td>1.1</td>
<td>0.88</td>
<td>0.96</td>
<td>0.95</td>
<td>0.1</td>
<td>0.65</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Locomotion</td>
<td>0.97</td>
<td>0.79</td>
<td>0.76</td>
<td>0.82</td>
<td>0.1</td>
<td>0.11</td>
<td>0.33</td>
<td>0.04</td>
</tr>
<tr>
<td>Sitting</td>
<td>1.9</td>
<td>1.3</td>
<td>2.0</td>
<td>1.4</td>
<td>0.1</td>
<td>0.50</td>
<td>&lt;0.001</td>
<td>0.80</td>
</tr>
<tr>
<td>Comfort behav.</td>
<td>0.13</td>
<td>0.07</td>
<td>0.08</td>
<td>0.05</td>
<td>0.01</td>
<td>0.05</td>
<td>&lt;0.001</td>
<td>0.06</td>
</tr>
<tr>
<td>Eating</td>
<td>7.2</td>
<td>8.0</td>
<td>7.2</td>
<td>8.1</td>
<td>0.2</td>
<td>0.72</td>
<td>&lt;0.001</td>
<td>0.91</td>
</tr>
<tr>
<td>Drinking</td>
<td>2.8</td>
<td>1.9</td>
<td>2.8</td>
<td>2.3</td>
<td>0.1</td>
<td>0.13</td>
<td>&lt;0.001</td>
<td>0.08</td>
</tr>
<tr>
<td>Urinate/defecate</td>
<td>0.16</td>
<td>0.34</td>
<td>0.32</td>
<td>0.36</td>
<td>0.03</td>
<td>0.09</td>
<td>0.02</td>
<td>0.41</td>
</tr>
<tr>
<td>Playing</td>
<td>0.08</td>
<td>0.05</td>
<td>0.13</td>
<td>0.11</td>
<td>0.03</td>
<td>0.14</td>
<td>0.28</td>
<td>0.69</td>
</tr>
<tr>
<td>Exploration floor</td>
<td>8.0</td>
<td>6.0</td>
<td>7.5</td>
<td>6.8</td>
<td>0.4</td>
<td>0.73</td>
<td>0.004</td>
<td>0.09</td>
</tr>
<tr>
<td>Nose body</td>
<td>1.9</td>
<td>1.4</td>
<td>1.8</td>
<td>1.7</td>
<td>0.1</td>
<td>0.37</td>
<td>0.004</td>
<td>0.08</td>
</tr>
<tr>
<td>Rooting</td>
<td>1.8</td>
<td>0.4</td>
<td>1.6</td>
<td>0.45</td>
<td>0.1</td>
<td>0.82</td>
<td>&lt;0.001</td>
<td>0.40</td>
</tr>
<tr>
<td>Rooting object</td>
<td>0.08</td>
<td>0.06</td>
<td>0.08</td>
<td>0.07</td>
<td>0.01</td>
<td>0.99</td>
<td>0.74</td>
<td>0.85</td>
</tr>
<tr>
<td>Chewing</td>
<td>5.8</td>
<td>3.5</td>
<td>5.6</td>
<td>3.4</td>
<td>0.2</td>
<td>0.41</td>
<td>&lt;0.001</td>
<td>0.86</td>
</tr>
<tr>
<td>Chewing toy</td>
<td>0.82</td>
<td>1.1</td>
<td>1.1</td>
<td>1.8</td>
<td>0.2</td>
<td>0.005</td>
<td>0.03</td>
<td>0.22</td>
</tr>
<tr>
<td>Nosing body</td>
<td>0.75</td>
<td>0.87</td>
<td>0.79</td>
<td>1.0</td>
<td>0.1</td>
<td>0.21</td>
<td>0.02</td>
<td>0.52</td>
</tr>
<tr>
<td>Nose contact</td>
<td>0.17</td>
<td>0.17</td>
<td>0.15</td>
<td>0.15</td>
<td>0.02</td>
<td>0.34</td>
<td>0.76</td>
<td>0.95</td>
</tr>
<tr>
<td>Belly nosing</td>
<td>0.03</td>
<td>0.13</td>
<td>0.02</td>
<td>0.09</td>
<td>0.03</td>
<td>0.37</td>
<td>0.002</td>
<td>0.40</td>
</tr>
<tr>
<td>Mounting</td>
<td>0.03</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.45</td>
<td>0.18</td>
<td>0.23</td>
</tr>
<tr>
<td>Fighting</td>
<td>0.05</td>
<td>0.03</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
<td>0.25</td>
<td>0.07</td>
<td>0.39</td>
</tr>
<tr>
<td>Head knock</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
<td>0.03</td>
<td>0.01</td>
<td>0.82</td>
<td>0.80</td>
<td>0.92</td>
</tr>
<tr>
<td>Biting</td>
<td>0.01</td>
<td>0.04</td>
<td>0.04</td>
<td>0.05</td>
<td>0.01</td>
<td>0.006</td>
<td>0.03</td>
<td>0.30</td>
</tr>
<tr>
<td>Fighting at feeder</td>
<td>0.03</td>
<td>0.05</td>
<td>0.05</td>
<td>0.06</td>
<td>0.01</td>
<td>0.30</td>
<td>0.19</td>
<td>0.97</td>
</tr>
<tr>
<td>Tail biting</td>
<td>0.05</td>
<td>0.18</td>
<td>0.07</td>
<td>0.17</td>
<td>0.02</td>
<td>0.70</td>
<td>&lt;0.001</td>
<td>0.51</td>
</tr>
<tr>
<td>Ear biting</td>
<td>0.08</td>
<td>0.14</td>
<td>0.11</td>
<td>0.18</td>
<td>0.02</td>
<td>0.03</td>
<td>0.004</td>
<td>0.86</td>
</tr>
<tr>
<td>Manip. other</td>
<td>0.17</td>
<td>0.40</td>
<td>0.20</td>
<td>0.40</td>
<td>0.04</td>
<td>0.70</td>
<td>&lt;0.001</td>
<td>0.73</td>
</tr>
</tbody>
</table>
Indirect genetic effects and behaviour

3.4. Tail damage scores

Pigs already showed tail damage from the moment of weaning, with an average tail damage score of 2.2 (Figure 2). During the nursery phase (wk 4 – 7) there was no difference between the IGEg groups for tail damage ($P = 0.93$), but a clear difference was present between barren and enriched pens (tail damage score nursery: barren $2.3 \pm 0.04$; enriched $1.8 \pm 0.04$; $P < 0.001$). During the finishing phase (wk 8 – 23) high IGEg pigs had a lower tail damage score (high $2.0 \pm 0.05$; low $2.2 \pm 0.05$; $P = 0.004$), and the positive effect of enrichment remained (mean tail damage score finishing: barren $2.6 \pm 0.05$; enriched $1.6 \pm 0.05$; $P < 0.001$). This resulted in an additive effect of IGEg group and straw enrichment on tail damage, without interactions between these two factors ($P = 0.79$).

3.5. Consumption of jute sacks

From week eight onward a jute sack was attached to the wall of each pen to limit tail biting behaviour (Figure 1). Chewing on a jute sack was indeed related to chewing on a tail, with a positive correlation between the consumption of jute sacks per pen and average tail damage on pen level ($r_p = 0.34$; $P = 0.003$). In pens with high IGEg pigs these sacks had to be replaced 30 % less often than in pens

![Figure 2. Tail damage score for high IGEg pigs in barren pens, high IGEg pigs in enriched pens, low IGEg pigs in barren pens, and low IGEg pigs in enriched pens. Note that the y-axis ranges from 1 – 3.5 while tail damage scores from individual pigs may range from 1 – 4.]
with low IGEg pigs. Over a period of 15 weeks, high IGEg pigs consumed $2.9 \pm 0.3$ jute sacks per pen, whereas low IGEg pigs consumed $4.2 \pm 0.3$ sacks per pen ($P = 0.002$). Pigs in barren pens consumed $4.3 \pm 0.3$ jute sacks whereas in enriched pens on average $2.8 \pm 0.3$ jute sacks were consumed ($P < 0.001$). There was no interaction between IGEg group and housing condition for the consumption of jute sacks ($P = 0.84$).

4. Discussion

We have investigated the behavioural consequences of a single generation of divergent selection for IGEg in pigs in two housing systems. The divergent IGEg groups showed structural differences in biting behaviours directed towards pen mates and to the physical environment during the finishing phase. This indicates that selection on IGEg may alter a range of behaviours, and even behaviours not related to group members, such as biting on objects in the environment. This suggests that selection on IGEg does not merely alter social interactions, but rather results in changes in an internal state of the animal from which differences in behaviour may arise.

4.1. Potential underlying mechanisms

The origin of biting behaviour may be found in amongst others aggression, frustration, stress, or maintenance of dominance relationships (Marler, 1976; Schröder-Petersen and Simonsen, 2001; Scott, 1948). Pigs of high IGEg were suggested to be better in establishing dominance relationships (Canario et al., 2012), and pigs selected for high IGEg showed subtle differences in aggressive behaviour (Camerlink et al., 2013). Although the expression of aggressive and competitive behaviours might have been tempered by ad libitum feeding (Camerlink et al., 2014), these behaviours did not seem the major factor underlying the effects of IGEg in the currently studied pigs. Aggression and competition in general, however, are associated with IGE in a wide range of taxa (reviewed by Wilson, 2013).

Another hypothesis is that IGEg might alter activity (Rodenburg et al., 2010), whereby the positive effect on the growth rate of others would occur due to apathy of the animal, resulting in a reduced number of social interactions, and
thus also a reduced negative impact on the growth rate of others. The activity level of high and low IGEg pigs did not differ in the current study, which suggests no such response to selection. The varying biting behaviours seem more to originate from frustration or stress. Pigs have a strong intrinsic need to root and forage, and when this need cannot find an outlet in the physical environment it may be redirected to group members (e.g. Schrøder-Petersen and Simonsen, 2001). Tail biting, ear biting, and chewing on distraction material may therefore have a similar motivational background. These behaviours have also been related to frustration, stress, and fearfulness (Taylor et al., 2010; Zupan et al., 2012). Additional behavioural and physiological data suggest that high IGEg pigs may be better capable of handling stressful situations and are less fearful (Camerlink et al., 2013; Reimert et al., 2013, 2014). Similarly, laying hens selected on IGEs for survival, which is directly related to cannibalistic pecking, were less sensitive to stress and were less fearful (reviewed in Rodenburg et al., 2010). Tail biting and cannibalistic pecking have similar underlying needs (e.g. urge to forage, feed or explore) and causes (e.g. stress or nutritional deficiencies). Though this concerns different species, and selection for IGE on different traits, the behavioural responses to selection have remarkable similarities which may suggest a similar mechanism in pigs and laying hens. Together, the various behaviours that are altered through selection on IGEg seem to reflect an internal state rather than solely social interactions.

4.2. The effect of selection

In this study, many behaviours have been tested for statistical significance, which increases the risk of false positives due to chance. However, we found a systematic pattern of less biting behaviour in high IGEg pigs, which was supported by extreme P-values that are unlikely to be chance results. We believe that the four significant results all relating to biting behaviour, with an average P-value of ~0.005 (biting, chewing toy, jute sacks consumed, and tail damage score) indicate a true effect. We did not observe differences between IGEg groups in tail biting behaviour itself, which might be due to the scan sampling method, whereby short lasting behaviours are easily missed. The higher tail damage in low IGEg pigs indicates that low IGEg pigs did spend more time on tail biting or were biting more fiercely. Biting behaviour, and especially tail biting, is considered an important animal welfare issue and our results suggest that selection on IGEg may contribute to a solution.
The potential effect of IGEg on harmful biting behaviour might have been underestimated in the current trial. The circumstances of the trial were more favourable compared to common (Dutch) intensive farming conditions (more space per animal), and control measures were taken to limit tail biting (daily treatment of wounded tails, provision of wood shavings and jute sacks, and the removal of animals with shortened tails). In particular, part of the disposition to bite may have been redirected to chewing on the jute sack (Fraser et al., 1991; Van de Weerd and Day, 2009). This together may have reduced tail biting and may have prevented a severe outbreak (Statham et al., 2009; Zonderland et al., 2008). Interference in possible underlying mechanisms of IGEs, for example changing resource availability, might alter the effect of selection (Arango et al., 2005; Wilson, 2013). With no interference in the cannibalistic pecking of laying hens, clear differences between high and low IGE selection lines were found (reviewed in Rodenburg et al., 2010). From a scientific perspective, measures to limit tail biting would ideally have been omitted, but this would go against ethical regulations of animal experiments. If biting behaviour would be one of the mechanisms underlying IGEg in pigs, then control measures may have reduced the expression and effect of selection.

4.3. Considerations for implementation

Previously, behavioural changes were suggested in a small experiment applying selection on IGEg in pigs (Rodenburg et al., 2010), and in a multiple-generation selected (considering DGE and IGE) experiment based on the performance of groups of half sibs (Gunsett, 2005). The current study is, however, the first large scale experiment evaluating the behavioural consequences of selection on IGEg in a large mammal. Knowledge on the mechanisms behind IGEg in pigs may contribute to the optimization of pig breeding and farming. For example, insight in which inherited behaviours affect growth rate of group mates may outline the potential possibilities, and potential profitability, of reducing or enhancing specific social interactions. Follow-up research under commercial conditions, and selection over multiple generations, would be essential to gain further insight in the magnitude and potential variability of the behavioural changes on the long term. If selection on high IGEg causes pigs to show less harmful biting behaviour, then over generations, other behaviours might emerge in relation to IGEg.
4.4. Benefits from both genetics and environment

G × E interactions may be present for pig production traits (Schinckel *et al.*, 1999), but are to date not shown for pig behaviour (e.g. Guy *et al.*, 2002). Little G × E interactions for pig behaviour were found in the current study, and it is therefore not expected that genetic selection on IGEg would alter behaviour differently in different housing conditions. Provision of straw resulted in more behaviour directed towards the environment, which is in accordance with literature (e.g. Fraser *et al.*, 1991). The reduction in damaging behaviour and the lower tail damage scores of pigs on straw clearly point out the potential of substrate to improve pig health and welfare. Tail damage was further reduced in pigs selected for high IGEg, which suggests that differences in the genetic disposition to perform tail biting remain present also when suitable substrate is provided. This shows that biting behaviour can be reduced from two approaches, namely by redirecting the biting behaviour towards the environment instead of conspecifics through the provision of suitable substrate, and by reducing the motivation to bite through selection on IGEg. Straw is often regarded the most suitable substrate to reduce tail biting (Van de Weerd and Day, 2009; Zonderland *et al.*, 2008), but selection on IGEg may give an additional reduction that is cumulative over generations, leading to a further increase in animal welfare.

Conclusion

Selection on high IGE for growth in pigs reduced biting behaviour, which was expressed in lower occurrences of aggressive biting, ear biting, biting on materials provided for chewing (including jute sacks), and less tail damage due to tail biting. The availability of straw in the pen reduced the expression of pen-mate directed behaviours. Hereby straw may redirect the biting behaviour to the environment, whereas selection for IGEg may reduce the disposition to bite. Both may therefore lead to improvements in animal welfare. We outlined some aspects for further research and would like to emphasize that the impact of selection for IGEs for production traits may reach further than solely social interactions.
Chapter 5

Acknowledgements

This research was part of the project ‘Seeking Sociable Swine’, which was financially supported by The Netherlands Organisation for Scientific Research (NWO) and the Dutch Ministry of Economic Affairs. We would like to thank the colleagues and students from the Adaptation Physiology Group of Wageningen University who helped during the experiment. All institutional and national guidelines for the care and use of laboratory animals were followed.
## Appendix

### Table A1. Ethogram

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General individual</strong></td>
<td></td>
</tr>
<tr>
<td>Sleeping</td>
<td>Lying without performing any other described behaviour, eyes closed</td>
</tr>
<tr>
<td>Lying inactive</td>
<td>Lying without performing any other described behaviour, eyes opened</td>
</tr>
<tr>
<td>Standing</td>
<td>Standing without performing any other described behaviour</td>
</tr>
<tr>
<td>Locomotion</td>
<td>Walking or running without performing any other described behaviour</td>
</tr>
<tr>
<td>Sitting</td>
<td>Sitting or kneeling without performing any other described behaviour</td>
</tr>
<tr>
<td>Comfort behaviour</td>
<td>Rubbing body against objects or pen mate, scratching body with hind leg or</td>
</tr>
<tr>
<td></td>
<td>stretching (part of) body</td>
</tr>
<tr>
<td>Eating feeder</td>
<td>Eating at feeder</td>
</tr>
<tr>
<td>Drinking</td>
<td>Drinking from drinking nipple</td>
</tr>
<tr>
<td>Urinate/defecate</td>
<td>Urinating or defecating</td>
</tr>
<tr>
<td><strong>Exploration</strong></td>
<td></td>
</tr>
<tr>
<td>Exploration floor</td>
<td>Sniffing, touching or scraping floor</td>
</tr>
<tr>
<td>Nosing object</td>
<td>Nosing above floor level</td>
</tr>
<tr>
<td>Rooting</td>
<td>Rooting pen floor or in wood shavings or straw</td>
</tr>
<tr>
<td>Rooting object</td>
<td>Rooting above floor level or object</td>
</tr>
<tr>
<td>Chewing</td>
<td>Non-feed chewing or chewing straw</td>
</tr>
<tr>
<td>Chewing toy</td>
<td>Chewing toy: chain with ball or jute sack</td>
</tr>
<tr>
<td><strong>Social</strong></td>
<td></td>
</tr>
<tr>
<td>Nosing head or body</td>
<td>Touching/snifffing any part of a pen mate except nose</td>
</tr>
<tr>
<td>Nose contact</td>
<td>Mutual nose contact</td>
</tr>
</tbody>
</table>
| Playing                    | Individual or group wise gambling, pivoting: running around the pen, some-
|                            | times with gently nudging of pen mates                                      |
| Belly nosing               | Rubbing belly of a pen mate with up and down snout movements               |
| Mounting                   | Standing on hind legs while having front legs on other pig's body          |
| **Aggression**             |                                                                            |
| Fighting                   | Ramming or pushing a pen mate with or without biting the pen mate. Can be |
|                            | either mutual or individual                                                |
| Head knocking              | Head knock given at place other than feeder                                 |
| Biting                     | Bite given at other place than feeder                                       |
| Fighting at feeder         | Push, head knock or bite given at feeder                                   |
| **Oral manipulation of group mates** |                                            |
| Tail biting                | Nibbling, sucking or chewing the tail of a pen mate                         |
| Ear biting                 | Nibbling, sucking or chewing the ear of a pen mate                          |
| Manipulating other         | Nibbling, sucking or chewing part of the body of a pen mate                |
References

Indirect genetic effects and behaviour


Peeters, K., Eppink, T.T., Ellen, E.D., Visscher, J., and Bijma, P. 2012. Indirect genetic effects for survival in domesticated chickens (Gallus gallus) are magnified in crossbred genotypes and show a parent-of-origin effect. Genetics. 192, 705-713.


