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

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Abstract

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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).

Figure 1. Fattening pig that bites in the tail of a pen mate.

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.

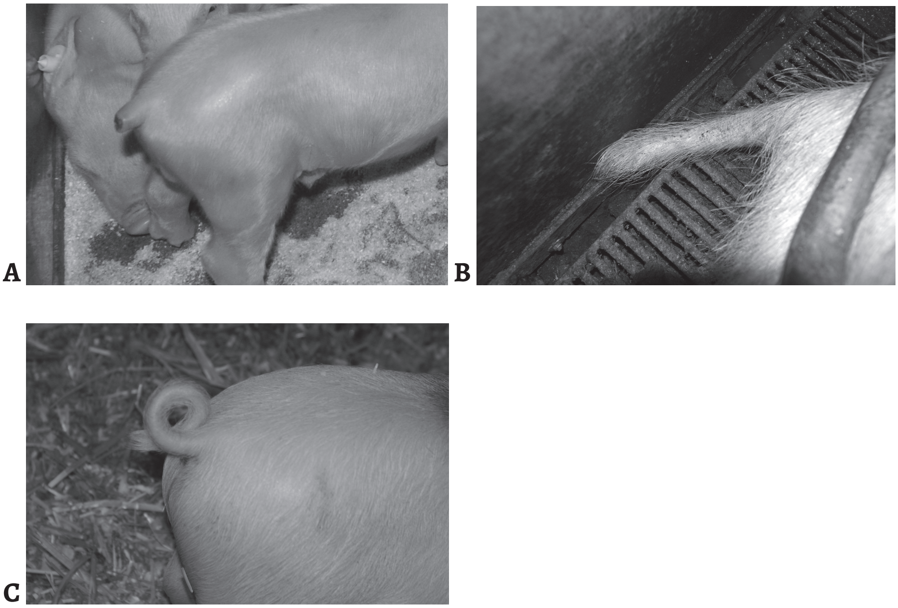


Figure 2. Pig tails from shortly docked to undocked. A: Recently docked tail of a young piglet, B: Half docked tail of a sow, C: Intact tail of a recently weaned piglet.

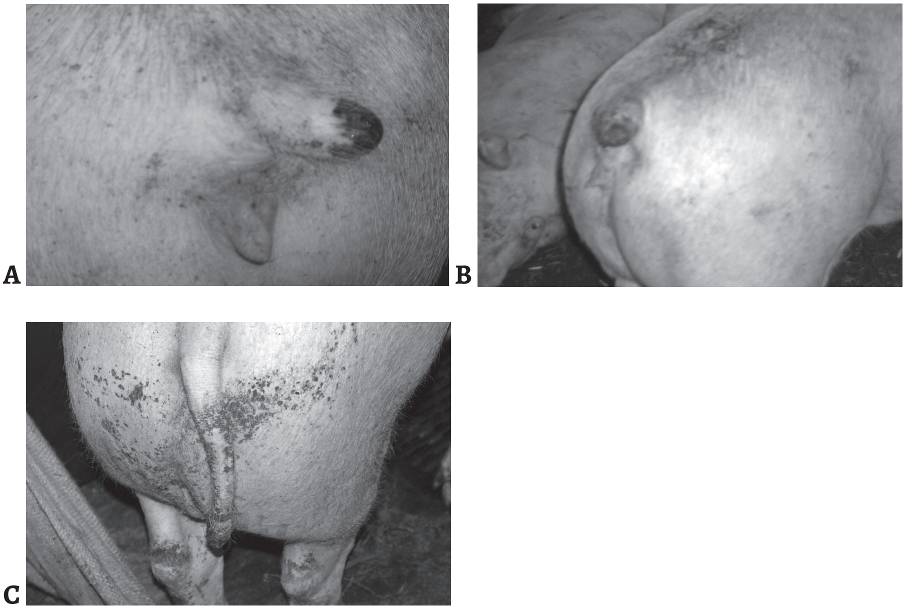


Figure 3. Tail wounds. A: Shortly docked pig with scabs and small wound, B: Shortly docked pig with largely damaged and swollen tail, but showing signs of healing, C: Pig with intact tail with wound, treated with tar.

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; Schrøder-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).

Tail biting 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

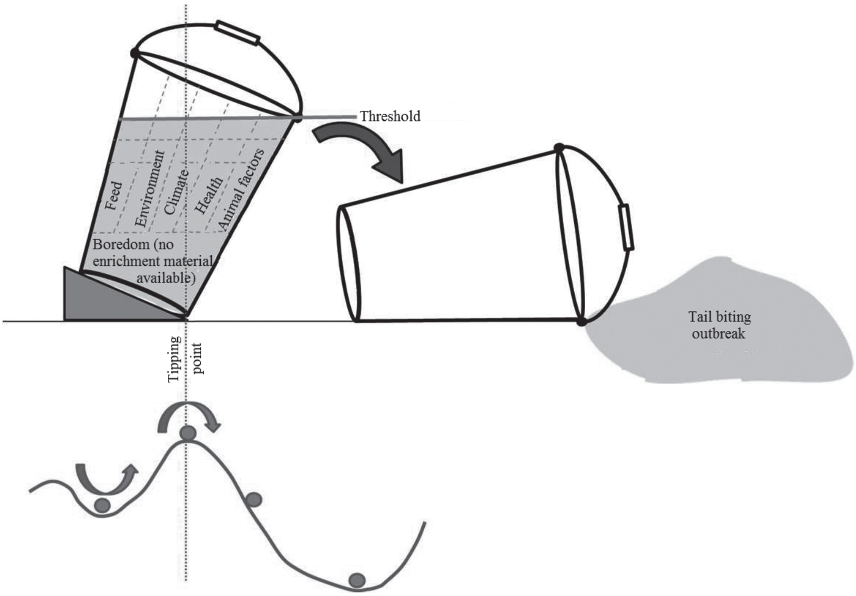


Figure 4. Metaphorical bucket filled with risk factors of tail biting that should be in balance. The factor boredom is, here, suggested as largest risk factor and relates to the unfulfilled need to explore and forage. The presence of too many risk factors will lead to the threshold and causes the bucket 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

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

Beattie, V.E., O'Connell, N.E., Kilpatrick, D.J., and Moss, B.W. 2000. Influence of environmental enrichment on welfare-related behavioural and physiological parameters in growing pigs. Anim. Sci. 70, 443-450.

Beattie, V.E., Sneddon, I.A., Walker, N., and Weatherup R.N. 2001. Environmental enrichment of intensive pig housing using spent mushroom compost. Anim. Sci. 72, 35-42.

Bijma, P., Muir, W.M., and Van Arendonk, J.A.M. 2007a. Multilevel selection 1: Quantitative genetics of inheritance and response to selection. Genetics. 175, 277-288.