Damaging behaviors in rearing gilts: the effect of jute sacks, and relations with production characteristics

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Abstract

Pigs may display biting behavior directed at pen mates, resulting in body damage such as tail wounds. We aimed to assess the suitability of jute sacks to reduce biting behaviors. Additionally, we assessed several phenotypic and genotypic characteristics of different types of tail biters.

Pigs originated from 72 litters and were housed in pens with jute sacks available (J) or in control pens (C). At weaning at 4 weeks of age, gilts and boars were separated and only gilts were further studied. Tail and ear damage were recorded at time of weaning, and during the weaner and rearing phase. Also sow damage was considered. Biting behaviors (tail, ear and other) were observed during the weaner and rearing phase. Weight was recorded at birth, weaning and end of the weaner phase, and average daily gain was calculated from birth till weaning and from weaning till 9 weeks. Furthermore, estimated breeding values (EBV) for litter size, litter birth weight, and growth (of ~25-105 kg and of life) were determined.

Jute sacks significantly reduced tail and ear damage measured at weaning (both $P < 0.001$) and they tended to reduce sow tail damage ($P = 0.09$). Jute sacks also reduced tail damage of gilts post-weaning ($P < 0.001-0.03$), with a five-fold reduction of the proportion of 13 week old gilts having a tail wound. All types of biting behavior directed at pen mates were up to 50 % lower in J pens (weaner: all $P < 0.001$, rearing: $P < 0.001-0.04$). Generally, higher genotypic litter size, litter birth weight, and growth seemed associated with higher levels of ear or tail biting behavior ($P = 0.002-0.09$), albeit dependent on treatment and phase. Also higher phenotypic litter sizes were associated with higher levels of biting behaviors ($P = 0.004-0.08$). High-tail biters and to a lesser extent also Medium-tail biters seemed to stem from larger litters ($P = 0.01-0.05$), to be heavier ($P = 0.03-0.04$), to grow faster ($P = 0.05-0.08$) and to display higher levels of all kinds of biting behavior directed to pen mates and the jute sack ($P < 0.001-0.10$) compared with Non-tail biters, dependent on treatment and phase of life. To conclude, jute sacks may profoundly reduce damaging behaviors and tail wounds in gilts. Furthermore, tail biting is associated with the production level of the gilts (both phenotypically and genetically) which suggests a role for breeding programs, and additional research focusing on metabolic demands of tail biting pigs.

**Key words:** pigs, tail biting, ear biting, enrichment, growth, litter size.

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1. Introduction

Tail biting is a common problem in pig husbandry systems (EFSA, 2007) and the behavior is known for its multifactorial background (Van Putten, 1969; Taylor et al., 2010). The effectiveness of enrichment to reduce tail biting has been extensively demonstrated (e.g. Beattie et al., 1996; Zonderland et al., 2008). Organic enrichment materials such as straw or compost are most promising to improve pig welfare (Beattie et al., 2001; Bracke et al., 2006) as they meet pigs’ requirements of proper enrichment, which has to be complex, changeable, destructible, manipulable and should contain some edible parts (Van de Weerd et al., 2005; Studnitz et al., 2007). Nevertheless, the majority of pig producers do not use these materials due to their labor requirements, hygienic consequences and costs (Tuyltens, 2005), and, therefore, other, preferably long-lasting, materials are used (mainly chains; Bracke et al., 2013). These materials may reduce tail biting on a short-term, but not on a long-term basis (Bracke et al., 2006; Zonderland et al., 2008) due to loss of attractiveness. Therefore, the search for other suitable enrichment is still ongoing. Jute sacks (also called burlap, hessian or gunny bags) may be suitable enrichment as they are made of firm plant material, chewable, easy to handle, and not too expensive. The main aim of our study was to assess the suitability of jute sacks to reduce tail biting and other damaging behaviors. We chose rearing gilts as subject of study as these pigs commonly have longer tails than finisher pigs, which may increase the risk of tail wounds. Additionally, we studied different types of tail biters (Non, Medium, High) (Brunberg et al., 2011) and assessed relationships between tail biting, other damaging behaviors and production characteristics. Knowledge about characteristics of these pig types may help in understanding the development of tail biting behavior.
2. Materials and Methods

The experiment was approved by the Animal Care and Use Committee of the Wageningen University.

2.1. Animals and Housing

2.1.1. Pre-weaning

Piglets (1033 in total) were born in two rounds from 72 sows (Topigs 20) at commercial farm “Van Beek SPF Varkens B.V. Lelystad”, The Netherlands. No tooth resection was performed, but tails were docked ± ½ in gilts (509 in total) and ± ¾ (i.e. ¼ tail remaining) in males (524 in total). Males were not castrated. Piglets were further subjected to standard procedures on farm. Piglets were kept in commercial farrowing pens (2.5 × 1.65 m, 54.7 % slats) with sow crate and either in pens with jute sack (J) or in control pens without a jute sack (also called burlap, hessian or gunny bags/sacks) (C) from birth onward (Figure 1). Distribution of litters over J (36 litters) and C (36 litters) was balanced for sow parity and round. Jute sacks (57 × 95 cm, ± 140 gr jute) (Van Heusden, Waardenburg, The Netherlands) were attached to the pen wall using a metal strip and with the open side downwards. The sow could not reach the jute sack. In the pre-weaning phase, one sack was used for two pens and therefore always two neighboring farrowing pens had a jute sack available (Figure 1). Control piglets could not see the jute sack. Temperature was set at 20 °C, and a solid floor (1.2 × 0.45 m) with heating lamp above it was available for the piglets. Commercial piglet pellets were fed ad libitum from 10 days of age until weaning and water was available through one nipple drinker. Daylight entered the farrowing pens and lamps were usually on from 10.00 h until 16.00 h.

2.1.2. Post-weaning

After weaning, at 4 weeks of age, male and female piglets were separated and only females were further studied until the age of 13 weeks. Gilts (346 in total) were first housed in weaner pens (2.95 × 1.45, ± 50 % slats) in groups of 8-10 (36 pens) depending on piglet size. The pens had partly barred pen walls and consequently gilts were able to make snout contact with neighboring gilts. All pens had a chain with a plastic toy. Treatment (J/C) remained for gilts the same throughout life. Jute sacks were from now on attached to the front side of the
pen in order to prevent neighboring pens from reaching the enrichment material (Figure 2). Jute sacks were replaced when < 30 cm was left, when the sack came off the pen wall (this occurred 20 times in total), and when the groups were relocated. A commercial pellet was provided *ad libitum* in a feeder containing two feeding places, and water was available through one nipple drinker. Ambient temperature was first set at 26 °C and gradually decreased to 21 °C. At approximately 9 weeks of age, gilts (309 in total) were, by group (36 pens), transferred to rearing pens (3.95 × 2.08 m, 40 % slats or 3.9 × 2.2, ± 50 % slats, equally divided over treatments) which were separated by partly barred walls. Feed was provided *ad libitum* in a feeder with two feeding places, and again one nipple drinker was available. Temperature started at 25 °C and was gradually decreased to 20 °C. Daylight entered the pens and lamps were usually on from 10.00 h until 16.00 h.
Figure 1. Jute sack attached to the side wall of a conventional farrowing pen.

Figure 2. Jute sack attached to the front wall of a weaner pen.
2.2. Damage scores

2.2.1. Tail damage scores

Tail damage scores (1. No tail damage, 2. Bite marks, 3. Small wound, 4. Medium wound, part of tail missing, 5. Severe wound, no tail is left) were based on procedures of Kritas and Morrison (2007) and Zonderland et al. (2008). Tail damage was recorded for the first time the day before weaning when piglets were lifted and the tail was kept between two fingers. Thereafter, tail damage was recorded when gilts were 9, 11, and 13 weeks of age. The observer entered the pen and kept the tail again between two fingers, but without lifting the gilts.

2.2.2. Ear damage scores

Ear damage was recorded together with tail damage scoring. At weaning, ears were kept between two fingers, but thereafter, no physical contact was necessary to record the damage. Only damage to the backside of the ears (both left and right) was recorded and ear damage was divided in top half (near the tip of the ear) and bottom half (near the base of the ear) (1. No ear damage, 2. Top or bottom lesions, 3. Top and bottom lesions, 4. Severe damage, part of ear is missing). Damage scores of both ears were recorded, but only maximum damage (either left or right ear) was used for further analysis.

2.2.3. Sow damage

The day before weaning, three sow damage scores, likely caused by the piglets, were recorded: tail damage, mammary gland package damage, and vulva damage. Tail damage scoring was identical to the previously described tail damage scoring system for gilts. The procedure of Anonymous (2010) was used to record damage to the mammary gland package (1. No damage, 2. Little, i.e. ≤ 2 packages damaged, 3. Medium, i.e. > 2 packages damaged or one deep wound present, 4. Severe, i.e. > 2 packages damaged and > 1 deep wounds). Vulva damage was adapted from Welfare Quality (2009) and assessed the presence and size of lesions (1. No damage, 2. Small lesions (≤ 2 cm), 3. Large lesions (> 2 cm)).

2.3. Behavioral observations

Behavioral observations were performed post-weaning in both weaner (6, 7 and 8 weeks of age) and rearing pens (11 weeks of age). Observations were done
from 08.00 – 11.30 h and 14.00 – 17.30 h. Each pen was observed 2 × 10-min during the morning and 2 × 10-min during the afternoon during two consecutive days, resulting in 80 min observations per gilt per week of observation. All occurrences of tail biting (chewing or forceful biting the tail of a pen mate), ear biting (chewing the ear of a pen mate), and other types of biting pen mates (chewing other parts of the body than the tail or ears, excluding aggressive bites) were recorded. Both biter and victim were documented. Furthermore, in pens with jute sacks, nosing (snout contact) or biting (chewing) the jute sack were recorded as well. A number was sprayed on the back of each gilt for proper identification using stock marker spray.

2.4. Production characteristics

Total (T) litter size, litter size excluding stillborn piglets (CS) and litter size excluding stillborn piglets and corrected for added or removed cross-fostered piglets (CSC) (173 piglets of which 73 were gilts) were determined. Weight was recorded at birth, at weaning (4 weeks of age), and at 9 weeks of age. Average daily gain (ADG) in grams per day was determined from birth until weaning, from weaning until 9 weeks of age and from birth until 9 weeks of age. The estimated breeding values (EBV) of all piglets were obtained via routine genetic evaluation using MIXBLUP in a multitrait model (Mulder et al., 2010) and they provide the deviation from the population average. EBV for litter size, litter birth weight and test daily gain (TDG) of ~25-105 kg and life daily gain (LDG) were obtained.

2.5. Statistical analysis

SAS version 9.2 (Statistical Analysis System Institute and Inc., 2002-2008) was used for all statistical analyses.

2.5.1. Gilt damage scores

To be able to test the effect of treatment (J/C) on tail and ear damage, first pen averages were calculated for damage scores of gilts only and for all piglets present (i.e. gilts and boars) at time of weaning (week 4). Thereafter, a general linear model was run with fixed effects of treatment and round (1/2). A similar model was used to test the effect of treatment on tail and ear damage scores when gilts were 9 weeks of age. Tail and ear damage scores of gilts during the rearing
phase (week 11 and 13) were first averaged per animal and then per pen, and thereafter subjected to the same procedure as described for week 9.

A generalized linear mixed model with a binary scale (0/1) and logit link function was used to test the effect of jute sack presence, per observation day (week 4, 9, 11 and 13), on number of pigs with a tail wound or mild to severe ear damage (top and bottom lesions). At weaning, fixed effects of treatment, sex, their interaction and round, and a random effect of pen (nested in treatment and round) were included in the model. Post-weaning, only gilts were studied and, therefore, sex and the interaction between sex and treatment were excluded from the model.

2.5.2. Sow damage

The effect of the presence of jute sacks on sow damage scores was tested with a generalized linear mixed model with a multinomial distribution and cumulative logit link function. Fixed effects of treatment (J/C) and round (1/2) were tested. A generalized linear mixed model, with a binary scale and logit link function, was used to test the effect of jute sacks on the presence of a tail wound or not, the presence of at least tail bite marks or not, medium damage to the mammary gland package (severe damage was not observed), and small to large vulva damage (only 4 sows had large lesions). Again fixed effects of treatment (J/C) and round (1/2) were included in the model.

2.5.3. Behavioral observations

To test the effect of treatment on behavioral data obtained in the weaner phase (week 6, 7, and 8), a mixed model was used with fixed effects of treatment (J/C) and round (1/2), and random effects of pig (nested in pen, round and treatment) and pen (nested in round and treatment). A similar model was run to test the effect of treatment on behavioral data obtained in the rearing phase (week 11), but without the random effect of pig as only one week was considered. Variables of which the residuals did not approach normality were square root transformed.

2.5.4. Estimated breeding values (EBV) and litter size

To assess relationships between EBV, and damaging behaviors of gilts, all damaging behaviors were averaged over gilts born in the same litter as they have the same EBV. Only litters of ≥ 5 gilts were included in the model, boars were
excluded. A general linear model, by treatment, was conducted with damaging behaviors as dependent variables, and EBV (one per model) was included as covariate together with the fixed effect of round (1/2). The same approach was used to assess relationships between total (T) litter size and litter size excluding stillborn piglets (CS), and damaging behaviors. Damaging behaviors were again considered as dependent variables, whereas litter size (T and CS, one per model) was considered as covariate. Here, litter size CSC was not considered as this variable was not based on original litters but on actual number of piglets present. If residuals of damaging behaviors (as averages from gilts of the same litter) did not approach normality, logarithmic transformation was applied.

2.5.5. (Non) Tail biters and their characteristics

To assess relationships between type of tail biter and other damaging behaviors, and production (weight and growth) variables, first a distinction was made between Non-tail biters, Medium-tail biters, and High-tail biters in both treatments (J/C) and in both phases in which damaging behaviors were observed (weaner and rearing phase). Considering the weaner phase, animal averages of damaging behaviors observed during week 6, 7 and 8, were used. The distinction between types of tail biter was based on tail biting performance in the particular phase and treatment group. Pigs that never displayed tail biting behavior were classified as Non-tail biters, pigs with tail biting levels between zero and the median of values (i.e. median of observed tail biting occurrences instead of average value or true median, as the presence of many non-tail biters resulted in a low average and true median) were classified as Medium-tail biters (J weaner: 0-2, J rearing: 0-3, C weaner: 0-2.67, C rearing: 0-5 tail biting occurrences during 80 min of observations), and pigs performing tail biting as much as the median of values or more were classified as High-tail biters (J weaner: 2-6.3, J rearing: 3-5, C weaner: 2.67-7, C rearing: 5-16 tail biting occurrences during 80 min of observations). We chose different classifications for each treatment as many types of enrichment reduce (more or less) tail biting behavior (e.g. Van de Weerd et al., 2006; Zonderland et al., 2008; Van de Perre et al., 2011) and likely also the level of tail biting of different types of tail biters. A different classification per phase was used as tail biting behavior in the weaner phase was averaged over more observation weeks, and tail biting behavior in the rearing phase was not, and furthermore, tail biting behavior may increase over time in all individuals (Ursinus et al., 2014) and therefore an update of classifications seems necessary.
To test the effect of tail biter category on displaying damaging behaviors and weight and growth variables, mixed models were used with fixed effects of type of tail biter (Non-tail biter, Medium-tail biter and High-tail biter) and round (1/2), and a random effect of pen (nested in round). Variables of which the residuals did not approach normality were square root transformed.

To assess the relationship between type of tail biter and litter size excluding stillborn and corrected for added or displaced cross-fostered piglets (CSC) (i.e. actual number of piglets present) two generalized linear mixed models with a binary scale (0/1) and logit link function were used and run by treatment (J/C) and by phase (weaner/ rearing). The binary scale of the first model consisted of Non/Medium-tail biters (= 0) and High-tail biters (= 1), whereas the binary scale of the second model consisted of Non-tail biters (= 0) and Medium/High-tail biters (= 1). Type of tail biter (0/1) was the dependent variable and litter size was added in the model as covariate together with the fixed effect of round. Pen (nested in round) was considered a random effect. Odds ratios (OR) were estimated to assess whether the likelihood of being classified as a High- or Medium/High-tail biter was either higher or lower given the litter size CSC (assessed as one unit offset from the covariate’s mean value).

3. Results

3.1. Effect of jute sacks

3.1.1. Gilt damage scores

Jute sack provision significantly reduced both tail and ear damage at weaning (i.e. 4 weeks of age) when considering all piglets present (gilts and boars) (Table 1). However, provision of a jute sack did not significantly affect tail or ear damage at weaning when considering gilts only. Post-weaning, jute sacks reduced tail and ear damage of gilts in the weaner phase (9 weeks of age). Furthermore, jute sacks reduced tail damage during the rearing phase (11 and 13 weeks of age), but not ear damage (Table 1). The provision of a jute sack did not reduce the proportion of pigs (gilts and boars) with a tail wound (mild, medium or severe) in week 4 (P = 0.50), 9 (P = 0.42) and 11 (P = 0.20), but profoundly decreased the proportion of gilts with a tail wound in week 13 (OR = 0.2, P = 0.01) (Figure 3). Furthermore, gilts were less likely to have a tail wound (OR = 0.3, P = 0.02),
but more likely to have an ear wound (OR = 1.9, P = 0.02) at time of weaning as compared to boars. Provision of a jute sack did not reduce the proportion of pigs with damage at the ear top and bottom or severe ear damage (score > 2) in week 4 (P = 0.14) and 13 (P = 0.37), but it tended to do so in week 9 (OR = 0.4, P = 0.05) and it was the opposite in week 11 (OR = 2.5, P = 0.04).