Bijma, P., Muir, W.M., Ellen, E.D., Wolf, J.B., and Van Arendonk, J.A.M. 2007b. Multilevel selection 2: Estimating the genetic parameters determining inheritance and response to selection. Genetics. 175, 289-299.

Blackshaw, J.K., 1981. Some behavioural deviations in weaned domestic pigs: persistent inguinal nose thrusting, and tail and ear biting. Anim. Prod. 33, 325-332.

Bolhuis, J.E., Van den Brand, H., Bartels, A.C., Oostindjer, M., Van den Borne, J.J.G.C., Kemp, B., and Gerrits, W.J.J. 2010. Effects of fermentable starch on behaviour of growing pigs in barren or enriched housing. Appl. Anim. Behav. Sci. 123, 77-86.

Boogaard, B.K., Boekhorst, L.J.S., Oosting, S.J., and Sørensen, J.T. 2011. Socio-cultural sustainability of pig production: Citizen perceptions in the Netherlands and Denmark. Livest. Sci. 140, 189-200.

Bos, B., Groot Koerkamp, P., Gosselink, J., and Bokma, S. 2008. Reflexive Interactive Design and its application in a project on sustainable dairy husbandry systems. In: 8th European IFSA Symposium, Clermont-Ferrand, France, pp. 87-99.

Bracke, M.B.M., De Lauwere, C.C., Wind, S.M.M., and Zonderland, J.J. 2013. Attitudes of Dutch Pig Farmers Towards Tail Biting and Tail Docking. J. Agric. Environ. Ethics. 26, 847-868.

Bracke, M.B.M., Hulsegge, B., Keeling, L., and Blokhuis, H.J. 2004. Decision support system with semantic model to assess the risk of tail biting in pigs: 1. Modelling. Appl. Anim. Behav. Sci. 87, 31-44.

Bracke, M., Vermeer, H., Bokma, M., Van der Peet, C., Bolhuis, L., and Leeijen, J. 2012. Checklist Aanpak staartbijten bij (biologische) varkens. Wageningen UR, Lelystad, The Netherlands.

Breuer, K., Sutcliffe, M.E.M., Mercer, J.T., Rance, K.A., Beattie, V.E., Sneddon, I.A., and Edwards, S.A. 2003. The effect of breed on the development of adverse social behaviours in pigs. Appl. Anim. Behav. Sci. 84, 59-74.

Breuer, K., Sutcliffe, M.E.M., Mercer, J.T., Rance, K.A., O'Connell, N.E., Sneddon I.A., and Edwards, S.A. 2005. Heritability of clinical tail-biting and its relation to performance traits. Livest. Prod. Sci. 93, 87-94.

Broom, D.M., 2006. Adaptation. Berl. Munch. Tierarztl. 119, 1-6.

Brunberg, E., Wallenbeck, A., and Keeling, L.J. 2011. Tail biting in fattening pigs: Associations between frequency of tail biting and other abnormal behaviours. Appl. Anim. Behav. Sci. 133, 18-25.

Carver, C.S. and Miller, C.J. 2006. Relations of serotonin function to personality: Current views and a key methodological issue. Psychiat. Res. 144, 1-15.

Cox, L.N. and Cooper, J.J., 2001. Observations on the pre- and post-weaning behaviour of piglets reared in commercial indoor and outdoor environments. Anim. Sci. 72, 75-86.

Dalfsen Declaration. 2013. www.wageningenur.nl, last accessed: 17-6-2014.

Day, J.E.L., Kyriazakis, I., and Lawrence, A.B. 1996. An investigation into the causation of chewing behaviour in growing pigs: The role of exploration and feeding motivation. Appl. Anim. Behav. Sci. 48, 47-59.

De Jonge, F.H., Tilly, S.L., Baars, A.M., and Spruijt, B.M. 2008. On the rewarding nature of appetitive feeding behaviour in pigs (Sus scrofa): Do domesticated pigs contrafreeload? 114: 359-372.

Delorme, R., Betancur, C., Callebert, J., Chabane, N., Laplanche, J.L., Mouren-Simeoni, M.C. Launay, J.M., and Leboyer, M. 2005. Platelet serotonergic markers as endophenotypes for obsessive-compulsive disorder. Neuropsychopharmacol. 30, 1539-1547.

Dijksma , 2014. Kamerbrief: Toezeggingen notaoverleg Dierenwelzijn. Directoraat-generaal Agro, Directie Dierlijke Agroketens en Dierenwelzijn, DGA-DAD/14042557, The Hague, The Netherlands.

EC. 2012. Communication from the commission to the European Parliament, the Council and the European Economic and Social Committee on the European Union Strategy for the protection and welfare of animals 2012-2015. European Commission, Brussels, Belgium.

EC. 2008. Council Directive 2008/120/EC of 18 December 2008: laying down minimum standards for the protection of pigs. The Council of the European Union, OJ. 18.2.2009, 47, 5-13.

Edwards, S.A., 2006. Tail biting in pigs: Understanding the intractable problem. Vet. J. 171, 198-199.

EFSA. 2007. Scientific report on the risks associated with tail biting in pigs and possible means to reduce the need for tail docking considering the different housing and husbandry systems. Annex to the EFSA J. 611, 1-13 (Question No EFSA-Q-2006-013, 1-98).

Eurostat. 2014. Production of meat: pigs. Epp.eurostat.ec.europa.eu, last accessed: 7-6-2014.

Fraser, D. 1987. Attraction to blood as a factor in tail-biting by pigs. Appl. Anim. Behav. Sci. 17, 61-68.

Fritschen, R. and Hogg, A. 1983. Preventing tail biting in swine (anti-comfort syndrome). Nebguide G 75-246 (Revised). Institute of Agriculture and Natural Resources, University of Nebraska, Lincoln, NE.

Hansen LL, Hagelsø AM, and Madsen A. 1982. Behavioural results and performance of bacon pigs fed "AD libitum" from one or several self-feeders. Appl. Anim. Ethol. 8, 307-333.

Hensler, J.G. 2010. Serotonin in mood and emotion. In: Müller, C.R. and Jacobs, B. (Editors). Handbook of the behavioural neurobiology of serotonin. Academic Press, London, pp. 367-378.

Hughes, B.O. and Duncan, I.J.H. 1988. The notion of ethological 'need', models of motivation and animal welfare. Anim. Behav. 36, 1696-1707.

Koolhaas, J.M., De Boer, S.F., Buwalda, B., and Van Reenen, K. 2007. Individual variation in coping with stress: A multidimensional approach of ultimate and proximate mechanisms. Brain Behav. Evol. 70, 218-226.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van der Vegt, B.J., Van Reenen C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W., and Blokhuis, H.J. 1999. Coping styles in animals: Current status in behavior and stress- physiology. Neurosci. Biobehav. R. 23, 925-935.

Korte, S.M., Prins, J., Vinkers, C.H., and Olivier, B. 2009. On the origin of allostasis and stress-induced pathology in farm animals: Celebrating Darwin's legacy. Vet. J. 182, 378-383.

Meindertsma, C. 2008. PIG 05049. Flocks, ISBN 9081241311.

Moinard, C., Mendl, M., Nicol, C.J., and Green, L.E. 2003. A case control study of on-farm risk factors for tail biting in pigs. Appl. Anim. Behav. Sci. 81, 333-355.

Muir, W.M., 2005. Incorporation of competitive effects in forest tree or animal breeding programs. Genetics. 170, 1247-1259.

Munsterhjelm, C., Brunberg, E., Heinonen, M., Keeling, L., and Valros, A. 2013. Stress measures in tail biters and bitten pigs in a matched case-control study. Anim. Welfare. 22, 331-338.

Noonan, G.J., Rand, J.S., Priest, J., Ainscow, J., and Blackshaw, J.K., 1994. Behavioural observations of piglets undergoing tail docking, teeth clipping and ear notching. Appl. Anim. Behav. Sci. 39, 203-213.

Penny, R.H., Walters, J.R., and Tredget, S.J., 1981. Tail-biting in pigs: a sex frequency between boars and gilts. Vet. Rec. 108, 35.

Rauw, W.M., Kanis, E., Noordhuizen-Stassen, E.N., and Grommers, F.J. 1998. Undesirable side effects of selection for high production efficiency in farm animals: A review. Livest. Prod. Sci. 56: 15-33.

Reimert, I., Rodenburg, T.B., Ursinus, W.W., Kemp, B., and Bolhuis, J.E. 2014. Responses to novel situations of female and castrated male pigs with divergent social breeding values and different backtest classifications in barren and straw-enriched housing. Appl. Anim. Behav. Sci. 151, 24-35.

Robert, S. and Martineau, G.P. 1994. Reconciling productivity and welfare in intensive pig husbandry: a challenge for the year 2000. Rev. Sci. Tech. OIE. 13: 99-117.

Rodenburg, T.B., Bijma, P., Ellen, E.D., Bergsma, R., De Vries, S., Bolhuis, J.E., Kemp, B., and Van Arendonk, J.A.M. 2010. Breeding amiable animals? Improving farm animal welfare by including social effects in breeding programmes. Anim. Welfare. 19, 77-82.

Rodenburg, T.B. and Koene, P. 2007. The impact of group size on damaging behaviours, aggression, fear and stress in farm animals. App. Anim. Behav. Sci. 103, 205-214.

Sällvik, K. and Walberg, K. 1984. The effects of air velocity and temperature on the behaviour and growth of pigs. J. Agr. Eng. Res. 30, 305-312.

Schrøder-Petersen, D.L. and Simonsen, H.B. 2001. Tail biting in pigs. Vet. J. 162, 196-210.

Schrøder-Petersen D.L., Simonsen H.B., and Lawson L.G. 2003. Tail-in-mouth behaviour among weaner pigs in relation to age, gender and group composition regarding gender. Acta Agric. Scand. A-AN 53: 29-34.

Sinisalo, A., Niemi, J.K., Heinonen, M., and Valros, A. 2012. Tail biting and production performance in fattening pigs. Livest. Sci. 143, 220-225.

Smulders, D., Verbeke, G., Mormède, P., and Geers, R., 2006. Validation of a behavioral observation tool to assess pig welfare. Physiol. Behav. 89, 438-447.

Sutherland, M.A. and Tucker, C.B., 2011. The long and short of it: A review of tail docking in farm animals. Appl. Anim. Behav. Sci. 135, 179-191.

Taylor, N.R., Main, D.C., Mendl, M., and Edwards, S.A. 2010. Tail-biting: a new perspective. Vet. J. 186, 137-147.

Taylor, N.R., Parker, R.M.A., Mendl, M., Edwards, S.A., and Main, D.C.J. 2012. Prevalence of risk factors for tail biting on commercial farms and intervention strategies. Vet. J. 194, 77-83.

Turner, S.P. 2011. Breeding against harmful social behaviours in pigs and chickens: State of the art and the way forward. Appl. Anim. Behav. Sci. 134, 1– 9.

Valros, A., Keeling, L., Thodberg, K., and Zanella, A.J. 2012. Tail biting and tail docking in the pig: Biological mechanisms, prevention, treatment and economic aspects. Slutrapport. Nordic Joint Committee for Agricultural and Food Research (NKJ), Oslo, Norway.

Van den Berg, J. 1982. Tail-biting in pigs. Causes, effects and prevention. Neth. J. Vet. Sci. 107, 735-743.

Van de Weerd, H.A., Docking, C.M., Day, J.E.L., Breuer, K., and Edwards, S.A. 2006. Effects of species-relevant environmental enrichment on the behaviour and productivity of finishing pigs. Appl. Anim. Behav. Sci. 99, 230-247.

Van Hierden, Y.M., De Boer, S.F., Koolhaas, J.M., and Korte, S.M. 2004. The control of feather pecking by serotonin. Behav. Neurosci. 118, 575-583.

Van Putten, G. 1969. An investigation into tail-biting among fattening pigs. Brit. Vet. Jl. 125, 511-517.

Van Reenen, C.G., O'Connell, N.E., Van Der Werf, J.T.N., Korte, S.M., Hopster, H., Jones, R.B., and Blokhuis, H.J. 2005. Responses of calves to acute stress: Individual consistency and relations between behavioral and physiological measures. Physiol. Behav. 85, 557-570.

Wallgren, P. and Lindahl, E. 1996. The influence of tail biting on performance of fattening pigs. Acta Vet. Scand. 37, 453-460.

Wood-Gush, D.G.M. and Vestergaard, K., 1989. Exploratory behavior and the welfare of intensively kept animals. J. Agric. Ethics. 2, 161-169.

Zonderland, J.J. 2010. Talking Tails: Quantifying the development of tail biting in pigs. Doctoral Thesis, Wageningen University, Wageningen, The Netherlands.

Zonderland, J.J., Bosma, B., and Hoste, R., 2011a. Financiële consequenties van staartbijten bij varkens. Rapport 543. Wageningen UR Livestock Research, Lelystad, The Netherlands.

Zonderland, J.J., Kemp, B., Bracke, M.B.M., Den Hartog, L.A., and Spoolder, H.A.M. 2011b. Individual piglets contribution to the development of tail biting. Animal 5, 601-607.