3.1.2. Sow damage scores

A jute sack provided to the piglets tended to reduce tail damage scores of sows (58 sows were used) measured at time of weaning as compared to sows kept in C pens (mean score J: 2.0 ± 0.14, C: 2.3 ± 0.09, with score 1 = 12 %, 2 = 62 %, 3 = 26 %, 4 = 0 %, 5 = 0 % of sows, P = 0.09). Treatment (J/C) did not affect mammary gland package of the sows (57 sows) (mean score J: 1.9 ± 0.15, C: 2.1 ± 0.12, with score 1 = 25 %, 2 = 51 %, 3 = 25 %, 4 = 0 % of sows, P = 0.35), nor vulva damage (56 sows) (mean score J: 1.3 ± 0.11, C: 1.5 ± 0.12, with score 1 = 66, 2 = 27 %, 3 = 7 % of sows, P = 0.24). Furthermore, treatment did not affect proportion of sows with a tail wound (mild, medium or severe) (P = 0.44) (Figure 4).

Nonetheless, J treatment reduced proportion of sows with tail damage (score > 2) (J: 76 %, C: 97 %, P ≤ 0.05). Treatment did not affect medium damage to the mammary gland package (P = 0.90) or small to large vulva damage (P = 0.26) (Figure 4).

Table 1. Tail and ear damage scores of pigs kept in pens with or without jute sacks, recorded at time of weaning and during the weaner and rearing phase.

<table>
<thead>
<tr>
<th>Damage score</th>
<th>Jute sack</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weaning (week 4)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail (gilt)</td>
<td>1.4 ± 0.05</td>
<td>1.5 ± 0.04</td>
<td>0.38</td>
</tr>
<tr>
<td>Ear (gilt)</td>
<td>1.7 ± 0.06</td>
<td>1.8 ± 0.05</td>
<td>0.36</td>
</tr>
<tr>
<td>Tail (all piglets)</td>
<td>1.4 ± 0.03</td>
<td>1.5 ± 0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Ear (all piglets)</td>
<td>1.7 ± 0.04</td>
<td>1.8 ± 0.04</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Weaner (week 9)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail</td>
<td>1.6 ± 0.05</td>
<td>1.8 ± 0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>Ear</td>
<td>1.7 ± 0.08</td>
<td>1.9 ± 0.09</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Rearing (week 11, 13)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail</td>
<td>1.5 ± 0.04</td>
<td>1.8 ± 0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ear</td>
<td>1.7 ± 0.04</td>
<td>1.7 ± 0.05</td>
<td>0.96</td>
</tr>
</tbody>
</table>

1 Tail damage scores: 1 (no damage) - 5 (severe damage).
2 Ear damage scores: 1 (no damage) - 4 (severe damage).
Figure 3. Percentage of gilts with a (small) tail wound at time of weaning (week 4), during the weaner phase (week 9) and the rearing phase (week 11 and 13) for gilts kept in pens with or without jute sacks. ** \( P = 0.01 \).

Figure 4. Percentage of sows with a tail wound, medium damage to the mammary gland package, and with small to large vulva lesions at time of weaning in pens with or without jute sacks available for the piglets.
3.1.3. Damaging behaviors

Jute sacks always profoundly reduced (36 – 50 %) frequencies of all types of biting behavior directed at pens mates as compared to C pens (Table 2). Furthermore, jute sacks were bitten for approximately 10 times per pig during 80 min of observations in both the weaner and rearing phase (Table 2).

3.2. Estimated breeding values

EBV (64 litters) for litter size, litter birth weight, TDG (~25-105 kg) and LDG per treatment (J/C) are presented in Table 3. The higher EBV for TDG ($\beta = 0.002, P = 0.04$) and EBV for litter birth weight ($\beta = 0.001, P = 0.002$) were in gilts kept in C pens, the more gilts displayed ear biting behavior during the weaner phase. A similar tendency was found for EBV for LDG and ear biting in gilts kept in C pens during the weaner phase ($\beta = 0.003, P = 0.09$). Moreover, the higher EBV for TDG ($\beta = 0.01, P = 0.09$) were in gilts kept in C pens, the more gilts tended to display tail biting behavior during the rearing phase. The higher EBV for litter size were in gilts kept in J pens, the more gilts tended to display tail biting behavior during the rearing phase ($\beta = 0.51, P = 0.06$). The higher EBV for litter birth weight ($\beta = 0.004, P = 0.07$) were in gilts kept in J pens, the more gilts tended to display ear biting behavior during the rearing phase.

3.3. Total (T) litter size and litter size excluding stillborn piglets (CS)

Litter size CS ($\beta = 0.15, P = 0.01$) was, in J pens, higher in gilts that displayed relatively much tail biting behavior during the rearing phase. T litter size ($\beta = 0.31, P = 0.01$) and litter size CS ($\beta = 0.25, P = 0.004$) were, in C pens, higher in gilts that displayed relatively much other (than tail or ear) biting behavior directed at pen mates during the rearing phase. T litter size ($\beta = 0.12, P = 0.08$) tended, in C pens, to be higher in gilts that displayed relatively much tail biting behavior during the rearing phase.
Table 2. Damaging behaviors performed by pigs in pens with jute sacks or control pens, and jute sack usage in pens with jute sacks.

<table>
<thead>
<tr>
<th>Behavior¹</th>
<th>Jute sack</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weaner (week 6, 7, 8)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 pens</td>
<td>19 pens</td>
<td></td>
</tr>
<tr>
<td>Tail biting</td>
<td>0.7 ± 0.08</td>
<td>1.1 ± 0.07</td>
</tr>
<tr>
<td>Ear biting</td>
<td>1.8 ± 0.11</td>
<td>3.4 ± 0.22</td>
</tr>
<tr>
<td>Other biting</td>
<td>1.6 ± 0.11</td>
<td>3.0 ± 0.24</td>
</tr>
<tr>
<td>Jute sack biting</td>
<td>9.6 ± 0.79</td>
<td>-</td>
</tr>
<tr>
<td>Jute sack snout contact</td>
<td>2.4 ± 0.11</td>
<td>-</td>
</tr>
<tr>
<td><strong>Rearing (week 11)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 pens</td>
<td>19 pens</td>
<td></td>
</tr>
<tr>
<td>Tail biting</td>
<td>0.5 ± 0.09</td>
<td>1.0 ± 0.19</td>
</tr>
<tr>
<td>Ear biting</td>
<td>1.7 ± 0.15</td>
<td>2.9 ± 0.22</td>
</tr>
<tr>
<td>Other biting</td>
<td>1.8 ± 0.17</td>
<td>3.5 ± 0.28</td>
</tr>
<tr>
<td>Jute sack biting</td>
<td>9.8 ± 1.46</td>
<td>-</td>
</tr>
<tr>
<td>Jute sack snout contact</td>
<td>1.2 ± 0.13</td>
<td>-</td>
</tr>
</tbody>
</table>

¹ Behaviors are given in frequencies per 80 min observations.

Table 3. Estimated breeding values (EBV) for gilts kept in pens with or without a jute sack available. EBV provide the deviation from the population average and can be either '-' or '+'.

<table>
<thead>
<tr>
<th>EBV</th>
<th>Jute sack</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>EBV litter birth weight (g)</td>
<td>-22.3 ± 15.77</td>
<td>-156.6 - 152.0</td>
</tr>
<tr>
<td>EBV litter size (n)</td>
<td>0.6 ± 0.13</td>
<td>-0.8 - 2.1</td>
</tr>
<tr>
<td>EBV TDG¹ (g)</td>
<td>8.7 ± 4.57</td>
<td>-62.6 - 52.4</td>
</tr>
<tr>
<td>EBV LDG¹ (g)</td>
<td>3.0 ± 3.22</td>
<td>-30.7 - 24.6</td>
</tr>
</tbody>
</table>

¹ TDG = Test daily gain of ~25-105 kg.
² LDG = Life daily gain.
3.4. (Non) Tail biters and their characteristics

3.4.1. Pens with jute sacks

Tail biters were not consistent in their tail biting behavior as no effect was present of type of tail biter as identified during the weaner period on tail biting observed during the rearing phase, nor was type of tail biter as identified during the rearing phase related to tail biting as observed during the weaner phase (Table 4).

Type of tail biter (Non-, Medium-, or High-tail biter) identified in J pens during the weaner phase was associated with weight at weaning and weight measured in week 9, where High-tail biters (16 gilts) were a bit heavier than Non-tail biters (52 gilts) (Table 4), and Medium-tail biters (95 gilts) tended to be heavier at time of weaning compared to Non-tail biters (not presented in Table 4). Accordingly, High-tail biters grew faster from birth till weaning compared to Non-tail biters, and from weaning till 9 weeks of age they tended to do so compared to both Non-tail biters and Medium-tail biters. Furthermore, High-tail biters tended to ear bite more, tended to bite more in other parts of the body during the weaner phase, and also tended to bite more in jute sacks during the weaner and the rearing phase compared to Non-tail biters. High-tail biters also tended to bite more in jute sacks during the weaner phase compared to Medium-tail biters. High-tail biters had, furthermore, a higher likelihood to stem from larger litters CSC (i.e. excluding stillborn piglets and also adjusted for added and removed cross-fostered piglets) (P ≤ 0.05, OR = 1.2). Similar results were observed when combining Medium- and High-tail biters, where Medium/High-tail biters had a higher likelihood to stem from a larger litter (CSC) (P = 0.04, OR = 1.2) than Non-tail biters.

Type of tail biter identified in J pens during the rearing phase was not significantly associated with any of the damaging behaviors observed, nor with weight or growth (Table 4). However, Non-tail biters (106 gilts) tended to nose the jute sack less during the rearing phase compared to Medium-tail biters (33 gilts) and High-tail biters (9 gilts). Additionally, High-tail biters (P = 0.01, OR = 1.5), but not Medium/High-tail biters (P = 0.40), again had a high likelihood to stem from a large litter CSC.
Table 4. Production levels and behaviors displayed by different types of tail biters (Non-, Medium-, and High-tail biter) as identified during the weaner and rearing phase in pens with a jute sack available.

<table>
<thead>
<tr>
<th></th>
<th>Non tail biter</th>
<th>Medium tail biter</th>
<th>High tail biter</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weaner phase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Production</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>1.2 ± 0.03</td>
<td>1.2 ± 0.03</td>
<td>1.3 ± 0.06</td>
<td>0.35</td>
</tr>
<tr>
<td>Weaning weight (kg)</td>
<td>7.0 ± 0.15</td>
<td>7.4 ± 0.12</td>
<td>7.8 ± 0.33</td>
<td>0.03</td>
</tr>
<tr>
<td>Weight at 9 weeks (kg)</td>
<td>22.7 ± 0.43</td>
<td>23.4 ± 0.39</td>
<td>25.3 ± 0.97</td>
<td>0.04</td>
</tr>
<tr>
<td>ADG Birth-weaning (g)</td>
<td>211.3 ± 4.57</td>
<td>223.7 ± 3.69</td>
<td>234.9 ± 8.91</td>
<td>0.05</td>
</tr>
<tr>
<td>ADG Weaning-9 weeks (g)</td>
<td>463.4 ± 9.91</td>
<td>470.5 ± 9.49</td>
<td>513.4 ± 20.13</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Behavior</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail biting rearing</td>
<td>0.3 ± 0.13</td>
<td>0.6 ± 0.12</td>
<td>0.7 ± 0.25</td>
<td>0.17</td>
</tr>
<tr>
<td>Ear biting weaner</td>
<td>1.5 ± 0.16</td>
<td>1.8 ± 0.13</td>
<td>2.3 ± 0.30</td>
<td>0.06</td>
</tr>
<tr>
<td>Ear biting rearing</td>
<td>1.5 ± 0.26</td>
<td>1.6 ± 0.22</td>
<td>2.4 ± 0.70</td>
<td>0.28</td>
</tr>
<tr>
<td>Other biting weaner</td>
<td>1.3 ± 0.13</td>
<td>1.6 ± 0.13</td>
<td>2.5 ± 0.56</td>
<td>0.08</td>
</tr>
<tr>
<td>Other biting rearing</td>
<td>1.5 ± 0.24</td>
<td>1.9 ± 0.25</td>
<td>2.3 ± 0.57</td>
<td>0.29</td>
</tr>
<tr>
<td>Jute sack biting weaner</td>
<td>9.6 ± 0.85</td>
<td>9.2 ± 0.56</td>
<td>11.8 ± 1.67</td>
<td>0.07</td>
</tr>
<tr>
<td>Jute sack biting rearing</td>
<td>8.9 ± 1.45</td>
<td>9.9 ± 0.98</td>
<td>12.7 ± 3.26</td>
<td>0.10</td>
</tr>
<tr>
<td>Jute sack nosing weaner</td>
<td>2.3 ± 0.21</td>
<td>2.4 ± 0.17</td>
<td>2.6 ± 0.38</td>
<td>0.79</td>
</tr>
<tr>
<td>Jute sack nosing rearing</td>
<td>1.1 ± 0.22</td>
<td>1.3 ± 0.14</td>
<td>0.9 ± 0.30</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Rearing phase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>1.2 ± 0.02</td>
<td>1.3 ± 0.04</td>
<td>1.3 ± 0.09</td>
<td>0.75</td>
</tr>
<tr>
<td>Weaning weight (kg)</td>
<td>7.3 ± 0.12</td>
<td>7.6 ± 0.18</td>
<td>7.6 ± 0.32</td>
<td>0.71</td>
</tr>
<tr>
<td>Weight at 9 weeks (kg)</td>
<td>23.6 ± 0.33</td>
<td>24.7 ± 0.57</td>
<td>24.6 ± 1.01</td>
<td>0.59</td>
</tr>
<tr>
<td>ADG Birth-weaning (g)</td>
<td>220.6 ± 3.64</td>
<td>228.5 ± 5.46</td>
<td>223.8 ± 9.04</td>
<td>0.77</td>
</tr>
<tr>
<td>ADG Weaning-9 weeks (g)</td>
<td>480.9 ± 7.30</td>
<td>501.5 ± 12.80</td>
<td>500.3 ± 23.72</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>Behavior</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail biting weaner</td>
<td>0.6 ± 0.09</td>
<td>0.9 ± 0.15</td>
<td>0.6 ± 0.25</td>
<td>0.19</td>
</tr>
<tr>
<td>Ear biting weaner</td>
<td>1.7 ± 0.11</td>
<td>2.0 ± 0.28</td>
<td>1.7 ± 0.47</td>
<td>0.37</td>
</tr>
<tr>
<td>Ear biting rearing</td>
<td>1.6 ± 0.19</td>
<td>1.8 ± 0.40</td>
<td>1.9 ± 0.61</td>
<td>0.82</td>
</tr>
<tr>
<td>Other biting weaner</td>
<td>1.6 ± 0.14</td>
<td>1.7 ± 0.21</td>
<td>1.2 ± 0.33</td>
<td>0.61</td>
</tr>
<tr>
<td>Other biting rearing</td>
<td>1.6 ± 0.18</td>
<td>2.4 ± 0.48</td>
<td>1.4 ± 0.38</td>
<td>0.23</td>
</tr>
<tr>
<td>Jute sack biting weaner</td>
<td>9.7 ± 0.57</td>
<td>9.2 ± 1.17</td>
<td>10.7 ± 1.10</td>
<td>0.70</td>
</tr>
<tr>
<td>Jute sack biting rearing</td>
<td>8.9 ± 0.87</td>
<td>12.3 ± 1.76</td>
<td>12.4 ± 5.12</td>
<td>0.19</td>
</tr>
<tr>
<td>Jute sack nosing weaner</td>
<td>2.4 ± 0.16</td>
<td>2.5 ± 0.26</td>
<td>3.0 ± 0.56</td>
<td>0.50</td>
</tr>
<tr>
<td>Jute sack nosing rearing</td>
<td>1.0 ± 0.11</td>
<td>1.6 ± 0.32</td>
<td>1.8 ± 0.52</td>
<td>0.08</td>
</tr>
</tbody>
</table>