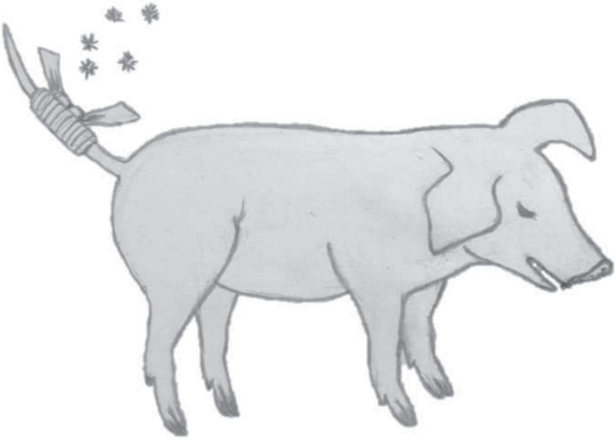
Zonderland, J.J., Schepers, F., Bracke, M.B.M., Den Hartog, L.A., Kemp, B., and Spoolder, H.A.M. 2011c. Characteristics of biter and victim piglets apparent before a tail-biting outbreak. Animal 5, 767-775.

Zonderland, J.J., Bracke, M.B.M., Den Hartog, L.A., Kemp, B., and Spoolder, H.A.M. 2010a. Gender effects on tail damage development in single- or mixed-sex groups of weaned piglets. Livest. Sci. 129, 151-158.

Zonderland, J., Bracke, M., De Lauwere, C. 2010b. 'Risicofactoren van staartbijten'. V-focus. 2, 45-46.

Zonderland, J.J., Wolthuis-Fillerup, M., Van Reenen, C.G., Bracke, M.B.M., Kemp, B., Den Hartog, L.A., and Spoolder, H.A.M. 2008. Prevention and treatment of tail biting in weaned piglets. Appl. Anim. Behav. Sci. 110, 269-281.

Zupan, M., Janczak, A.M., Framstad, T., and Zanella, A.J. 2012. The effect of biting tails and having tails bitten in pigs. Physiol. Behav. 106, 638-644.



2

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 m^2, 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 m^2) 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 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.

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

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

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: $r = 0.39\text{-}0.92$, all $P < 0.001$) and with tail posture scores (phase 2-3: $r = 0.18\text{-}0.55$, with $P < 0.01\text{-}0.001$), 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 ± 0.01, range: 0-3.37, n = 987 piglets, B: 0.25 ± 0.03, n = 240 piglets, E: 0.25 ± 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 ± 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 ± 0.07, E: 2.1 ± 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 ± 0.02, phase 2: 0.17 ± 0.02, phase 3: 0.19 ± 0.02 % of observations)

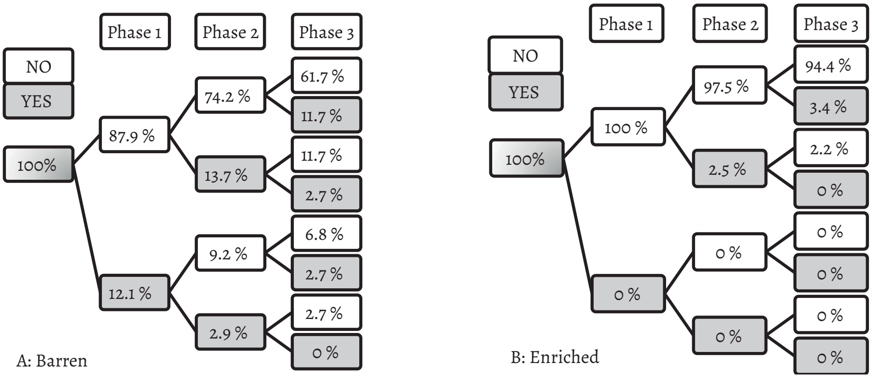


Figure 1. Percentage of pigs identified as (non) tail biter during phases 1-3 post-weaning, where pigs performing tail biting more than once during scan sampling in the particular phase were classified as tail biters. A: Barren housed pigs, B: Enriched housed pigs. Please note: some animals died or were 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.

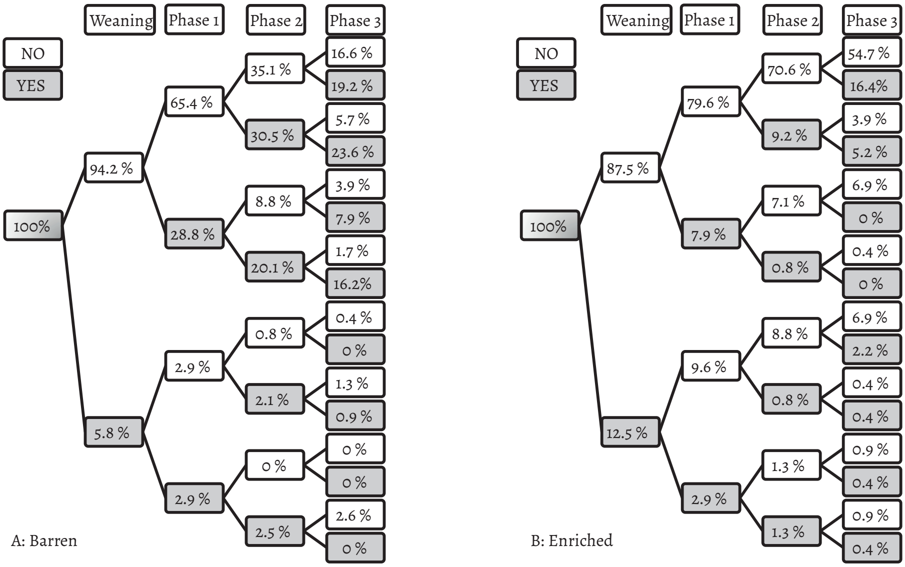


Figure 2. Percentage of pigs identified as (non) tail bite victim at time of weaning and phases 1-3 post-weaning, where pigs that had at least once a wound during the particular phase were classified as victims of tail biting. A: Barren housed pigs, B: Enriched housed pigs. Please note: some animals 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.

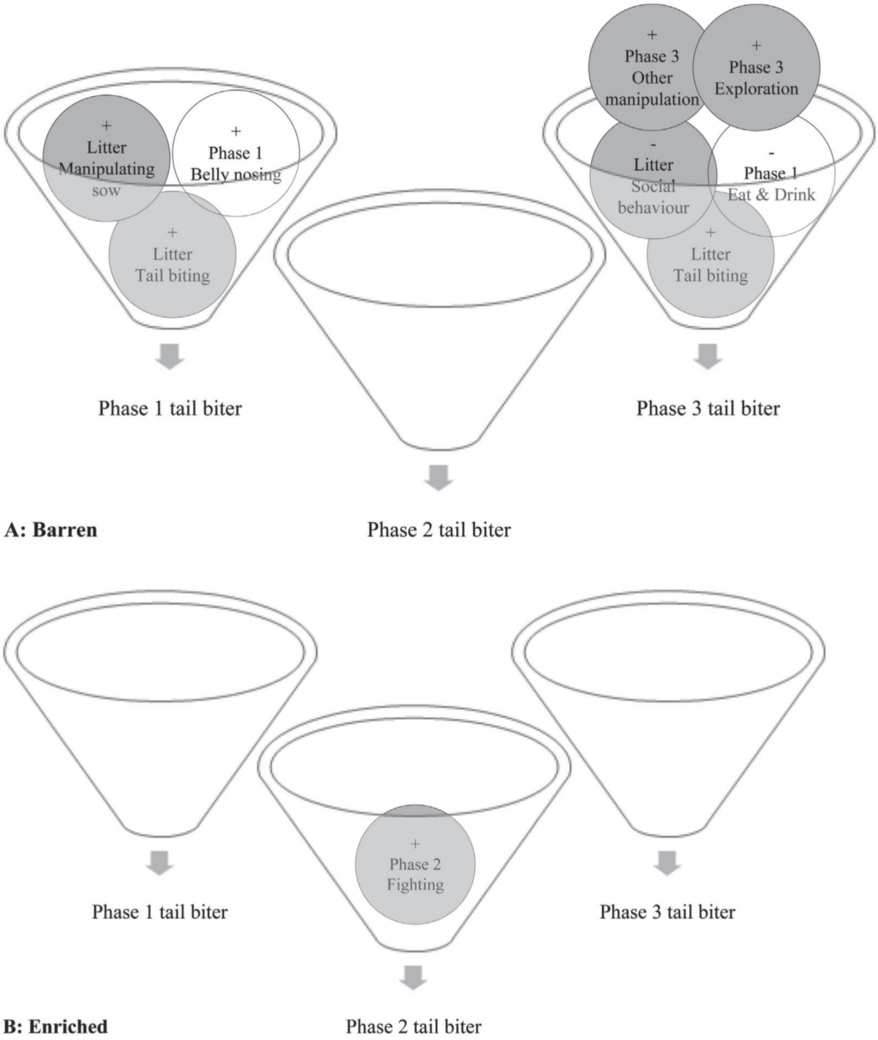
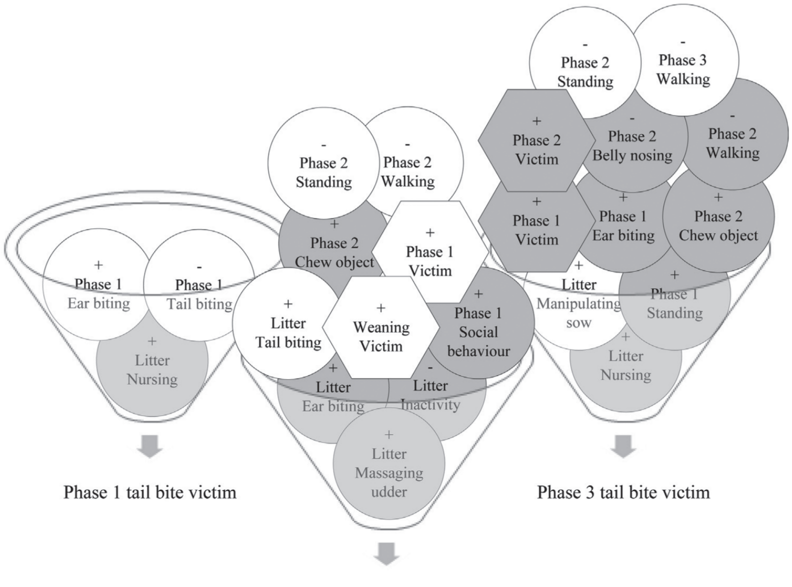
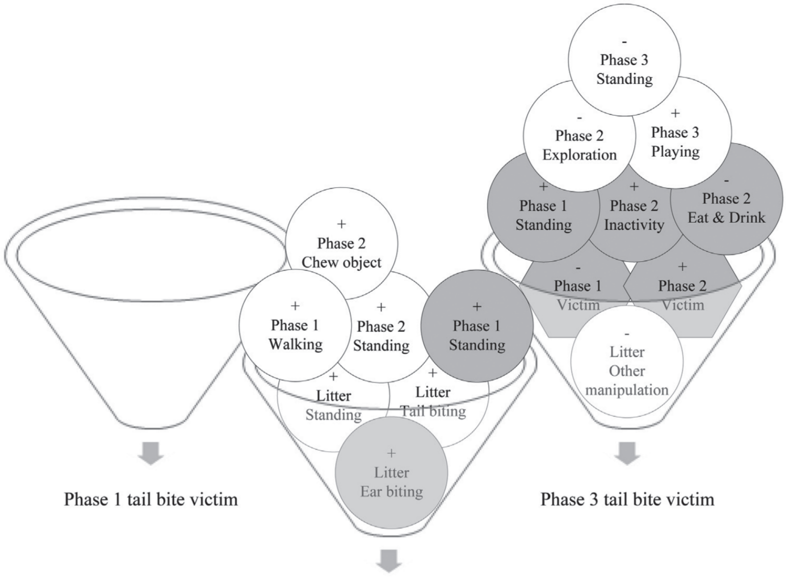


Figure 3. Funnels presenting behaviours observed during the pre-weaning period (average of the whole litter) and phases 1-3 (individual pig), that are likely to predict tail biters in phase 1-3 post-weaning. Grey shapes indicate significant relationships, white shapes indicate tendencies, '+' indicates 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.



A: Barren



B: Enriched

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

3B). 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.

	Phase 1 Tail biting			**Phase 2 Tail biting**			**Phase 3 Tail biting**		
	Phase 1	*Phase 2*	*Phase 3*	*Phase 1*	*Phase 2*	*Phase 3*	*Phase 1*	*Phase 2*	*Phase 3*
Tail biting		-0.01	-0.25			-0.13			
Eating and drinking	-0.24	0.31+	0.07	**0.34***	-0.04	-0.26	0.02	-0.08	-0.13
Walking	-0.06	-0.31+	0.04	0.18	-0.11	0.02	0.04	**0.57*****	-0.02
Standing	-0.06	-0.20	0.09	-0.07	-0.03	-0.11	0.05	**0.40***	-0.07
Inactive	0.17	-0.10	-0.01	**-0.33***	-0.28+	-0.11	-0.11	-0.30+	-0.11
Fighting	0.17	-0.04	0.20	0.07	-0.03	0.05	-0.18	0.12	-0.19
Ear biting	0.11	0.09	0.09	0.02	0.28	0.17	0.00	**-0.42****	-0.02
Belly nosing	0.03	-0.13	-0.29+[α]	-0.01	0.03	0.03	-0.03	0.17	0.20
Other manipulation	0.07	0.01	-0.09	0.09	**0.41****	-0.02	0.12	-0.12	0.22
Mounting	0.10	**-0.35***[α]	0.08	0.19	0.18	-0.06	-0.04	-0.18	-0.10
Exploration	-0.18	0.18	0.13	**0.37***	0.31+	0.26	0.15	0.21	0.02
Chewing objects	0.09	-0.16	0.09	**0.43****	**0.50****	0.27+	-0.24	0.08	-0.05
Social	-0.02	-0.24	-0.11	0.12	0.09	0.22	-0.18	0.12	0.21
Playing	-0.16	0.19	0.10	0.23	0.10	-0.28+	-0.04	**0.33***	0.05

[α] 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.