1 Data presented as raw means. Means lacking a common letter differ by P < 0.05 (a/b) or P < 0.10 (y/z).
2 Type of tail biter as identified during the weaner phase.
3 Behaviors are given in frequencies per 80 min observations.
4 Type of tail biter as identified during the rearing phase.
### Table 5. Production levels and behaviors displayed by different types of tail biters (Non-, Medium-, and High-tail biter) as identified during the weaner and rearing phase in control pens.\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Non tail biter</th>
<th>Medium tail biter</th>
<th>High tail biter</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weaner phase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Production</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>1.2 ± 0.05</td>
<td>1.2 ± 0.03</td>
<td>1.2 ± 0.06</td>
<td>0.88</td>
</tr>
<tr>
<td>Weaning weight (kg)</td>
<td>7.5 ± 0.23</td>
<td>7.5 ± 0.12</td>
<td>7.9 ± 0.33</td>
<td>0.83</td>
</tr>
<tr>
<td>Weight at 9 weeks (kg)</td>
<td>22.3 ± 0.72</td>
<td>23.0 ± 0.30</td>
<td>23.3 ± 0.83</td>
<td>0.82</td>
</tr>
<tr>
<td>ADG Birth-weaning (g)</td>
<td>229.9 ± 7.25</td>
<td>227.2 ± 3.69</td>
<td>242.7 ± 10.19</td>
<td>0.71</td>
</tr>
<tr>
<td>ADG Weaning-9 weeks (g)</td>
<td>435.2 ± 17.03</td>
<td>456.1 ± 6.52</td>
<td>458.0 ± 17.66</td>
<td>0.67</td>
</tr>
<tr>
<td><strong>Behavior</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail biting rearing</td>
<td>1.0 ± 0.59</td>
<td>0.9 ± 0.15</td>
<td>1.7 ± 0.61</td>
<td>0.14</td>
</tr>
<tr>
<td>Ear biting weaner</td>
<td>2.0 ± 0.26(^a)</td>
<td>3.6 ± 0.20(^b)</td>
<td>5.1 ± 0.65(^c)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ear biting rearing</td>
<td>3.5 ± 0.62</td>
<td>2.8 ± 0.23</td>
<td>3.4 ± 0.78</td>
<td>0.38</td>
</tr>
<tr>
<td>Other biting weaner</td>
<td>1.8 ± 0.28(^a)</td>
<td>3.1 ± 0.33(^b)</td>
<td>3.9 ± 0.44(^b)</td>
<td>0.002</td>
</tr>
<tr>
<td>Other biting rearing</td>
<td>3.0 ± 0.60</td>
<td>3.4 ± 0.32</td>
<td>5.0 ± 1.11</td>
<td>0.43</td>
</tr>
<tr>
<td><strong>Rearing phase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Production</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>1.2 ± 0.03</td>
<td>1.3 ± 0.04</td>
<td>1.1 ± 0.06</td>
<td>0.68</td>
</tr>
<tr>
<td>Weaning weight (kg)</td>
<td>7.6 ± 0.13</td>
<td>7.7 ± 0.20</td>
<td>7.0 ± 0.40</td>
<td>0.65</td>
</tr>
<tr>
<td>Weight at 9 weeks (kg)</td>
<td>23.5 ± 0.32</td>
<td>23.6 ± 0.47</td>
<td>21.8 ± 0.81</td>
<td>0.54</td>
</tr>
<tr>
<td>ADG Birth-weaning (g)</td>
<td>232.2 ± 4.27</td>
<td>233.2 ± 5.66</td>
<td>218.2 ± 13.36</td>
<td>0.65</td>
</tr>
<tr>
<td>ADG Weaning-9 weeks (g)</td>
<td>466.6 ± 7.12</td>
<td>468.8 ± 9.91</td>
<td>433.3 ± 15.94</td>
<td>0.68</td>
</tr>
<tr>
<td><strong>Behavior</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail biting weaner</td>
<td>1.1 ± 0.11(^a)</td>
<td>1.2 ± 0.14(^a)</td>
<td>2.1 ± 0.79(^b)</td>
<td>0.02</td>
</tr>
<tr>
<td>Ear biting weaner</td>
<td>3.3 ± 0.24(^a)</td>
<td>3.5 ± 0.28(^a)</td>
<td>5.6 ± 1.41(^b)</td>
<td>0.04</td>
</tr>
<tr>
<td>Ear biting rearing</td>
<td>2.6 ± 0.27</td>
<td>3.3 ± 0.36</td>
<td>3.9 ± 1.39</td>
<td>0.20</td>
</tr>
<tr>
<td>Other biting weaner</td>
<td>3.0 ± 0.24</td>
<td>3.2 ± 0.68</td>
<td>3.3 ± 0.86</td>
<td>0.76</td>
</tr>
<tr>
<td>Other biting rearing</td>
<td>2.9 ± 0.33(^a)</td>
<td>4.5 ± 0.53(^b)</td>
<td>3.4 ± 1.03(^b)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

1 Data presented as raw means. Means lacking a common letter differ by P < 0.05 (a/b/c).
2 Type of tail biter as identified during the weaner phase.
3 Behaviors are given in frequencies per 80 min observations.
4 Type of tail biter as identified during the rearing phase.
3.4.2. Control pens

Tail biters seemed also in C pens not consistent in their tail biting behavior as no effect was present of type of tail biter as identified during the weaner period on tail biting observed during the rearing phase (Table 5). However, type of tail biter as identified during the rearing phase was significantly related to tail biting as observed during the weaner phase, where High-tail biters displayed also during the weaner phase the most tail biting behavior compared to Non- and Medium-tail biters.

Type of tail biter (Non-, Medium-, or High-tail biter) identified in C pens during the weaner phase was not significantly associated with weight or growth of the animals (Table 4). However, Non-tail biters (32 gilts) displayed less ear biting and other biting behaviors during the weaner phase compared to Medium-tail biters (132 gilts) and High-tail biters (19 gilts). High-tail biters, furthermore, displayed more ear biting and tended to display more other biting behavior compared to Medium-tail biters. Litter size CSC did not affect the likelihood of being a High-tail biter (P = 0.59) or a Medium/High tail biter (P = 0.66).

Type of tail biter identified in C pens during the rearing phase was also not significantly associated with weight or growth of the animals (Table 5). However, High-tail biters (9 gilts) displayed more tail biting and ear biting behavior during the weaner phase as compared to Non-tail biters (95 gilts) and Medium-tail biters (57 gilts). Furthermore, Medium-tail biters displayed more biting in other parts of the body than in ears or tails during the rearing phase compared to Non-tail biters. Litter size CSC did not affect the likelihood of being a High-tail biter (P = 0.57) or a Medium/High tail biter (P = 0.48) as identified in the rearing phase.

4. Discussion

4.1. Jute sack usage

The provision of a jute sack (J) significantly reduced all types of damaging biting behaviors of breeding gilts directed at their pen mates, with levels of biting behaviors being reduced in J pens up to 50% compared to control (C) pens. Consequently, tail damage was at all times lower in pigs from J pens compared to C pens, with the proportion of 13 week old gilts having a tail wound being decreased from ±15% in C pens to ±2% in J pens. The jute sack provided
remained attractive throughout the experiment, as illustrated by the similar number of interactions (± 10 times per 80 min) of gilts with the jute sack in both the weaner and rearing phase. The relatively high and sustaining level of interest in the jute sack by the pigs gives the jute sack a head start compared to many other enrichment materials that seem not attractive at all such as a chain or plastic pipe attached to a chain (Bracke et al., 2007; Van de Weerd et al., 2003). However, previously a decreased interest in a jute sack was observed over time, but the pigs studied were kept in straw-bedded pens before onset of the study (Van de Weerd et al., 2003) which may have affected their interest in other ‘simpler’ objects. Biting pen mates was not fully eliminated in J pens and provision of a jute sack could not reduce biting behavior as much as straw bedding can (± 4 times lower, Camerlink et al., accepted). Nevertheless, also in pens with straw some biting in pen mates occurs and, also tail damage is observed (Ursinus et al., 2014). Moreover, absolute differences in tail damage scores between both treatments (J/C) would probably have been more pronounced when studying pigs with long instead of half-long tails as was the case in the current study. Early ear damage was also reduced by the presence of a jute sack, but later in life treatment did not affect ear damage scores. This may implicate that although ear biting behavior was much lower in J pens compared to C pens, ear damage as scored in our study may also have stemmed from aggressive encounters as pigs may target the ears during fights (D’Eath, 2002; McGlone, 1985).

Pre-weaning, both gilts and boars were present, whereas post-weaning only gilts were studied. At time of weaning (4 weeks of age), gilts were less likely to have a tail wound compared to boars. This difference in tail wounds may have been caused by the difference in tail length, where boars had shorter tails than gilts. Consequently, tail docking in the first week of life likely inflicted a larger wound in boars (i.e. a pigtail is a bit broader at the root compared to the end) which may have resulted in a higher attractiveness (Fraser, 1987a) for other piglets to chew on during the first weeks of life. Nonetheless, similar results were found previously where males had more tail damage (Penny et al., 1981), and females likely displayed the damaging behavior more (Zonderland et al., 2010). Females respond differently in novelty tests than (castrated) males (Reimert et al., 2013, 2014) and it could be that they differ from males in motivation for explorative behavior too. In other studies, no difference between males and females was found with respect to tail biting (Blackshaw, 1981; Ursinus et al., 2014).
Sow damage scores at time of weaning were not affected by treatment except for a tendency for a higher tail damage in the pens without a jute sack available for the piglets. As a limited number of sows were considered for analysis, a lack of power may have resulted in an underestimation of differences in sow damage in J and C pens (specifically tail and vulva damage). It may be worthwhile to consider sow damage in future studies regarding biting behavior of piglets as, seemingly, not all biting behavior is directed to litter mates or pen fixtures, but also to the dam. Additionally, damaged sows may be more attractive for other sows to victimize (vulva biting: Van Putten and Van de Burgwal, 1990) when put back in the group after weaning.

4.2. Different types of pigs and their characteristics

Gilts were not likely to be consistent in their tail biting behavior, although High-tail biters kept in C pens during the rearing phase were likely to have also bitten more on tails during the weaner phase. Also in a previous study (Ursinus et al., 2014) we observed inconsistency in tail biting behavior, which suggests that intrinsic motivations to bite may fluctuate over time.

Generally, High-tail biters and to a lesser extent also Medium-tail biters, also performed relatively high levels of ear and other biting behavior directed at pen mates. However, these findings were less pronounced in J pens, which is likely related to the relatively high levels of interacting with the jute sack by the (Medium/) High-tail biters. This difference between different treatments supports the notion that the environment may affect relations between behaviors displayed (Bell and Sih, 2007; Pearish et al., 2013). Previously, a distinction could be made between tail biters, ear biters, and biters that displayed both tail and ear biting behavior (Blackshaw, 1981). Furthermore, it has been suggested that pigs that display high levels of tail biting behavior may be most specialized in biting pen mates, whereas pigs that display medium levels of tail biting may display all kinds of abnormal behaviors directed at pen mates and pen fixtures (Brunberg et al., 2011). Our results do not support a clear distinction between Medium- and High-tail biters, as Medium-tail biters also displayed relatively high levels of biting behaviors and High-tail biters seemed also highly interested in the jute sack. It has been suggested that tail biting pigs can be divided in ‘two stage’ (gradually developing), ‘sudden-forceful’ (related to resources), and ‘obsessive’ (fanatical) tail biters (Taylor et al., 2010), and that the tail biting behavior of these distinct types
of tail biters may have different motivational backgrounds. However, reliably distinguishing between such differences in tail biters is difficult (Brunberg et al., 2011) when starting observations post-weaning, as tail biting occurs already pre-weaning, and, moreover, the number of obsessive tail biters is likely low (Ursinus et al., 2014).

Nevertheless, also the current study points to the existence of different motivational backgrounds of tail biting as especially high levels of tail biting were related to several of the estimated breeding values (EBV) and phenotypic, weight, growth and litter size. A high EBV for litter birth weight was associated with higher levels of ear biting behavior, but not tail biting behavior. A high EBV for growth (test daily gain of ~25-105 kg or life daily gain) was also associated with relatively high levels of ear biting behavior and to a lesser extent with tail biting behavior. Also phenotypic weight and growth was highest in gilts identified as High-tail biters, but only early in life and only in J pens. Additionally, original litter size (total or excluding stillborn piglets) seemed higher in (mainly C housed) gilts that displayed relatively high levels of biting behaviors. Also litter size corrected for cross-fostering was found to be highest in gilts identified as High-tail biters. Collectively, these results may suggest that selection for heavier piglets, a higher growth, and larger litters may result in higher levels of aberrant behavior, albeit partly dependent on treatment (J/C) and phase of life. Our results seem, therefore, in line with previously proposed associations between biting behavior and genetics (Breuer et al., 2003, 2005), and tail biting and weight (Breuer et al., 2005). Tail biting was found to be genetically correlated with lean tissue growth rate (positive) and back fat thickness (negative), and tail biters seemed to be the larger pigs (Breuer et al., 2005) instead of the smaller pigs which was suggested by others (Beattie et al., 2005; Wallgren and Lindahl, 1996). The associations found here between biting behavior and a higher weight, growth and larger litters may be related to metabolic demands of these pigs. Differences in metabolic demands may affect foraging behavior (Day et al., 1995) and thereby may also affect the level of tail biting behavior (Fraser, 1987b). High-tail biters may, thus, have a higher (unfulfilled) nutritional demand compared to pigs expressing less or no tail biting behavior. For instance, tail biting increases when pigs were fed a diet with low levels of tryptophan (i.e. precursor of serotonin; Azmitia, 2010) (Martínez-Trejo et al., 2009), and recently, tail biting has been associated with low blood serotonin levels (Ursinus et al., accepted). Altogether it seems plausible that a mismatch between the high level of production and metabolic demand of pigs...
can result in (temporary) high levels of tail biting behavior. It must be noted, however, that especially tail biting in J pens showed a relationship with growth, which may suggest that these tail biters have a specific metabolic motivation to start tail biting, whereas tail biters from C pens likely have a broader motivation and are driven more by boredom.

Conclusions

The provision of a jute sack can reduce tail, ear, and other biting behavior of gilts directed to pen mates for up to half as much compared to gilts kept in barren pens. Consequently, in pens with a jute sack available, tail damage was also lower and a five-fold reduction was observed in the proportion of 13 week old gilts having a tail wound. Furthermore, displaying high levels of tail biting behavior was generally related to displaying higher levels of all kinds of biting behavior, a relatively high genotypic and phenotypic growth, and originating from a large litter. However, relationships between tail biting and other measured variables varied between pigs kept in pens with or without jute sacks, which suggests that boredom rather than a metabolic motivation plays the largest role in pigs kept in barren pens. Nonetheless, our results may imply a role for future breeding programs, and, additionally, more research seems essential with respect to the metabolic demands of highly tail biting pigs.

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Chapter 6

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7

General discussion
Chapter 7

General discussion

The main aim of this thesis was to identify characteristics of pigs that relate to the development of oral manipulative behaviours directed to conspecifics, such as tail biting, under different housing conditions. This thesis shows that most pigs are inconsistent in displaying tail biting behaviour over different phases of production (Chapters 2 and 6). Additionally, the occurrence of tail biting was difficult to predict based on pigs' preceding behaviours displayed in their resident pen (Chapter 2). Behavioural predictors of tail biting were, however, seen at group level. Tail biting pigs had a higher chance to originate from litters that performed relatively high levels of tail biting compared to other litters. Tail biting behaviour was, thus, observed already pre-weaning and also tail damage was present at time of weaning. Additionally, on group level, problems with tail biting were preceded by higher activity levels and more oral manipulative behaviours directed at pen mates and enrichment materials. Also during an episode of tail biting, activity levels and such oral manipulations appeared to be higher at pen level (Chapter 2). During an episode of tail biting, tail biters were generally more orally manipulative towards pen mates (Chapter 6). Furthermore, subjecting pigs to a novelty test revealed that tail biters seemed more fearful than non-tail biters (Chapter 4). Tail biters also differed from other pigs in blood serotonergic measures: they had lower blood serotonin storage, but not continuously so and mainly during episodes of life during which they had been observed tail biting. In Chapter 3, it was found that blood and brain serotonergic measures were correlated and both associated with the level of exploration of pigs in a novel object test. Together with other correlations between behavioural responses and brain serotonin measures, the experiment in Chapter 3 suggested that the more fearful a pig, the lower its blood serotonin level and hippocampal serotonin turnover.

A pig's genetic background also seems to be associated with its tendency to show biting behaviours (Chapters 5 and 6). Pigs with an estimated relatively negative indirect genetic effect for growth, i.e. an unfavourable heritable effect on the growth of their pen mates, caused more tail damage (Chapter 5). Furthermore, these pigs displayed higher levels of oral manipulations directed at pen mates and enrichment materials provided, including a jute sack, and jute usage was higher in these pigs. In Chapter 6, both phenotypic growth rate and genetic potential of pigs for production characteristics seemed associated with
the frequency of tail biting behaviour displayed, i.e. pigs with high production levels showed more tail biting. Finally, it was found that straw-bedding (Chapters 2 and 5) and jute sacks (Chapter 6) strongly reduced biting behaviours and other damaging behaviours directed at pen mates and, consequently, tail damage.

In the current chapter (i.e. Chapter 7), it is firstly described how tail biting is associated with other behaviours of pigs at individual and group level, both before and during episodes of tail biting. Subsequently, relations between serotonergic measures and behavioural responses of pigs and their propensity to show tail biting are discussed, followed by the possible impact of genetics and personality on tail biting. Thereafter, the effect of enrichment materials is highlighted and a conceptual framework of biting behaviours is proposed that may be useful in future actions with respect to the prevention or reduction of biting behaviours. Further implications for both practice and research are provided and the discussion ends with the main conclusions.