	Phase 1 Tail damage			**Phase 2 Tail damage**			**Phase 3 Tail damage**		
	Phase 1	*Phase 2*	*Phase 3*	*Phase 1*	*Phase 2*	*Phase 3*	*Phase 1*	*Phase 2*	*Phase 3*
Tail damage		**0.46****	0.20			**0.47****			
Tail biting	**0.31***	0.23	-0.15	0.25	0.15	-0.22	0.22	0.13	0.01
Eating and drinking	**0.38***	0.30+	0.08	0.13	0.17	0.14	-0.01	-0.09	0.15
Walking	-0.10	**-0.32***	0.04	0.24	-0.08	-0.03	**0.33***	0.07	0.25
Standing	-0.13	-0.12	-0.24	-0.03	**-0.37***	-0.18	0.03	-0.23	-0.05
Inactive	-0.06	-0.16	-0.08	-0.17	-0.11	-0.21	-0.21	0.08	-0.11
Fighting	**0.32***	0.21	**0.34***	0.27+	-0.06	-0.21	**0.39***	-0.25	-0.05
Ear biting	-0.05	0.07	-0.13	-0.03	0.16	-0.14	-0.06	-0.09	0.13
Belly nosing	-0.29	0.09	-0.04	-0.12	-0.08	-0.07	-0.12	-0.22	-0.17
Other manipulation	-0.09	0.24	-0.22	-0.21	0.27+	-0.06	0.05	**0.34***	-0.14
Mounting	-0.13	0.05	0.06	0.06	-0.14	-0.23	0.00	0.13	-0.07
Exploration	0.02	0.16	0.20	0.05	0.16	0.28+	0.21	0.06	0.14
Chewing objects	**0.32***	0.18	0.10	**0.45****	0.30+	0.20	**0.43****	**0.39***	0.11
Social	-0.02	-0.17	0.05	0.27+	0.00	0.05	0.12	-0.05	-0.07
Playing	-0.01	-0.13	-0.16	0.30+	0.01	-0.01	0.01	-0.10	0.01

+ 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.

	Phase 1 Tail biting[a]			**Phase 2 Tail biting**			**Phase 3 Tail biting**		
	Phase 1	*Phase 2*	*Phase 3*	*Phase 1*	*Phase 2*	*Phase 3*	*Phase 1*	*Phase 2*	*Phase 3*
Tail biting		-0.20	0.08			0.14			
Eating and drinking	0.01	0.03	0.21	-0.14	0.08	-0.17	-0.28+	0.07	0.03
Walking	0.12	0.21	0.17	0.18	-0.10	-0.05	-0.19	-0.06	-0.04
Standing	0.17	0.16	0.04	0.00	-0.09	0.03	-0.30+	-0.29+	-0.20
Inactive	-0.19	-0.06	-0.05	-0.16	0.08	0.12	0.03	0.22	-0.14
Fighting	-0.12	0.04	0.30+	**0.35***	-0.13	-0.18	0.00	-0.18	0.21
Ear biting	-0.21	0.10	0.04	0.04	-0.02	-0.19	0.02	-0.28+	0.13
Belly nosing	-0.01	-0.07	0.06	0.11	0.08	0.20	0.23	-0.11	0.12
Other manipulation	-0.22	**0.37***	-0.10	**0.32***	0.00	0.10	-0.20	**0.33***	-0.14
Mounting	0.10	-0.04	0.00	0.02	-0.06	-0.03	0.18	0.12	-0.08
Exploration	0.19	-0.02	-0.06	0.10	-0.11	-0.07	0.04	-0.25	0.15
Chewing objects	-0.04	0.00	-0.02	-0.02	0.01	0.16	-0.03	-0.18	0.02
Social	-0.17	0.11	0.10	**0.33***	0.06	0.21	-0.02	0.18	-0.15
Playing	0.24	0.03	-0.09	0.25	-0.15	0.04	0.13	-0.11	**0.36***

+ 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.

	Phase 1 Tail damage			**Phase 2 Tail damage**			**Phase 3 Tail damage**		
	Phase 1	*Phase 2*	*Phase 3*	*Phase 1*	*Phase 2*	*Phase 3*	*Phase 1*	*Phase 2*	*Phase 3*
Tail damage		0.06	-0.05			**0.32***			
Tail biting	**-0.33***[α]	0.03	**-0.37***	0.06	0.08	0.08	0.03	0.04	**0.32***
Eating and drinking	0.07	**-0.32***	-0.09	0.10	0.22	0.30+	-0.16	0.14	-0.08
Walking	0.26	-0.13	-0.07	0.10	0.06	0.04	0.16	-0.13	-0.16
Standing	-0.04	-0.03	-0.11	0.02	-0.05	-0.10	-0.06	-0.04	0.03
Inactive	0.02	0.15	0.08	-0.27+	**-0.31***	**-0.40****	-0.16	0.02	0.14
Fighting	-0.12	-0.27+	-0.04	0.01	0.22	0.02	-0.14	-0.14	0.17
Ear biting	-0.01	0.03	-0.10	-0.21	-0.06	0.00	-0.09	-0.04	0.18
Belly nosing	-0.05	0.06	0.07	-0.13	-0.03	0.03	-0.01	0.18	-0.02
Other manipulation	0.28+	0.01	0.17	0.06	0.11	-0.08	-0.01	0.14	**-0.36***
Mounting	-0.24	0.09	0.08	0.08	0.22	-0.24	-0.13	0.20	-0.14
Exploration	-0.03	-0.05	-0.03	**0.35***	0.29+	**0.38***	0.22	-0.08	-0.14
Chewing objects	0.05	0.08	0.04	0.19	0.05	0.19	0.15	-0.04	0.15
Social	0.20	0.00	**-0.31***	0.26	0.08	-0.06	0.25	-0.13	-0.26
Playing	-0.31+	-0.01	0.02	-0.07	**0.46****	**0.62*****	0.09	0.11	**0.41****

[α] 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 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; Schrøder-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 Schrøder-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 Schrøder-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 post-weaning, 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 post-weaning. 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.

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References

Anonymous. 2001. Scientists' assessment of the impact of housing and management on animal welfare. J. Appl. Anim. Welf. Sci. 4, 3-52.

Beattie, V.E., Breuer, K., O'Connell, N.E., Sneddon, I.A., Mercer, J.T., Rance, K.A., Sutcliffe, M.E.M., and Edwards, S.A. 2005. Factors identifying pigs predisposed to tail biting. Anim. Sci. 80, 307-312.

Beattie, V.E., Walker, N., and Sneddon, I.A. 1995. Effect of rearing environment and change of environment on the behaviour of gilts. Appl. Anim. Behav. Sci. 46, 57-65.

Beattie, V.E., Walker, N., and Sneddon, I.A. 1996. An investigation of the effect of environmental enrichment and space allowance on the behaviour and production of growing pigs. Appl. Anim. Behav. Sci. 48, 151-158.

Bernstein, J.Y. and Watson, M.W. 1997. Children who are targets of bullying: A victim pattern. J. Interpers. Violence. 12, 483-498.

Blokhuis, H.J., Ekkel, E.D., Korte, S.M., Hopster, H., and Van Reenen, C.G. 2000. Farm animal welfare research in interaction with society. Vet. Q. 22, 217-222.

Bolhuis, J.E., Parmentier, H.K., Schouten, W.G.P., Schrama, J.W., and Wiegant, V.M. 2003. Effects of housing and individual coping characteristics on immune responses of pigs. Physiol. Behav. 79, 289-296.

Bolhuis, J.E., Schouten W.G.P., Schrama J.W., and Wiegant V.M. 2006. Effects of rearing and housing environment on behaviour and performance of pigs with different coping characteristics. Appl. Anim. Behav. Sci. 101: 68-85.

Bracke, M.B.M., Hulsegge, B., Keeling, L., and Blokhuis, H.J., 2004a. Decision support system with semantic model to assess the risk of tail biting in pigs: 1. 'Modelling'. Appl. Anim. Behav. Sci. 87, 31-44.

Bracke, M.B.M., Hulsegge, B., Keeling, L., and Blokhuis, H.J. 2004b. Decision support system with semantic model to assess the risk of tail biting in pigs: 2. 'Validation'. Appl. Anim. Behav. Sci. 87, 45-54.

Bracke, M.B.M., Spruijt, B.M., and Metz, J.H.M. 1999. Overall animal welfare reviewed. Part 3: Welfare assessment based on needs and supported by expert opinion. Neth. J. Agricultural Sci. 47, 307-322.

Bracke, M.B.M., Zonderland, J.J., Lenskens, P., Schouten, W.G.P., Vermeer, H., Spoolder, H.A.M., Hendriks, H.J.M., and Hopster, H. 2006. Formalised review of environmental enrichment for pigs in relation to political decision making. Appl. Anim. Behav. Sci. 98, 165-182.

Breuer, K., Sutcliffe, M.E.M., Mercer, J.T., Rance, K.A., Beattie, V.E., Sneddon, I.A., and Edwards, S.A. 2003. The effect of breed on the development of adverse social behaviours in pigs. Appl. Anim. Behav. Sci. 84, 59-74.

Brunberg, E., Wallenbeck, A., and Keeling, L.J. 2011. Tail biting in fattening pigs: Associations between frequency of tail biting and other abnormal behaviours. Appl. Anim. Behav. Sci. 133, 18-25.

Camerlink, I. and Turner, S.P. 2013. The pig's nose and its role in dominance relationships and harmful behaviour. Appl. Anim. Behav. Sci. 145, 84-91.

Camerlink, I., Turner, S.P., Bijma, P., and Bolhuis, J.E. 2013. Indirect genetic effects and housing conditions in relation to aggressive behaviour in pigs. PLoS One 8: 6.

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.

Cox, L.N. and Cooper, J.J. 2001. Observations on the pre- and post-weaning behaviour of piglets reared in commercial indoor and outdoor environments. Anim. Sci. 72, 75-86.

EFSA. 2007. Scientific report on the risks associated with tail biting in pigs and possible means to reduce the need for tail docking considering the different housing and husbandry systems. Annex to the EFSA J. 611, 1-13 (Question No EFSA-Q-2006-013, 1-98).

Elliott, L., Coleman, M., Shiel, A., Wilson, B.A., Badwan, D., Menon, D., and Pickard, J. 2005. Effect of posture on levels of arousal and awareness in vegetative and minimally conscious state patients: A preliminary investigation. J. Neurol. Neurosur. Ps. 76, 298-299.

Feddes, J.J.R., Fraser, D., Buckley, D.J., and Poirier, P. 1993. Electronic sensing of non-destructive chewing by growing pigs. Trans. Am. Soc. Agric. Eng. 36, 955-958.

Fraser, D. 1987a. Attraction to blood as a factor in tail-biting by pigs. Appl. Anim. Behav. Sci. 17, 61-68.

Fraser, D. 1987b. Mineral-deficient diets and the pig's attraction to blood: implications for tail-biting. Can. J. Anim. Sci. 67, 909-918.

Fritschen, R. and Hogg, A. 1983. Preventing tail biting in swine (anti-comfort syndrome). Nebguide G 75-246 (Revised). Institute of Agriculture and Natural Resources, University of Nebraska, Lincoln, NE.

Huey, R.J. 1996. Incidence, location and interrelationships between the sites of abscesses recorded in pigs at a bacon factory in Northern Ireland. Vet. Rec. 138, 511-514.

Hunter, E.J., Jones, T.A., Guise, H.J., Penny, R.H.C., and Hoste, S. 2001. The relationship between tail biting in pigs, docking procedure and other management practices. Vet. J. 161, 72-79.

Jaeger, F. 2013. The project "intact curly tails" in pigs - Are we just before a breakthrough? Tierarztl. Umschau 68, 3-11.

Keeling, L.J., Wallenbeck, A., Larsen, A., and Holmgren, N. 2012. Scoring tail damage in pigs: An evaluation based on recordings at Swedish slaughterhouses. Acta Vet. Scand. 54, 32.

Kleinbeck, S.N. and McGlone, J.J. 1993. Pig tail posture: a measure of stress. Texas Tech. Univ. Agric. Sci. Tech. Rep. No. T-5-327, 47-48.

Korte, S.M., Prins, J., Vinkers, C.H., and Olivier, B. 2009. On the origin of allostasis and stress-induced pathology in farm animals: Celebrating Darwin's legacy. Vet. J. 182, 378-383.