Tail biting in all its (in)consistency

Individual tail biting behaviour displayed by pigs was generally inconsistent over time (Chapters 2 and 6). Inconsistency in tail biting behaviour means that tail biters observed in one phase of life were not necessarily also tail biters in earlier or later stages of life. In Chapter 2, 480 fattening pigs were classified into either non-tail biters or tail biters (i.e. based on at least two tail biting incidences displayed) during three post-weaning phases which were roughly comparable to the weaner, grower and finisher stages under commercial conditions. Tail biters were highly inconsistent in their tail biting behaviour over these three phases post-weaning (Chapter 2). It may be possible that our methodology of classifying tail biters based on behavioural scan sampling affected the results, as behaviours such as tail biting are relatively short-lasting. However, in Chapter 6, pigs - on a commercial farm - were continuously observed and also in this study most pigs were inconsistent in their tail biting behaviour over different phases of life. Moreover, in another study on a different commercial farm in which continuous behavioural recordings were used, we again found no consistency in tail biting behaviour over time (unpublished results). In practice, however, the problem of tail biting is often generalized to the existence of 'problem pigs', without taking the possible existence of variability within pigs
into consideration. Also in most scientific studies, tail biters have been identified without assessing the long term (in)consistency in tail biting behaviour of these pigs (e.g. Brunberg et al., 2011; Zonderland et al., 2011b). Therefore, it seems that the tendency to show tail biting is often implicitly assumed to be a stable trait characteristic of pigs. The findings from Chapters 2 and 6, however, indicate that the propensity to display tail biting behaviour may be a more temporary and state-like rather than a trait-like characteristic of pigs. Nevertheless, tail biters may vary in frequency and intensity of tail biting behaviour they display (Brunberg et al., 2011) or, hypothetically, by their underlying motivation to perform the behaviour (Taylor et al., 2010). Three types of tail biting have been proposed that may differ in their underlying motivation: 1. a gradually developing type of tail biting (two-stage or chronic), 2. a type of tail biting which may be performed by pigs to gain access to a resource (e.g. food), or 3. an obsessive type of tail biting (i.e. >1.5 – 25% of the observed time) in which pigs actively search for tails to bite in (see review of Taylor et al., 2010). It may be hypothesized that pigs that obsessively search for tails and spend much time on tail biting may remain more consistent in their tail biting behaviour once started. These types of pig usually need to be removed from the pens to prevent further damage to pen mates (Taylor et al., 2010). Indeed, in Chapter 6, possible evidence was found for a difference in consistency of tail biting behaviour according to type of tail biting. In this chapter, breeding gilts were classified into non-tail biters, medium-tail biters and high-tail biters. The high-tail biters identified during the rearing phase (i.e. at 11 weeks of age) and kept in barren pens, were consistent in their tail biting behaviour as they displayed the behaviour also more during the preceding weaner phase than the other two categories of pigs. The existence of intra-individual variability in behaviour of animals has been discussed in literature (MacDonald et al., 2006; Stamps et al., 2012) and it was suggested that one individual may be more predictable with respect to a given behaviour than others (Stamps et al., 2012). Consequently, intra-individual variability may explain the inconsistency in tail biting behaviour. It is possible that variability in behaviour may be a trait characteristic of animals itself (La Guardia and Ryan, 2007; Stamps et al., 2012), but with respect to tail biting, variability may also be dependent on external factors resulting in a more state-like variation in behaviour displayed by individuals: 1. tail biters may be attracted to the tails of victim pigs due to the presence of scabs or wounds that are tasty for them (Fraser, 1987a), 2. tail biting may be socially-facilitated (Schrøder-Petersen and Simonsen, 2001), 3. victims may behave in such a way that they are
an easy target (Schröder-Petersen and Simonsen, 2001; Valros et al., 2012), or 4. underlying motivations may vary (e.g. fluctuating metabolic demands; Day et al., 1996; Fraser, 1987b).

Post-weaning victims of tail biting (i.e. pigs with a (mild) tail wound) identified in Chapter 2 were consistently victims throughout life. At the same time, no clear behavioural pattern was associated with becoming a victim, although victims displayed higher levels of chewing objects (i.e. jute sack or chain with ball). Also the limited number of pigs in the pens may have played a role in the choice of victim(s). Others described that victims may visit a feeder more often already several weeks prior a tail bite outbreak (i.e. at time of first treatment for tail damage) compared to other pigs (Wallenbeck and Keeling, 2013), and that victims were more aggressive and chased other pen mates more compared to tail biters shortly prior to a tail bite outbreak (i.e. when significantly damaged tails were present) (Zonderland et al., 2011b). It is likely that especially shortly prior to a tail bite outbreak, tail biting was already ongoing, and the aggressive behaviours and changes in feeder visits may have been a result of being tail bitten rather than its cause (Wallenbeck and Keeling, 2013). However, the tails of pigs that frequently visit the feeder may also be easy targets for tail biters (Wallenbeck and Keeling, 2013). To the best of our knowledge, no other longitudinal studies have been done so far that examined the consistency of being a tail biting victim. To sum up, except for the high-tail biters in Chapter 6, tail biting behaviour was inconsistently displayed by pigs and if this also holds for pigs in other studies then the tendency to display tail biting behaviour may, in many pigs, be a temporary state rather than a trait.

**Behavioural predictors of tail biting behaviour**

Individual tail biters were more likely to stem from litters in which relatively high levels of tail biting behaviour was displayed during the farrowing period (i.e. 0-4 weeks of age) (Chapter 2). Previously, it has been noted that tail-in-mouth behaviour displayed by piglets may precede tail biting at a later age (Schröder-Petersen et al., 2003). However, in the current study, tail biting behaviour displayed by piglets already resulted in tail damage at time of weaning (Chapters 2 and 6). This was not expected as tail damage is usually considered a problem only starting to occur post-weaning (e.g. Wallgren and Lindahl, 1996;
Tail damage at time of weaning might also be present on other (commercial) farms and in other studies, but it is probably minor and likely remains, therefore, unnoticed. The fact that tail damage was clearly observed in the piglets in Chapters 2 and 6 may be due to the methodology of lifting the piglets and scoring the tail while holding it between the fingers, which is not done in practice. Tail biters that were observed when significantly damaged tails were present, displayed also higher levels of tail biting behaviour six days earlier (Zonderland et al., 2011a). However, no earlier behavioural observations were presented by Zonderland et al. (2011a) to show that these tail biters differed already earlier in life in their (tail biting) behaviour. Chapters 2 and 6 showed that tail biting behaviour was largely inconsistent over different life phases, at least post-weaning. This may also be the reason that it was impossible to predict tail biting behaviour by other behaviours displayed by individual pigs in the resident pen (Chapter 2). Thus, tail biting in individual pigs was not predicted by the time they spent on general behaviours, activity or other types of biting behaviour (Chapter 2). However, in Chapter 6, high-tail biters identified during the rearing phase and kept in barren pens displayed also higher levels of tail biting and ear biting behaviour in the weaner phase (Chapter 6), which suggests that only these extreme tail biters have a long-term tendency to bite in pen mates. According to Zonderland et al. (2011a) almost all barren housed pigs within a pen (> 80 %) started tail biting shortly prior to the presence of significantly damaged tails, making the prediction of tail biting at the individual level based on behaviours displayed earlier in life difficult. If such a high proportion of pigs within a pen has started tail biting it is likely that many of them were attracted to wounded tails (Fraser, 1987a), were socially facilitated to perform tail biting (Schröder-Petersen and Simonsen, 2001), or that the problem - e.g. high temperatures (Smulders et al., 2008) - may have held for the whole pen. Also in our study, although no such high numbers of tail biters were identified within one pen in the same phase (Chapter 2), tail biting could not be predicted with individual behaviours displayed earlier in life. To the best of our knowledge, no other studies focussed on individual behaviour in the resident pen that could predict tail biting behaviour in a different phase of life. Beattie et al. (2005) subjected piglets before weaning individually to a rope test when confined in the creep area and they found that ear biting behaviour post-weaning rather than tail biting was positively correlated with time spent in contact with the rope. Although tail biting could not be predicted on the individual level in this thesis, tail biting problems could be predicted by
behaviours observed in the resident pen, on group level (Chapter 2). General activity levels including pig-, pen-, and object-directed manipulative behaviours seemed higher in pens with higher levels of tail biting behaviour and tail damage (Chapter 2). Also others found that groups of pigs in problem pens were more restless shortly before presence of significant tail damage (Statham et al., 2009; Zonderland et al., 2011b) and that use of objects provided in the pen can predict or notice biting problems (Van de Perre et al., 2011). Furthermore, it has been found that pigs in tail biting pens visited the feeder shorter, seemed to display more foraging behaviours (not related to the feeder), and seemed more aggressive to pen mates well before the onset of a tail biting outbreak (i.e. at time of the presence of bloody tails) (Wallenbeck et al., 2010). Collectively, our results suggest that tail biting is difficult to predict at the individual level by observing behaviour in the resident pen. At pen level, however, increased general activity levels and pig-, pen-, and object-directed oral manipulative behaviours can predict later problems with tail biting and tail damage in a group.

**Behavioural associations during an episode of tail biting**

All tail biters described in Chapter 6 generally displayed more of all kinds of oral manipulative behaviours directed at pen mates during the episode in which they were identified as tail biters. Jute sack usage also tended to be highest in high-tail biters, suggesting that the pigs distributed their oral manipulations over pen mates and the jute sack. In Chapter 2 no such associations were found between individual behaviours and tail biting during the life stage in which tail biting was observed. This difference in results of Chapters 2 and 6 may be due to the different types of pigs used (fattening pigs versus breeding gilts, respectively), the methodological approach (behavioural scan sampling versus continuous behavioural recordings), different phases of life identified (i.e. shorter in Chapter 6 compared to Chapter 2), and the circumstances (experimental farm versus commercial farm). The results found in Chapter 6 are in line with previous research where tail biters spent more time interacting with a chain or rubber hoses (Zonderland et al., 2011b) and also performed more other manipulative behaviours directed at pen mates (Brunberg et al., 2011) during the phase in which tail biting was observed. Also when subjecting pigs to an individual rope test during the same period in which tail biting was observed, tail biting was positively correlated
with interacting with this rope (Beattie et al., 2005). Brunberg et al. (2011) found, furthermore, a difference between low performers (i.e. pigs that displayed some tail biting behaviour, comparable to our medium-tail biters in Chapter 6), and high performers (i.e. pigs that displayed tail biting the most, comparable to our high-tail biters in Chapter 6), with high performers being more specialized in manipulative behaviours directed at pen mates and low performers directing the behaviour to both pen mates and pen fixtures. This difference between low and high performers in manipulative behaviours displayed did not seem to be present in the pigs of Chapter 6 when comparing medium-tail biters and high-tail biters, but we included only jute sack interactions and not all pen directed interactions as was done by Brunberg et al. (2011). To sum up, tail biting may be accompanied by a (temporary) higher motivation to display all kinds of oral manipulations directed to pen mates and pen fixtures or objects.

**Tail biting and blood serotonin**

Biting behaviours directed at pen mates can be largely reduced through the provision of enrichment materials (Chapters 2, 5 and 6). Enrichment materials can be used to fulfil a pig’s behavioural need to explore and forage and possibly reduce frustration if present (Hughes and Duncan, 1988; Wood-Gush and Vestergaard, 1989). Exploration and foraging behaviours were found to be increased during times of a metabolic imbalance (Day et al., 1996; Fraser, 1987b). Possibly, therefore, the temporary higher levels of tail biting (Chapters 2 and 6) and other oral manipulative behaviours directed at pen mates and the jute sack (Chapter 6) may have stemmed from a temporary metabolic imbalance. Tail biting has been associated with mineral deficiency (Fraser, 1987b), but the metabolic demand for amino acids may also vary between (Kampman-Van de Hoek et al., 2013) and within pigs (Le Floc’h et al., 2004). For instance, levels of essential amino acids in blood were lower in pigs fed ad libitum, but with limited feeding places (as was the case in our experiments) compared to pigs that could eat simultaneously, but in meals (Palander et al., 2013). Additionally, immune system activation can affect amino acid metabolism by redirecting amino acids to immunological processes (Le Floc’h et al., 2004). This mechanism may also be relevant in the context of tail biting in pigs: Palander et al. (2013) found that pigs from tail biting pens had lower amino acid levels in their blood. The lowest
amino acid levels were observed in victim pigs, which was likely due to immune activation caused by being bitten (Palander et al., 2013).

In Chapter 4, blood serotonergic changes were found in tail biters. Tail biters seemed to have, temporary, lower blood platelet serotonin (5-HT) concentrations and they also tended to have higher 5-HT uptake velocities in blood platelets (Chapter 4). Serotonin has been associated with many biological processes, with 5-HT in the brain being involved in neurotransmission, and 5-HT in blood with vasoactivity, blood clotting (Mück-Šeler and Pivac, 2011), and gut functioning (Souza da Silva et al., 2014). Although peripheral and central 5-HT systems are considered separate systems (Chou et al., 1983; Yatham et al., 2000), in humans with mental disorders (e.g. obsessive compulsive disorder (OCD) or depression) and in other animals displaying aberrant behaviours (e.g. feather pecking in laying hens) changes in the 5-HT system in both brain (e.g. humans: Barton et al., 2008; Zitterl et al., 2008, and laying hens: Van Hierden et al., 2004) and blood (e.g. humans: Coppen et al., 1976; Delorme et al., 2005, and laying hens: Bolhuis et al., 2009; De Haas et al., 2013) were found. Also in pigs, relations between tail biting behaviour and 5-HT measures in the brain have been postulated (Valros et al., 2013). Tail biters in the study of Valros et al. (2013) had higher levels of the 5-HT metabolite 5-HIAA in the brain compared to other pigs, and in these tail biters, 5-HIAA tended to be positively correlated with blood levels of the essential amino acid and 5-HT precursor tryptophan (Azmitia, 2010) and with ratios of tryptophan levels to large neutral amino acids (LNAA) and to branched chain amino acids (BCAA) in the blood (i.e. amino acids that compete with tryptophan to cross the blood-brain-barrier; e.g. Van der Mast and Fekkes, 2000; Meeusen and Watson, 2007). Those ratios were also positively correlated with 5-HT levels in the brains of tail biters, suggesting changes in tryptophan uptake pattern (Valros et al., 2013). It has been hypothesized that an altered 5-HT system is part of an underlying trait which may predispose humans and animals to develop mental problems or aberrant behaviours, respectively (see review D’Souza and Craig, 2010). Nevertheless, blood 5-HT measures of tail biting pigs seemed lowered temporarily only, and mostly during phases of life during which they displayed tail biting behaviour (Chapter 4). Also in humans, whole blood 5-HT concentrations may be temporarily low, for instance during an episode of depression, but seem normal after recovery of the depression, either when medically treated or not (Coppen et al., 1976). Possible explanations for temporary changes in blood 5-HT measures may be the availability of the essential amino
acid tryptophan (Sève et al., 1991) in the diet to synthesize 5-HT (Azmitia, 2010) and physiological processes that use tryptophan and 5-HT (e.g. Guzik et al., 2006; Shen et al., 2012). Lower blood platelet 5-HT levels were for instance found in pigs fed a diet with slowly fermentable fibres, which suggested that 5-HT synthesis and/or release in the intestine was affected by this diet (Souza da Silva et al., 2014). Excesses of released 5-HT by enterochromaffin cells located in the gut (see review of Sanger, 2008) are transported to blood platelets for storage (Keszthelyi et al., 2009). Part of the dietary tryptophan is transported to the brain, as tryptophan can cross the blood-brain-barrier, and is synthesized into 5-HT for neurotransmission (e.g. Dingerkus et al., 2012; Mück-Šeler and Pivac, 2011). Tryptophan is necessary for growth in animals (Segall and Timiras, 1976), including pigs (Ball and Bayley, 1984; Shen et al., 2012) and tryptophan demand is increased at times of stress (Joseph and Kennett, 1983; Maes, 1995) and immune activation (Le Floc’h et al., 2011) due to e.g. disease. It can be hypothesized that with increased demand for tryptophan (and 5-HT), as a compensation animals increase exploration and foraging behaviours. Pigs fed a diet deficient in tryptophan indeed displayed increased levels of exploration during an open field test compared to pigs fed a diet with adequate levels of tryptophan (but not compared to pigs fed a diet with excessive tryptophan levels) (Meunier-Salaün et al., 1991). Consequently, an insufficient tryptophan availability may also affect oral manipulations in pigs. Indeed, it has been found that higher levels of tryptophan in the diet can decrease tail and ear biting behaviour (Martínez-Trejo et al., 2009). It can be speculated that pigs with a temporarily lowered 5-HT storage have an increased motivation to explore and forage due to a heightened metabolic demand for tryptophan, and consequently start tail biting. Also shortage in other nutrients may potentially result in an increased motivation to explore and forage, and form a risk for tail biting behaviour to emerge. Thus, pigs showing tail biting behaviour seem to have temporary lower blood 5-HT levels than non-tail biters, and further experimental studies should reveal whether this reflects an altered 5-HT or tryptophan metabolism in these animals.
To produce or not to produce?

It can be postulated that tail biting pigs have a heightened metabolic demand which seems to be supported by phenotypic production measures of the gilts studied in Chapter 6. In the pens provided with jute sacks, high-tail biters identified during the weaner phase were heaviest and seemed to grow fastest, whereas the birth weights of non-tail biters, medium-tail biters and high-tail biters were comparable. Furthermore, in the pens with jute sacks, tail biters were more likely to originate from large litters. These findings seem in line with a higher direct genetic (DGE) potential for growth, a higher litter birth weight and larger litter size in pigs that displayed ear biting and/or tail biting behaviours (albeit confounding factors such as maternal effects may have played a role here) (Chapter 6). In pigs, genomic regions were identified associated with tail biting behaviour (Wilson et al., 2012) and tail biting seems heritable, at least in some breeds (Breuer et al., 2005). In a study where pigs were selected for (non) predisposition to perform harmful social behaviours such as tail biting, it was found that non-predisposed pigs had a lower phenotypic growth (O’Connell et al., 2005). Additionally, tail biting seems genetically unfavourably associated with selection for lean tissue growth (Breuer et al., 2005). Thus, fast growing pigs may be most at risk to develop tail biting, which is confirmed by the data from Chapter 6. Others have suggested, however, that tail biters are typically the smaller, slower growing pigs (see review Schrøder-Petersen and Simonsen, 2001). Both small pigs and pigs with a fast growth may, however, have a high metabolic demand as compared to the average pigs. In smaller pigs this demand may stem from e.g. a disease or a low feed intake caused by a difficulty in obtaining a feeding place (see review Schrøder-Petersen and Simonsen, 2001), and in larger pigs this higher demand may stem from a high protein deposition in the body due to growth (e.g. Le Floc’h et al., 2011). As pointed out previously, tryptophan is necessary for growth, and indeed an association between tryptophan availability and growth was found in pigs (Ball and Bayley, 1984; De Ridder et al., 2012; Shen et al., 2012).

As explained above, biting behaviours tended to be related to the genetic production potential of pigs (Chapter 6), i.e. direct genetic effects for production traits were associated with tail biting. Additionally, a link between indirect genetic effects for growth and oral manipulative behaviours and tail damage was found in Chapter 5. An indirect genetic effect is a heritable effect of one individual on the trait values of another individual (Griffing, 1967; Moore
et al., 1997). Pigs seem to have a heritable influence on their group members’ growth (Bergsma et al., 2008). In Chapter 5 it was found that pigs selected for a relatively negative indirect genetic effect on the growth of their pen mates displayed more ear biting behaviour and more biting on enrichment materials, they had a higher ‘consumption’ of jute sacks (i.e. chewing and tearing the sack off), and caused more tail damage compared to pigs that had a relatively positive indirect genetic effect for growth. Possibly, pigs with a negative IGE may cause a reduced growth in pen mates due to their biting behaviours towards pen mates. Oral manipulations and biting of pen mates may result in a reduced growth in the victim pig (Camerlink et al., 2012; Smulders et al., 2006; Wallgren and Lindahl, 1996). This reduced growth may, for example, stem from an increased stress metabolism in victims (Smulders et al., 2006) as being bitten causes pain (Fritchen and Hogg, 1983), and a heightened inflammatory response due to tail wounds (Heinonen et al., 2010). The associations between (tail) biting and direct genetic effects for production traits (Chapter 6) and indirect genetic effects for growth (Chapter 5) seem to support previous notions that the tendency to display damaging behaviours such as tail biting in pigs is heritable and can be reduced by selection (see review Turner, 2011). Although tail biting behaviour was observed to be state-like instead of trait-like in most tail biters - which was also supported by the temporary changes in blood serotonin -, underlying production traits (e.g. growth potential) that possibly increase a pig’s metabolic demand may also play a role in the development of damaging behaviours.

A tail biters personality?

Although tail biting behaviour is temporarily seen in most individual pigs (Chapters 2 and 6), some of our results (Chapters 5 and 6) and those of others (Breuer et al., 2005; O’Connell et al., 2005) also indicate that tail biting may be mediated by genetic factors, including personality traits (Koolhaas et al., 2007; Korte et al., 2009). It has been hypothesized that coping strategies of pigs may be associated with tail biting (Koolhaas et al., 2007; Korte et al., 2009). According to this hypothesis, pigs acting more in a bold way when challenged, i.e. with an active coping strategy, are more likely to display tail biting behaviour (Koolhaas et al., 2007; Korte et al., 2009). In pigs, a pre-weaning back test may provide an indication of their coping strategy (Hessing et al., 1994; Bolhuis et al., 2000) and
behavioural responses displayed during this test were heritable (estimates of 0.37-0.56; Iversen et al., unpublished results; Velie et al., 2009). Pigs are generally classified in high resisters (HR) (i.e. active copers) and low resisters (LR) (i.e. reactive copers), where the first category vocalizes and struggles in order to escape and the latter one remains quietly on its back (Bolhuis et al., 2000; Melotti et al., 2011). According to the results of Chapter 4, the factor back test activity (i.e. behaviour observed in the back test summarized in one factor by means of a PCA) was not associated with tail biting behaviour displayed in different phases of life. However, an overall (i.e. based on the whole post-weaning period) tendency was observed for tail biters behaving more actively during the back test which would be in line with the hypothesis postulated by Koolhaas et al. (2007) and Korte et al. (2009). Therefore, it was also tested if a qualitative classification of pigs in HR or LR pigs would support this hypothesis, but results did not reveal consistent relationships between coping strategy and tail biting or tail damage (both continuously and as classifications into (non) tail biters and (non) victims) (Figure 1). Collectively, the results point out that coping strategies of pigs as measured with a back test were not related to tail biting, or to being a victim of tail biting (Chapter 4). In contrast, behaviour displayed in a novel object test was related to tail biting (Chapter 4), at least in barren housed pigs, as tail biters spent less time near an introduced novel object and more time near the walls of the arena and also were more often seen standing alert compared to non-tail biters. These results may suggest that barren housed tail biters are more fearful when individually subjected to a novel situation outside their resident pen (Chapter 4). In line with this, the pigs with a relatively negative IGE on the growth of their pen mates not only showed more oral manipulative behaviours and inflicted more tail damage, but they also were suggested to be more fearful during novelty tests (Reimert et al., 2014) and to show physiologically more signs of stress (i.e. they had generally higher leukocyte, lymphocyte and haptoglobin concentrations) (Reimert et al., under revision). Also in humans and other animals, higher levels of fearfulness and anxiety have been associated with mental disorders (e.g. OCD; Leplow et al., 2002) and damaging behaviours (e.g. feather pecking in laying hens; Rodenburg et al., 2010). Moreover, as pointed out previously, these mental disorders and damaging behaviours were also associated with a changed blood 5-HT system (De Haas et al., 2013; Delorme et al., 2005). Similarly, in Chapter 4 a relationship in pigs between tail biting and 5-HT was found. These findings, therefore, may suggest that, similar to feather pecking in laying hens (Bolhuis et al., 2009; Rodenburg et
Figure 1. Percentage of low resisters (LR) or high resisters (HR) as determined by a back test at two weeks of age, classified as tail biters or victims of tail biting. Three consecutive phases post-weaning were considered, here indicated with 1-3. TB = Tail biter, V = Victim. A: Barren housed tail biters. B: Enriched housed tail biters. C: Barren housed victims. D: Enriched housed victims. * P < 0.05.

al., 2010), tail biting in pigs, 5-HT and fearfulness are interrelated (Chapter 4). In Chapter 3, both blood and brain (i.e. right hippocampal area) 5-HT were related to exploration of a novel arena. Together with other relationships observed between brain 5-HT measures and behavioural responses to novelty - i.e. time spent on locomotion, standing alert and a tendency for time spent nosing the novel object - the results suggested a relationship between peripheral and central 5-HT and the level of fearfulness in the pigs (Chapter 3). Pigs that seemed more fearful during the novelty test (at 11 weeks of age) had lower (basal) levels of 5-HT in the blood (at 13 weeks of age), and a lower (basal) serotonin turnover and higher 5-HT concentration in the right hippocampus (at 19 weeks of age). Furthermore, 5-HT measures in blood were related to 5-HT measures in the hippocampal area in the brain (i.e. a brain structure involved in, amongst others, mediating the stress response (Siegford et al., 2008). Fearfulness has been suggested to be a trait in animals (Boissy, 1995), and also in pigs fearfulness seemed to be an underlying (personality) trait as behaviour and 5-HT measures were correlated over time (Chapter 3). Fear measured during novelty tests may also reflect a certain state independent of an animal's personality trait ‘fearfulness’, as the degree of fear may vary during different conditions. An animal's personality may consist of several dimensions (e.g. coping strategy and fearfulness) which may interact
with each other (Koolhaas et al., 2007; Van Reenen et al., 2005). In the present thesis, no interaction effects of the putative personality dimensions fearfulness and coping strategy on the chance to be a (non) tail biter or (non) victim in phase 1-3 post-weaning were found (unpublished data). Taken together, the present findings suggest that fearfulness, but not coping strategy, may be related to tail biting in pigs, at least in barren housing conditions. It remains, however, debatable whether tail biting in itself or fear expressed by tail biting pigs can be considered a stable (personality) trait or a temporary state, or a combination of both.

The perpetual effect of enrichment materials

Enrichment materials such as straw-bedding (Chapters 2 and 5) and jute sacks (Chapter 6) largely reduced biting behaviours and tail damage. In literature, the effect of enrichment on the level of oral manipulative behaviours directed at pen mates and pen fixtures has been largely acknowledged (e.g. Fraser et al., 1991; Van de Weerd et al., 2003; Zonderland et al., 2008). Over 60% of pigs kept on straw-bedding (Chapter 2) never developed a tail wound post-weaning (4-23 weeks of age) whereas this was only 17% in barren housed pigs, which would have been even less if these barren housed pigs were not provided with curative enrichment (2 handfuls of wood shavings and a jute sack) to prevent escalation of ongoing tail biting behaviour. Also a jute sack provided in the pen from birth onward (Chapter 6) was highly efficient and resulted in up to 50% less biting behaviours displayed during the weaner and rearing phase, and a five-fold reduction of tail wounds in 13-week old gilts. Furthermore, the jute sacks remained interesting for the gilts as 13-week old gilts still interacted as much with the sack as earlier in life (Chapter 6). Others did not find lasting (they tested for five days) interest by pigs in jute sacks (Van de Weerd et al., 2003), but this may have been caused by the pigs’ previous experience with straw-bedding in this study.

Although the results of Chapters 2, 5 and 6 again stress the importance of enrichment materials for pigs to be able to explore and forage, the results also clearly show that providing enrichment is not always sufficient in preventing biting behaviours and consequently tail damage. This is in line with previous findings in outdoor kept pigs in which also fairly large numbers of pigs with tail damage were observed (14-20%) (Walker and Bilkei, 2006). Walker and Bilkei
(2006) noted that the group of outdoor pigs suffered from respiratory disease and possibly limited feeding places were available as it was winter, but also genetics may have played a role here. Also in our study (Chapters 2, 4 and 5) some health issues were recorded, with one group of pigs facing a respiratory disease and in another group some pigs were lost due to meningitis. Although not all pigs were clinically ill, subclinical diseases may have been present. Most victims in enriched housing were found during the final phase post-weaning (i.e. 16-23 weeks of age), and in that period also associations between tail biting and blood 5-HT measures in these enriched housed pigs were found (Chapter 4), possibly reflected changed metabolic demands (see previously). In the experiments, relationships between behaviour, physiology and tail biting seemed similar in pigs kept in barren and enriched housing (i.e. altered blood 5-HT system, the level of oral manipulations/exploration at pen level, and indirect genetic effects) (Chapters 2, 4, 5, and 6). However, some relations between tail biting and other pig characteristics were not the same in pigs kept in different housing systems (Chapters 2, 4 and 6). For example, the presumed relationship between tail biting and fearfulness was only found in pigs kept in barren pens and not in enriched pens (Chapter 4). Similarly, the relationship between phenotypic weight/growth and tail biting was only demonstrated in gilts kept in pens with jute sacks and not in gilts kept in pens without jute sacks (Chapter 6). Although these differences between housing conditions in relationships between pig characteristics of differently housed pigs may partly stem from the low numbers of tail biters observed in enriched housing, they may also be related to different underlying motivations to tail bite (as pointed out by Taylor et al., 2010) in these different housing systems. Most tail biters in barren housing will possibly display biting behaviours directed to pen mates as they are not able to sufficiently perform other exploration behaviours (Hughes and Duncan, 1988; Wood-Gush and Vestergaard, 1989). In enriched housing, pigs have ample opportunity to perform exploratory behaviours providing that fresh substrates are given daily (see e.g. Bracke, 2007), but other problems potentially leading to tail biting may still remain, such as a nutritional deficiency (Meunier-Salaün et al., 1991; Martínez-Trejo et al., 2009), stress (Munsterhjelm et al., 2013), suboptimal climate (Sällvik and Walberg, 1984; Scheepens et al., 1991), or poor health (Taylor et al., 2012). Since relations between behavioural (Bell and Sih, 2007; Pearish et al., 2013) and perhaps also physiological characteristics of an individual can be affected by the environment, it may be important to assess relationships between pig characteristics separately for different housing systems to be able
to find the correct underlying problem of tail biting behaviour in these systems. Enrichment largely meets the need to explore and forage in pigs, but apparently in both barren and enriched housed systems pigs are present with underlying problems other than those related to the absence or presence of enrichment material *per se* that may lead to an enhanced motivation to explore and forage.

**Proposed conceptual framework of biting behaviours**

The findings in the current study strongly support earlier proposals of (tail) biting behaviours being related to the motivation to explore and forage (e.g. EFSA, 2007; Wood-Gush and Vestergaard, 1989; Zonderland, 2010). The results from the previous chapters also show that biting is not just a matter of housing conditions, a pig's experiences or genetics but rather a combination of factors that can be different for individual pigs. A similar suggestion by Fuller (1962, cited in Van Putten, 1969) was that factors involved in discomfort can be cumulative, but the outcome depends on the threshold of an individual which in turn relates to an animal's condition and its personal level of nervousness. Previously, Zonderland (2010) developed an aetiology (i.e. causation) model of a tail biting outbreak to explain that the motivation to explore can lead to tail biting behaviour, and to show that behavioural changes or ‘arousal’ following tail biting possibly leads to more tail biting (see Figure 2). However, the motivation to explore is closely related to the motivation to forage (e.g. Day *et al.*, 1996) and both can be affected by numerous factors as outlined in the previous sections (e.g. enrichment or changed 5-HT system). Edwards (2006) suggested that findings in both literature and practice point in the direction of malfunctioning of the pig's protein metabolism playing a role in tail biting. Our results (i.e. the blood 5-HT system and production measures) seem to support this notion - although protein metabolism as such was not studied and other metabolic problems may play a role too - and therefore an adjusted conceptual framework is proposed in Figure 3 which considers the possible background of the motivation to explore and forage. The main purpose of this framework is to show that underlying traits and states of pigs both can modulate the motivation to explore and forage and both can lead to all kinds of biting behaviours. This implies that tail biting pigs not necessarily continue the display of this damaging behaviour throughout life and, additionally, pigs that may be predisposed to develop tail biting behaviour
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(e.g. by a higher level of fearfulness) can be prevented from doing so when the combination of environmental conditions such as feed quality, housing, and management are optimal. The conceptual framework shows, furthermore, that tail biting is not the only oral manipulative behaviour that should be prevented, also other biting behaviours likely stem from a heightened motivation to explore and forage and can lead to body damage in the victim pigs. Taken together, tail biting pigs seem to bite due to a heightened motivation to explore and forage. The motivation to explore and forage may be increased due to numerous factors which are likely a combination of both temporary states and stable traits.

**Figure 2.** Aetiology model of a tail biting outbreak. The exploration motivation can lead to tail biting behaviour in the tail biter, and tail damage in the victim of tail biting. Group arousal may affect the level of exploration motivation. An increased chance is indicated with ‘+’ and a decreased chance with ‘-’. Key points where preventive measures can be undertaken were indicated with ‘a’, ‘b’ and ‘c’ (Zonderland, 2010).
Figure 3. Conceptual framework of the motivation to explore and forage affected by different (combinations of) states and traits in pigs. Biting behaviours may follow when an increased motivation to explore and forage has developed and if feeding and/or exploring enrichment proves to be insufficient. After biting has developed, other pigs may start inspective biting behaviours. Negative feedback loops are indicated with ‘−’. Before and at times when biting behaviours occur, groups of pigs in pens may become more active. 5-HT = serotonin.
Recommendations for future practice and research

Tail biting behaviour already occurs in the farrowing stable and can lead to small tail wounds at the time of weaning (Chapter 2). Therefore, it is necessary to start with the provision of enrichment materials to be able to fulfil the need to explore and forage (Hughes and Duncan, 1988; Wood-Gush and Vestergaard, 1989) already early in life. Straw-bedding (Chapter 2) and also jute sacks (Chapter 6) can largely reduce biting behaviours and, consequently, tail damage in pigs. Jute sacks may, however, be less effective than substrates such as straw, as pigs with tail wounds were much more prevalent in the barren pens with jute sacks compared to the straw-bedded pens in Chapter 2. When providing jute sacks from birth onward, breeding gilts had a five-fold reduction of tail damage compared to gilts kept in commercially barren pens with a chain and plastic toy only. Early experience with enrichment can affect behaviours of pigs at later age (Melotti et al., 2011; Munsterhjelm et al., 2009; Telkänranta et al., 2014), and possibly the presence of jute sacks may be most effective when provided from birth onward. Straw-bedding, and to a large extent also jute sacks, meet the requirements of proper enrichment materials for pigs (i.e. complex, changeable, destructible, manipulable and edible; Studnitz et al., 2007; Van de Weerd et al., 2005). Nonetheless, tail damage is still observed in enriched pens, either with straw-bedding or with jute sacks. When providing jute sacks it may be possible that not all pigs can sufficiently display exploration and foraging behaviours at the same time (i.e. pigs prefer to synchronize their behaviours, including explorative behaviours; Docking et al., 2008), and, additionally, pigs may lose their interest in an object on the long-run as it is no longer novel (Gifford et al., 2007). In Chapter 6, pigs were studied until 13 weeks of age, and, consequently, it is unknown if jute sacks maintain their efficiency in preventing tail damage at later age. Therefore, extra measures to prevent biting behaviours may be necessary when using point source enrichment such as jute sacks. In practice, it would be advisable to provide continuous access to chewing materials (such as a jute sack) and a little extra enrichment (e.g. handful of straw or feed scattered on the floor) twice daily. Zonderland et al. (2008) showed for instance that adding a minimum amount of long straw twice daily (20 g per pig per day) can be highly efficient in preventing tail biting behaviour if no other measures are taken. Combining several types of enrichment may be effective to fulfil the basic behavioural need of pigs to explore and forage.
As noted previously, also when using straw-bedding, still some pigs develop (tail) biting behaviours due to e.g. health problems or an inadequate climate (Taylor et al., 2012). It is, therefore, important to detect emerging behavioural problems early. This can be achieved by regularly observing a group of pigs in a pen as a whole as pigs in (future) problem pens are generally more active and display more pig and pen or object directed behaviours (Chapter 2). In some but not all cases tail biters are those pigs displaying more of these pig or pen/object directed behaviours (Chapter 6). It has to be recalled that most tail biters are inconsistent over time in their biting behaviours (Chapters 2 and 6) and observing pigs seems most effective if executed regularly and in every stage of their lives. Additionally, a hanging tail or a tail tucked between the legs can indicate ongoing problems with tail biting (Chapter 2), and can predict tail damage in the near future (2-3 days) if not yet present (Zonderland et al., 2009).

Previously, it was observed that much more pigs (28 %) lacked the curl in the tail during a tail bite outbreak (i.e. at time of the presence of bloody tails) compared to the preceding period (5 %) (Wallenbeck et al., 2010). To sum up, firstly, it is important to provide sufficient enrichment materials before biting behaviours directed to pen mates have developed, and secondly, early detection of emerging problems with biting behaviours (by observing the pigs regularly) is important to be able to respond timely and adequately by optimizing for instance housing or climatic conditions, management strategies or the feed provided, or by solving health problems.

The results of the current study suggested that although 5-HT measures can fluctuate in pigs (state-like), a fearful personality (trait-like) seems to predispose animals to have or develop changes in their 5-HT system and to develop tail biting (Chapters 3 and 4). This suggests that tail biting pigs may have a physiological susceptibility for the tendency to start biting which possibly can be solved by optimizing nutrition. This hypothesis seemed to be supported by phenotypic and genotypic characteristics of the pigs, where pigs displaying more biting behaviours seemed to produce more, i.e. mainly in terms of growth. If biting behaviours are indeed related to a physiological problem it may be possible that optimizing feed or providing feed supplements may largely solve the problem. Previous research already suggested the potential of feed in affecting behaviours: increasing the level of the 5-HT precursor tryptophan in the pig’s diet may reduce tail biting behaviour (Martínez-Trejo et al., 2009). Moreover, other measures may affect peripheral 5-HT levels in pigs as well. Research is necessary to assess the
effects of such interventions on both biting behaviours and 5-HT in pigs as it is currently unknown if 5-HT has a causal role in the development of tail biting behaviour or merely reflects another underlying problem that is not solved by increasing blood 5-HT levels as such.

**Main conclusions**

The current thesis shows that tail biting behaviour starts early in life, is inconsistent over time and difficult to predict on the basis of individual behavioural data. Nevertheless, tail biting may be predicted by: 1. higher levels of tail biting behaviour observed in the litter pre-weaning, 2. higher levels of activity in a group of pigs post-weaning, and 3. higher levels of pig-, pen-, and object-directed oral manipulative behaviours in a group of pigs post-weaning. Furthermore, tail biting may be associated with a temporarily higher motivation to orally manipulate pen mates and objects in the pen, and a temporary change of blood serotonin. This suggests that in many pigs tail biting is possibly related to a temporary underlying (physiological) problem and, consequently, seems to have a state-like origin although trait characteristics also seem to play a role, with a more fearful personality and higher production level being predisposing traits for developing tail biting. Additionally, pigs with a negative indirect genetic effect on the growth of pen mates were generally more orally manipulative towards pen mates and pen fixtures or objects (i.e. mainly jute sacks) and caused a higher level of tail damage. Enrichment, both straw-bedding and jute sacks, largely reduced, but not fully eliminated, tail biting and tail damage. It thus seems highly important to provide pigs with sufficient enrichment materials to keep problems with behaviours like tail and other biting directed to pen mates as low as possible. To conclude, tail biting, and other types of biting behaviours, seem to be affected by both state- and trait-like characteristics, making it a complex problem to overcome. Consequently, the tale of tail biting, and also of other biting behaviours, would benefit from a better understanding of the physiological processes involved in (tail) biting behaviour, and from combining future scientific and societal programmes that aim to optimize the genetic makeup, management, feeding, and housing conditions of pigs.
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Chapter 7


Summary
Summary

Tail biting behaviour is a long-existing and common problem in pig husbandry and is known for its multifactorial background. Risk factors seem plenty and vary from a suboptimal climate to the lack of enrichment materials. To diminish this problem, about 50 years ago, the practice of tail docking was implemented. Although tail docking largely reduces tail damage and accompanying economic losses, it cannot fully prevent tail biting behaviour and will not solve the underlying problem that causes a pig to start tail biting. Additionally, society becomes more and more aware of mutilations like tail docking and has started to express its dissatisfaction. Therefore, the need to understand, prevent, and reduce damaging behaviours such as tail biting is currently on top of the agendas of various stakeholders that are directly or indirectly associated with the pig industry (e.g. ministries, society, and farmers). Consequently, the main aim of this thesis was to identify biological characteristics of pigs that relate to oral manipulative behaviours such as tail biting.

The first experiment (Chapter 2) aimed to assess the consistency of tail biting and tail damage and to find behavioural predictors of this unwanted behaviour. A total of 480 pigs were followed from birth to slaughter (23 weeks). Piglets were born in conventional barren farrowing pens with farrowing crates and were weaned at 4 weeks of age. Thereafter, they were kept either in a standard pen or a pen with straw-bedding (6 pigs per pen). Each pen (barren and enriched) received a jute sack from 8 weeks onwards. Behaviour was observed both pre-weaning and post-weaning; pre-weaning behaviour was averaged per litter, whereas post-weaning behaviour was considered both per individual and averaged per pen. Additionally, tails were checked for damage caused by biting behaviours at time of weaning and thereafter once a week. Both behaviour and tail damage were averaged per pig over three phases post-weaning, roughly according to the commercial weaner, grower and finisher phase. Each tail received a score ranging from 1 (i.e. no tail damage) to 4 (i.e. (small) tail wound). Tail biting behaviour was evidently present prior to weaning and 9 % of the piglets already had a (small) tail wound at time of weaning. Although tail biting pigs were more likely to originate from litters that displayed tail biting already relatively much, tail biting behaviour post-weaning was highly inconsistent. Post-weaning tail damage, however, was consistent over time. Tail biting and tail damage were best predicted by behaviours at pen level and less by behaviours
at the individual level. Pens with a higher activity, and more pig and pen-directed manipulative behaviours were likely to have or develop problems with tail biting. Straw-bedding largely reduced tail biting behaviour and tail damage, although it could not fully eliminate it.

Individual behavioural characteristics as measured in Chapter 2 did not reliably predict tail biting behaviour. Nonetheless, it was hypothesized that individual (neuro)physiological characteristics are associated with damaging behaviours such as tail biting. Serotonergic (5-HT) measures in both blood and brain of humans and other animals have been associated with mental disorders (e.g. obsessive compulsivity and depression) and aberrant behaviours (e.g. feather pecking in laying hens). Studying tail biting behaviour requires a high number of animals, whereas brain research is a rather delicate procedure. Therefore, in Chapter 3 we aimed to assess possible correlations between blood and brain 5-HT measures of pigs. Additionally, it was tested whether both blood and brain 5-HT measures were related to behavioural responses of pigs to novelty. In total 31 pigs were subjected to a combined novel environment and novel object test and their behaviour was recorded continuously. Whole blood 5-HT, platelet 5-HT level, and platelet 5-HT uptake velocity were determined at 13 weeks of age. Levels of 5-HT, one of its metabolites and 5-HT turnover were determined at 19 weeks of age in the frontal cortex, hypothalamus and hippocampus. The behaviour of the pigs clearly differed between the novel environment and novel object test and suggested that the novel object test was more fear provoking. Both brain and blood 5-HT measures were evidently related to the behaviour in the novel object test, but not in the novel environment test. Interestingly, blood and hippocampal (i.e. a brain structure involved in, amongst others, mediating the stress response) 5-HT measures were interrelated and both related to explorative behaviours displayed during the novel object test. Altogether, findings suggested that pigs reacting more fearful may have lower 5-HT storage in the blood, and, additionally, a lower 5-HT turnover and higher 5-HT concentration in the (right) hippocampus.

In Chapter 4, it was investigated whether tail biting in pigs can be associated with blood 5-HT measures and with behavioural and physiological responses to novelty. It has been hypothesized that tail biters may act more in a bold way during challenging situations. The same 480 pigs as described in Chapter 2 were used and relations between pig characteristics were assessed by housing system (either barren or enriched). Type of housing post-weaning largely affected the prevalence of tail biting behaviour and possibly underlying
motivations to display the behaviour may also be different under different circumstances. Pre-weaning, piglets were subjected to a back test (~14 days of age) to obtain an indication of a pig’s preferred coping strategy. One week later, piglets were subjected to a small novel environment test. Post-weaning, the pigs were subjected to a similar combined novel environment and novel object test as described in Chapter 3, but only the novel object test was considered for analysis as the novel environment test may be less fear provoking, at least at this age. Additionally, salivary cortisol was measured around the post-weaning novelty test to assess adrenal responses to the test. Blood 5-HT measures were determined at week 8, 9 and 22. Within the different post-weaning phases studied, tail biting pigs often had a lower blood 5-HT storage and tended to have a higher 5-HT uptake velocity in blood platelets than non-tail biters. Furthermore, barren housed pigs that displayed tail biting behaviour appeared to have a higher level of fearfulness, but no indications for a relationship between tail biting tendencies with a pig’s coping strategy nor its adrenal response was found. Blood 5-HT could not predict future tail biting, but nonetheless tail biting and a low blood 5-HT seemed associated within the phase of life during which the damaging behaviour occurred. These results suggest that not only tail biting behaviour is inconsistent and consequently can occur temporarily in pigs, but possibly also blood 5-HT system functioning may be temporarily changed in these pigs.

Pigs may affect each other’s production level through behavioural interactions. The effect of one pig on another pig may be heritable and is then called an indirect genetic effect. In Chapter 5, it was aimed to assess whether selection for indirect genetic effects on growth of pen mates may alter the behaviour of pigs. The same pigs as described in Chapters 2 and 4 were used here as well. A 2 × 2 factorial design was applied, pigs were housed either barren or straw-enriched and pens consisted either of pigs with a relatively positive indirect genetic effect on the growth of their pen mates, or a relatively negative effect. Results showed that pigs with an estimated relatively negative effect on the growth of their pen mates displayed more biting behaviours, caused more tail damage and used (‘consumed’) more jute sacks post-weaning. No interactions between housing and indirect genetic effects were found, suggesting that the beneficial effect of selection on a high social genetic effect for growth on tail biting and tail damage was additive to that of straw-bedding. Additionally, in previous research, pigs with a negative indirect genetic effect on the growth of their pen mates seemed also more fearful during novelty tests and seemed from a physiological (i.e. immunological) perspective more affected by stress.
Consequently, pigs may potentially be selected for displaying less damaging behaviours directed at pen mates.

Chapters 2 and 5 proved that straw-bedding was highly efficient in reducing tail biting and tail damage. Nonetheless, straw-bedding is in many systems difficult to apply. Therefore, an alternative and still efficient enrichment material is required. In Chapter 6, the effect of jute sacks on damaging behaviours was studied as jute sacks seemed to be a potentially good enrichment material for pigs and the sacks seem easy to handle and not too expensive for farmers. Jute sacks can be bitten, nosed, transformed, broken and, as it is plant material, pieces torn from the sack can likely be safely swallowed. Additionally, relationships between tail biting and phenotypic and genetic production characteristics were explored. In total 1033 piglets from 72 litters on a commercial breeding farm were either provided with a jute sack or not from birth onward. Post-weaning (at 4 weeks), gilts and boars were separated and only breeding gilts were further followed as the gilts had half-long tails and boars had shortly docked tails. Jute sacks evidently reduced (up to 50%) all biting behaviours and a five-fold reduction in tail wounds was observed in 13-week old gilts. Additionally, phenotypic and genetic (albeit weakly) associations pointed in the direction of higher production being related to more (tail) biting behaviours. Consequently, combining sufficient enrichment provision with genetic selection may reduce injurious biting behaviours in pigs.

The problem of biting behaviours in pigs directed at pen mates is not as straightforward as previously expected as both the behaviour itself and also changed neurophysiological characteristics may be temporary present. This is in line with the notion that tail biting has a multifactorial background. A heightened motivation to explore and forage presumably underlies (tail) biting behaviours in pigs and likely originates from a combination of multiple states and traits. Factors possibly associated with these states and traits are: availability of enrichment materials, functioning of the 5-HT system, fearfulness, and phenotypic and genetic production characteristics such as growth which may coincide with metabolic demands.

To conclude, biting behaviours seem to originate from a heightened motivation to explore and forage and this heightened motivation may stem from a variety of states and traits in the pig. Consequently, the tale of tail biting, and also of other biting behaviours, would benefit from a better understanding of the physiological processes involved in (tail) biting behaviour, and from combining future scientific and societal programmes that aim to optimize the genetic makeup, management, feeding, and housing conditions of pigs.
Samenvatting
Staartbijten is een veel voorkomend probleem in de varkenshouderij en bestaat al geruime tijd. Het probleem van bijten door het ene varken in de staart van een ander varken heeft een multifactoriële achtergrond. Er zijn vele risicofactoren geïdentificeerd die mogelijk kunnen leiden tot staartbijten, variërend van een suboptimaal klimaat in de stal tot het afwezig zijn van wroet- en kauwmateriaal (verrijkingsmateriaal) in de hokken. Ongeveer 50 jaar geleden is er begonnen met het couperen van de varkensstaarten om op die wijze de staartbijtproblematiek te verminderen. Het couperen van de staarten kan de schade aan slachtoffers en de bijkomende economische verliezen beperken. Het couperen pakt echter het onderliggende probleem niet aan en geeft geen garantie dat staartbijten niet meer voorkomt. Bovendien wordt de maatschappij zich steeds meer bewust van de ingrepen die gebruikt worden in de intensieve veehouderij, waaronder het staartcouperen bij biggen, en groeit de maatschappelijke weerstand tegen deze ingrepen. Het is daarom des te belangrijker om staartbijten en ander beschadigend gedrag geuit door varkens beter te begrijpen, te voorkomen en te verminderen. Verschillende stakeholders die direct of indirect betrokken zijn bij de varkenshouderij (zoals ministeries, maatschappelijke organisaties, en veehouders) hebben dit vraagstuk momenteel hoog op de agenda staan. Het onderzoek in dit proefschrift had als doel om biologische karakteristieken van varkens te identificeren die gerelateerd zijn aan hun neiging om beschadigende orale manipulatieve gedragingen gericht op hun hokgenoten te vertonen, in het bijzonder staartbijten.

Het eerste experiment (Hoofdstuk 2) had als doel om de consistentie van het staartbijtgedrag en staartschade door de tijd heen te evalueren en gedragsvoorspellers van bijterij te identificeren. In totaal werden 480 varkens gevolgd van geboorte tot slacht (23 weken). Biggen werden geboren in conventionele onverrijkte kraamhokken met kraamboxen voor de zeugen. De biggen werden op een leeftijd van 4 weken gespeend. Na het spenen werd de helft van de biggen in standaard hokken gehuisvest en de andere helft in hokken met een strobed (6 biggen per hok). Vanaf een leeftijd van 8 weken kregen de biggen per hok (standaard en verrijkt) een jutezak ter beschikking. Gedrag werd zowel voor als na het spenen geobserveerd. Voor het spenen werd er per toom een gemiddelde genomen van elk type gedraging. Na het spenen werd het gedrag zowel op individueel niveau als per hok bekeken. Verder werden op het
Samenvatting

moment van spenen en daarna wekelijks de staarten gecontroleerd op schade veroorzaakt door bijterij. Van elke big werd voor gedrag en staartschade een gemiddelde bepaald tijdens elk van 3 opeenvolgende fasen die vergelijkbaar zijn met de praktijk: gespeende biggen (tot ± 25 kg), en de vroege (tot ± 70 kg) en late vleesvarkensfase. Staartscores varieerden van score 1 (geen staartschade) tot aan score 4 (een (kleine) wond). Staartbijtgedrag was duidelijk aanwezig voor het spenen en 9 % van de 480 biggen had al een (kleine) staartwond op moment van spenen. Een deel van de varkens die staartbeten na het spenen, kwam uit tomen waarin relatief veel staartbijten optrad. De varkens vertoonden dit gedrag echter niet consistent gedurende de hele periode na het spenen tot aan slacht. In elke fase stopten varkens met staartbijten en ontstonden ook nieuwe staartbijters. Staartschade was na het spenen wel consistent door de tijd heen. Zowel staartbijten als staartschade kon het beste worden voorspeld aan de hand van gedragingen geobserveerd op hokniveau en niet of minder goed op basis van individuele gedragingen. Meer problemen met staartbijterij ontstonden in die hokken waarin de varkens relatief actief waren en waarin meer orale manipulatieve gedragingen gericht op hokgenoten en de hokinrichting voorkwamen. Aanwezigheid van een strobed verminderde het staartbijtgedrag en staartschade aanzienlijk, maar volledige eliminatie van schadelijk gedrag werd niet bereikt.

Individuele gedragskarakteristieken zoals beschreven in Hoofdstuk 2 konden staartbijten niet voorspellen. Een andere hypothese was dat (neuro)fysiologische karakteristieken geassocieerd zouden kunnen zijn met beschadigend gedrag zoals staartbijten. Serotonerge (5-HT) kenmerken in zowel het bloed als de hersenen van mensen en andere dieren zijn geassocieerd met mentale aandoeningen (bv. obsessieve compulsiviteit en depressie) en afwijkende gedragingen (bv. verenpikken in leghennen). Het bestuderen van staartbijtgedrag vereist een groot aantal dieren, terwijl voor hersenonderzoek een delicate aanpak nodig is. Dit was de aanleiding voor het onderzoek zoals beschreven in Hoofdstuk 3. Hierin werden correlaties tussen 5-HT in het bloed en 5-HT in de hersenen van varkens beschreven. Daarnaast werd er onderzocht of 5-HT zowel in het bloed als de hersenen, geassocieerd kon worden met gedragingen van de varkens zoals geuit tijdens een gedragstest op een leeftijd van 11 weken. In die test werden de varkens (31 in totaal) individueel blootgesteld aan een onbekende omgeving (arena) en na 5 minuten ook aan een onbekend voorwerp. Het gedrag werd continu geobserveerd. Op een leeftijd van 13 weken
werd 5-HT bepaald in volbloed en per bloedplaatje. Daarnaast werd de opname snelheid van 5-HT in bloedplaatjes bepaald. Op een leeftijd van 19 weken werden in de hersenen 5-HT en 5-HIAA (een metabooliet van 5-HT) niveaus gemeten en werd de turnover ratio van 5-HT naar 5-HIAA bepaald. De hersendelen gebruikt voor deze metingen waren de frontale cortex, de hypothalamus en de hippocampus. Het gedrag van de varkens verschilde significant tussen de eerste en tweede helft van de gedragstest, en het aanbieden van een onbekend voorwerp leek angstiger voor varkens dan alleen het blootgesteld worden aan een nieuwe omgeving. Zowel 5-HT in het bloed als in de hersenen konden worden geassocieerd met het gedrag van de varkens tijdens de test met het onbekende voorwerp, maar niet met het gedrag tijdens het deel van de test waarin ze alleen blootgesteld waren aan de onbekende omgeving. Serotonin in het bloed en in de hippocampus (een hersendeel dat betrokken is bij o.a. het mediëren van de stress reactie) waren onderling gerelateerd en beiden konden worden gecorreleerd aan exploratief gedrag geuit tijdens de test met het onbekende voorwerp. Tezamen suggereerden de resultaten dat varkens die angstiger reageerden in die test een lagere 5-HT opslag in het bloed hadden en daarnaast een lagere 5-HT turnover ratio en een hoger 5-HT niveau in de (rechter) hippocampus.

In Hoofdstuk 4 werd onderzocht of staartbijten in varkens geassocieerd kon worden met 5-HT in het bloed en met gedrags- en fysiologische reacties op gedragstesten waarin onbekendheid voor de situatie een rol speelde. Voor dit experiment werden dezelfde varkens als beschreven in Hoofdstuk 2 gebruikt en relaties tussen karakteristieken van de varkens werden per huisvestingssysteem (verrijkt of standaard) bekeken. Voordat de biggen gespeend werden, werden ze blootgesteld aan een rugtest (± 14 dagen oud) om een indicatie te verkrijgen van het type aanpassingsreactie (coping strategie) van de biggen. Een week later werden de biggen blootgesteld aan een onbekende arena. Na het spenen werden de biggen (13 weken oud) blootgesteld aan eenzelfde test als beschreven in Hoofdstuk 3, waarbij de dieren eerst alleen in een onbekende arena werden geplaatst en daarna ook een onbekend voorwerp aangeboden kregen. Alleen de tweede helft van de test, dus met het onbekende voorwerp, werd gebruikt voor verdere analyses aangezien dit deel van de test als angstiger werd beschouwd door de varkens dan het eerste deel van de test, in elk geval rond deze leeftijd. Daarnaast werd de hoeveelheid cortisol in speeksel rondom deze test bepaald om de reactie van de bijnieren op deze test te meten. Bloed serotonine waardes werden bepaald op een leeftijd van 8, 9 en 22 weken. Binnen de
verschillende levensfases na het spenen bleek dat varkens die staartbeten over het algemeen lagere 5-HT opslag niveaus in het bloed hadden met tegelijkertijd een tendens voor een hogere opnamesnelheid van 5-HT in bloedplaatjes vergeleken met varkens die niet staartbeten. Daarnaast gedroegen varkens die uit een onverrijkt hok kwamen en staartbeten zich angstiger tijdens de test met het onbekende voorwerp. Geen relaties werden gevonden tussen staartbijten en de coping strategie van varkens en ook niet met de hoeveelheid cortisol in speeksel. Het was niet mogelijk om aan de hand van 5-HT in bloed individuele verschillen in staartbijten te voorspellen, maar gedurende een periode waarin een varken staartbeet was het aannemelijk dat het dier ook een lager 5-HT niveau in het bloed had. Deze resultaten suggereren dat zowel staartbijten als een afwijkend 5-HT systeem tijdelijk van aard kunnen zijn.

Varkens kunnen mogelijk het productieniveau van hun hokgenoten beïnvloeden door middel van hun gedrag. Het effect van één varken op een ander varken kan erfelijk zijn en als dit het geval is dan wordt dit een indirect genetisch effect genoemd. In Hoofdstuk 5 werd onderzocht of selectie voor een indirect genetisch effect op de groei van hokgenoten het gedrag van de varkens beïnvloedt. Dit experiment beschrijft dezelfde varkens als ook gebruikt in de Hoofdstukken 2 en 4. Een 2 × 2 factoriële proefopzet werd toegepast: varkens werden na het spenen verrijkt (strobed) of onverrijkt gehuisvest en een hok bestond volledig uit biggen met een relatief positief of negatief indirect effect op de groei van hun hokgenoten. De resultaten lieten zien dat varkens met een geschat negatief effect op de groei van hun hokgenoten meer bijterij in het algemeen uitten, meer staartschade veroorzaakten en meer jutezakken verbruikten. Geen statistische interactie tussen type huisvesting en het indirecte genetische effect op de groei van hokgenoten werd gevonden. Dit betekent dat de effecten van deze twee factoren op bijterij en staartschade additief zijn. Eerder werd beschreven dat varkens met een negatief indirect genetisch effect op de groei van hun hokgenoten angstiger leken tijdens gedragstesten waarin onbekendheid voor de situatie de hoofdrol speelde. Daarnaast leken deze varkens fysiologisch (immunologisch) gezien meer beïnvloed te worden door stress dan varkens met een positief genetisch effect op de groei van hun hokgenoten. Deze resultaten suggereren dat varkens geselecteerd zouden kunnen worden op het verminderd uiten van beschadigend gedrag gericht op hun hokgenoten.

Hoofdstukken 2 en 5 laten zien dat een strobed uiterst effectief is om staartbijten en staartschade te verminderen. Het gebruik van stro is in de
praktijk echter moeilijk te implementeren. Daarom is een alternatieve verrijking voor de varkens wenselijk. In Hoofdstuk 6 werd het effect van jutezakken op het beschadigende gedrag bestudeerd, omdat jutezakken een potentieel goed verrijkingsmateriaal voor varkens zouden kunnen zijn en ze bovendien gemakkelijk en goedkoop in gebruik lijken. Varkens kunnen in de jutezakken bijten, snuffelen, ze kunnen het vervormen, kapot maken en doordat het gemaakt is van plantaardig materiaal ontstaat er hoogstwaarschijnlijk geen fysiologisch probleem als stukjes doorgeslikt worden. Naast het effect van jutezakken werden ook potentiële relaties tussen staartbijten en fenotypische en genotypische productiekenmerken bestudeerd. In totaal werden 1033 biggen uit 72 tomen op een commercieel opfokbedrijf geboren en de helft van de tomen werd voorzien van een jutezak vrijwel direct na de geboorte terwijl de andere helft geen verrijking kreeg. Na het spenen (op 4 weken leeftijd) werden de geltjes en beertjes gescheiden en alleen de opfokgeltjes deden verder nog mee in het onderzoek. Geltjes hadden half gecoupeerde staartjes, beertjes kort gecoupeerde staartjes. Jutezakken reduceerden overduidelijk (tot 50 %) alle typen bijterij en uiteindelijk werd er een vijfvoudige vermindering van staartwonden gezien op het moment dat de geltjes 13 weken oud waren. Daarnaast wezen zowel fenotypische als genetische kenmerken (ook al waren relaties met deze laatsten zwak) in de richting van een positief verband tussen productieniveau van de geltjes en bijterij. Het combineren van toereikend verrijkingsmateriaal met genetische selectie zou een effectieve strategie kunnen zijn om beschadigend gedrag van varkens te verminderen.

Een belangrijke vinding was dat zowel het beschadigende gedrag zelf als de bijbehorende neurofysiologische karakteristieken maar tijdelijk bij individuele varkens aanwezig waren. Dit is overigens in lijn met de notie dat staartbijten een multifactoriële achtergrond zou hebben. Een verhoogde motivatie om te exploreren en foerageren lijkt de onderliggende factor van (staart)bijterij in varkens te zijn en deze wordt wellicht veroorzaakt door een combinatie van meerdere ‘states’ (toestanden) en ‘traits’ (kenmerken). Factoren die mogelijk geassocieerd zijn met dergelijke states en traits zijn: beschikbaarheid van verrijkingsmateriaal, functioneren van het 5-HT systeem, angstigheid en fenotypische en genetische productiekenmerken zoals groei die mogelijk verband houden met de metabolische behoeften van het varken.

Aan de hand van dit proefschrift kan geconcludeerd worden dat bijterij in varkens lijkt voort te komen uit een verhoogde motivatie om te exploreren en
te foerageren. Deze verhoogde motivatie is mogelijk ontstaan uit een combinatie van verschillende states en traits. Voor een beter begrip van staarbijten, en ook van ander bijtgedrag geuit door varkens, is het daarom belangrijk dat meer inzicht wordt verkregen in de fysilogische processen die betrokken zijn bij het uiten van beschadigend gedrag. Daarnaast is het van belang dat toekomstige wetenschappelijke en maatschappelijke programma's zich verenigen, zodat er gezamenlijk gewerkt kan worden aan het optimalizeren van de fokkerij van het varken, het management systeem op het varkensbedrijf, de voeding en de huisvestingscondities waarin de varkens leven.
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Dankwoord

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“Pigs are fabulous animals and you will think so too if you have the chance to meet them in a proper way, thanks piggies!”
Curriculum Vitae
Curriculum Vitae

Winanda Wilhelmina (or Nanda) Ursinus was born – as the youngest of three – in Lienden, the Netherlands, on the 29th of August, 1981. In 1997 she started with her first animal-oriented education at Groenhorst College in Barneveld. During this agricultural vocational education and training she did her internships at divergent companies and a non-profit organisation: Petshop De Zwaan, Horse-riding school Opelino, Dog and Cat Boarding House Ingen (where she kept working in her spare time till 2007), Sheep Farm Leeuwma, Animal Hospital Heslinga, and Bird shelter Hoogland. After obtaining her Animal Care and Production degree with a specialisation in management in 2001, she started at HAS Den Bosch in ‘s-Hertogenbosch. At this University of Professional Education she obtained her Animal Health Care and Production degree in 2004. She completed her first bachelor internship at Horse-riding school Opelino in Lienden, where she studied the dominance hierarchy of horses and the behavioural development of a foal. For the second internship she went to New Zealand to the Portobello Marine Laboratory of University of Otago in Dunedin. There, she collaborated in a study on the diet of feral New Zealand sea lions. Back in the Netherlands she finished her bachelor thesis at Corus IJmuiden while studying the effect of packing on the quality of solid canned dog food and thereby focussing on vitamins in dog food in relation to dog health. In 2004, she started her Master of Science programme in Animal Sciences and Aquaculture, with a specialisation in Preventive Animal Health and Welfare, at Wageningen University. She completed her major thesis at Ouwehands Zoo by studying aggression, the social structure, and the living environment of Barbary macaques. Thereafter, she started her minor thesis for the Dutch Sea Mammal Research Company, and was stationed at Boudewijn Seapark in Bruges, Belgium. Here, she studied the use of masking noise to reduce the echolocation efficiency of bottlenose dolphins in order to prevent bycatch of dolphins in trawl nets. After graduation in 2006, she started working at Wageningen University and Research Centre, first in Wageningen (Wageningen University) and later in Lelystad (Wageningen UR Livestock Research). Her projects were all related to animal welfare (mainly cows and pigs) and system innovations (mainly using the Reflexive Interactive Design method). In 2010, she started her PhD thesis on (mal) adaptation in pigs at the Adaptation Physiology Group of Wageningen University (Wageningen) and Animal behaviour and Welfare Group of Wageningen UR
Livestock Research (Lelystad). Currently, she still studies (tail) biting behaviours in pigs and is employed at the Adaptation Physiology Group of Wageningen University.
Publication list


Scientific articles

Refereed Journal Articles


Camerlink, I., Ursinus, W.W., Bijma, P., Kemp, B., and Bolhuis, J.E. Indirect genetic effects for growth rate in domestic pigs alter aggressive and manipulative biting behaviour. Accepted for publication in Behav. Genet.

Ursinus, W.W., Van Reenen, C.G., Reimert, I., and Bolhuis, J.E. Tail biting in pigs: blood serotonin and fearfulness as pieces of the puzzle? Accepted for publication in PLoS One.

Reimert, I., Rodenburg, T.B., Ursinus, W.W., Kemp, B., and Bolhuis, J.E. Selection on social breeding values for growth, environmental enrichment and coping style affect the immune status of pigs. Under revision in PLoS One.


Ursinus, W.W., Reimert, I., Van Reenen, C.G., Kemp, B., and Bolhuis, J.E. Immunological characteristics of tail biting in pigs. In prep.

Camerlink, I., Ursinus, W.W., Bijma, P., and Bolhuis, J.E. Welfare assessment of pigs selected for indirect genetic effects for growth and housed in barren and straw enriched pens. In prep.

Conference contributions


Reports and brochures


Publication list

economically, ecologically and socially sustainable pig production and steps towards realisation (brochure). Wageningen UR Livestock Research, Lelystad.

Miscellaneous


November 6, Baarn Nederland.
Education Certificate
# WIAS Training and Supervision Plan

<table>
<thead>
<tr>
<th>Description</th>
<th>Year</th>
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<tbody>
<tr>
<td><strong>The Basic Package (3.00 ECTS)</strong></td>
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<tr>
<td>WIAS Introduction Course, Wageningen, The Netherlands</td>
<td>2010</td>
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<tr>
<td>Course Ethics and Philosophy in Life Sciences, Wageningen, The Netherlands</td>
<td>2012</td>
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<tr>
<td><strong>International Conferences (7.60 ECTS)</strong></td>
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<tr>
<td>Knowing Animals: cross-fertilisation between natural and social sciences for understanding the quality of life of animals, Florence, Italy</td>
<td>2009</td>
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<td>60th Annual Meeting of the European Association for Animal Production (EAAP), Barcelona, Spain</td>
<td>2009</td>
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<tr>
<td>KSI Conference 2009: 1st European Conference on Sustainability Transitions, Amsterdam, The Netherlands</td>
<td>2009</td>
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<tr>
<td>44th International Society of Applied Ethology (ISAE), Uppsala, Sweden</td>
<td>2010</td>
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<tr>
<td>10th Endo-Neuro-Psycho Meeting, Lunteren, The Netherlands</td>
<td>2012</td>
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<td>Minding Animals Conference 2012, Utrecht University, The Netherlands</td>
<td>2012</td>
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<tr>
<td>46th International Society of Applied Ethology (ISAE), Vienna, Austria</td>
<td>2012</td>
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<tr>
<td>47th International Society of Applied Ethology (ISAE), Florianopolis, Brazil</td>
<td>2013</td>
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<tr>
<td>International Conference on Individual Differences, Groningen, The Netherlands</td>
<td>2013</td>
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<tr>
<td><strong>Seminars and Workshops (3.40 ECTS)</strong></td>
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<tr>
<td>Nordic Joint Committee for Agricultural Research (NKJ) meeting, Newcastle, England</td>
<td>2010</td>
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<td>Symposium ‘Scientific research in Animal Welfare: Do we make a difference?’ Wageningen, The Netherlands</td>
<td>2011</td>
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<tr>
<td>WIAS Science Day, Wageningen, The Netherlands</td>
<td>2011-2014</td>
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<td>NKJ meeting, Uppsala, Sweden</td>
<td>2011</td>
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<tr>
<td>Symposium ‘Prodromi: met meer comfort naar meer rendement’ Wijchen, The Netherlands</td>
<td>2011</td>
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<tr>
<td>NKJ meeting, Porvoo, Finland</td>
<td>2012</td>
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<tr>
<td>Workshop Insights Discovery, Lelystad, The Netherlands</td>
<td>2012</td>
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</tbody>
</table>
20th Annual Meeting of the Netherlands Society for Behavioural Biology (NVG), Soesterberg, The Netherlands 2012
Interactive masterclass ‘Zicht op staartbijten’, Sterksel, The Netherlands 2013

Presentations (12.00 ECTS)
Oral presentation, Knowing Animals, Florence, Italy 2009
Oral presentation, EAAP, Barcelona, Spain 2009
Oral presentation, NKJ, Uppsala, Sweden 2011
Oral presentation, Themadag Adaptatie Varken, Utrecht, The Netherlands 2011
Poster presentation, WIAS Science Day, Wageningen, The Netherlands 2012
Oral presentation, Institute for Pig Genetics, Beuningen, The Netherlands 2012
Oral presentation, Institute for Pig Genetics, Wageningen, The Netherlands 2012
Oral presentation, Minding Animals Conference, Utrecht, The Netherlands 2012
Oral presentation, 46th ISAE, Vienna, Austria 2012
Oral presentation, WIAS Science Day, Wageningen, The Netherlands 2013
Oral presentation, 47th ISAE, Florianopolis, Brazil 2013
Poster presentation, WIAS Science Day, Wageningen, The Netherlands 2014

In-Depth Studies (11.90 ECTS)
WIAS Advanced Statistics Course: Design of Experiments, Wageningen, The Netherlands 2010
Statistics for the Life Sciences, Wageningen, The Netherlands 2010
Welfare discussion group meetings, Wageningen, The Netherlands 2010-2012
Epigenesis and epigenetics, Wageningen, The Netherlands 2011
Advanced Course Infection Biology, Utrecht, The Netherlands 2012
Optional assignment by course Trends in stress biology: Interpretation of animal stress responses, Hobro, Denmark 2013

Statutory Courses (0.00 ECTS)
Use of Laboratory Animals, Wageningen, The Netherlands 2005

Professional Skills Support Courses (5.70 ECTS)
Writing for Academic Publication, Lelystad, The Netherlands 2009
Project Management Basis, Lelystad, The Netherlands 2009
### Education Certificate

- **PhD Competence assessment, Wageningen, The Netherlands** 2010
- **WGS course: Communication in Interdisciplinary Research, Wageningen, The Netherlands** 2012
- **Supervising MSc Thesis students, Wageningen, The Netherlands** 2012
- **Career Perspectives, Wageningen, The Netherlands** 2014

### Research Skills Training (6.00 ECTS)
- Preparing own PhD research proposal: WIAS proposal 2010

### Didactic Skills Training (10.75)
- Supervising 7 MSc students, Wageningen, The Netherlands 2010-2014
- **Lecture MSc course Health, Welfare and Management, Wageningen, The Netherlands** 2012
- Supervising BSc students in course Introduction in Animal Sciences, Wageningen, The Netherlands 2012

### Management Skills Training (2.30 ECTS)
- Wageningen Centre for Animal Welfare and Adaptation (CAWA): 2012-2013
  - Organizing start-up meeting and posting news & announcements, Wageningen, The Netherlands
- Assisting at ‘Open Innovatiedagen Varkens Innovatie Centrum’, Sterksel, The Netherlands 2013

### Education and Training Total

| **Education and Training Total** | **62.65 ECTS** |

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1. 1 ECTS credit equals a study load of approximately 28 hours.
Colophon

The research described in this thesis was financially supported by the Dutch Ministry of Economic Affairs.

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Printed by: GVO drukkers & vormgevers B.V. | Ponsen & Looijen, Ede, The Netherlands
A tale too long for a tail too short?

Identification of characteristics in pigs related to tail biting and other oral manipulations directed at conspecifics

Winanda Wilhelmina Ursinus

2014