LaFreniere, P. 2013. Children's play as a context for managing physiological arousal and learning emotion regulation. Psychol. Topic. 22, 2, 183-204.

Martin, P. and Bateson, P., 1993. Measuring behaviour: An introductory guide. second ed., Cambridge University Press, Cambridge, UK.

Melotti, L., Oostindjer, M., Bolhuis, J.E., Held, S., and Mendl, M. 2011. Coping personality type and environmental enrichment affect aggression at weaning in pigs. Appl. Anim. Behav. Sci. 133, 144-153.

Moinard, C., Mendl, M., Nicol, C.J., and Green, L.E. 2003. A case control study of on-farm risk factors for tail biting in pigs. Appl. Anim. Behav. Sci. 81, 333-355.

Munsterhjelm, C., Peltoniemi, O.A.T., Heinonen, M., Hälli, O., Karhapää, M., and Valros, A. 2009. Experience of moderate bedding affects behaviour of growing pigs. Appl. Anim. Behav. Sci. 118, 42-53.

Munsterhjelm, C., Simola, O., Keeling, L., Valros, A., and Heinonen, M. 2013. Health parameters in tail biters and bitten pigs in a case-control study. Animal 7, 814-821.

Oostindjer, M., Van den Brand, H., Kemp, B., and Bolhuis, J.E. 2011. Effects of environmental enrichment and loose housing of lactating sows on piglet behaviour before and after weaning. Appl. Anim. Behav. Sci. 134, 31-41.

Penny, R.H., Walters, J.R., and Tredget, S.J. 1981. Tail-biting in pigs: a sex frequency between boars and gilts. Vet. Rec. 108, 35.

Pütz, S., Jaeger, F., Wieland, C., Rohn, K., and Kaes, S. 2011. Overcoming the problem of tail biting in pigs. A contribution to solving the problem in its entirety. Tierarztl. Umschau 66, 349-354.

Reimert, I., Rodenburg, T.B., Ursinus, W.W., Duijvesteijn, N., Camerlink, I., Kemp, B., and Bolhuis, J.E. 2013. Backtest and novelty behavior of female and castrated male piglets with diverging social breeding values for growth. J. Anim. Sci. 91, 4589-4597.

Rodenburg, T.B. and Koene, P. 2007. The impact of group size on damaging behaviours, aggression, fear and stress in farm animals. Appl. Anim. Behav. Sci. 103, 205-214.

Sällvik, K. and Walberg, K. 1984. The effects of air velocity and temperature on the behaviour and growth of pigs. J. Agr. Eng. Res. 30, 305-312.

Schmolke, S.A., Li, Y.Z., and Gonyou, H.W. 2003. Effect of group size on performance of growing-finishing pigs. J. Anim. Sci. 81, 874-878.

Schrøder-Petersen D.L., Simonsen H.B., and Lawson L.G. 2003. Tail-in-mouth behaviour among weaner pigs in relation to age, gender and group composition regarding gender. Acta Agric. Scand. A-AN 53: 29-34.

Schrøder-Petersen, D.L., and Simonsen, H.B. 2001. Tail biting in pigs. Vet. J. 162, 196-210.

Smulders, D., Hautekiet, V., Verbeke, and G., Geers, R. 2008. Tail and ear biting lesions in pigs: An epidemiological study. Anim. Welfare 17, 61-69.

Statham, P., Green, L., Bichard, M., and Mendl, M. 2009. Predicting tail-biting from behaviour of pigs prior to outbreaks. Appl. Anim. Behav. Sci. 121, 157-164.

Sutherland, M.A. and Tucker, C.B. 2011. The long and short of it: A review of tail docking in farm animals. Appl. Anim. Behav. Sci. 135, 179-191.

Taylor, N.R., Main, D.C.J., Mendl, M., and Edwards, S.A. 2010. Tail-biting: A new perspective. Vet. J. 186, 137-147.

Turner, S.P. 2011. Breeding against harmful social behaviours in pigs and chickens: State of the art and the way forward. Appl. Anim. Behav. Sci. 134, 1– 9.

Valros, A., Keeling, L., Thodberg, K., and Zanella, A.J. 2012. Tail biting and tail docking in the pig: Biological mechanisms, prevention, treatment and economic aspects. Slutrapport. Nordic Joint Committee for Agricultural and Food Research (NKJ), Oslo, Norway.

Van de Weerd, H.A., Docking, C.M., Day, J.E.L., Breuer, K., and Edwards, S.A. 2006. Effects of species-relevant environmental enrichment on the behaviour and productivity of finishing pigs. Appl. Anim. Behav. Sci. 99, 230-247.

Van de Weerd, H.A., Docking, C.M., Day, J.E.L., and Edwards, S.A. 2005. The development of harmful social behaviour in pigs with intact tails and different enrichment backgrounds in two housing systems. Anim. Sci. 80, 289-298.

Van den Berg, J. 1982. Tail-biting in pigs. Causes, effects and prevention. Neth. J. Vet. Sci. 107, 735-743.

Van Putten, G. 1969. An investigation into tail-biting among fattening pigs. Brit. Vet. Jl. 125, 511-517.

Vanheukelom, V., Driessen, B., Maenhout, D., and Geers, R. 2011. Peat as environmental enrichment for piglets: The effect on behaviour, skin lesions and production results. Appl. Anim. Behav. Sci. 134, 42-47.

Wallenbeck, A. and Keeling, L.J., 2013. Using data from electronic feeders on visit frequency and feed consumption to indicate tail biting outbreaks in commercial pig production. J. Anim. Sci. 91, 2879-2884.

Wallgren, P. and Lindahl, E. 1996. The influence of tail biting on performance of fattening pigs. Acta Vet. Scand. 37, 453-460.

Wolke, D., Woods, S., and Samara, M. 2009. Who escapes or remains a victim of bullying in primary school? Brit. J. Dev. Psychol. 27, 835-851.

Wood-Gush, D.G.M. and Beilharz, R.G. 1983. The enrichment of a bare environment for animals in confined conditions. Appl. Anim. Ethol. 10, 209-217.

Yoneyama, S. and Naito, A. 2003. Problems with the Paradigm: the school as a factor in understanding bullying (with special reference to Japan). Brit. J. Sociol. Educ. 24, 315-330.

Zonderland, J.J. 2010. Talking Tails: Quantifying the development of tail biting in pigs. Doctoral Thesis, Wageningen University, Wageningen, The Netherlands.

Zonderland, J.J., Bracke, M.B.M., Den Hartog, L.A., Kemp, B., and Spoolder, H.A.M. 2010. Gender effects on tail damage development in single- or mixed-sex groups of weaned piglets. Livest. Sci. 129, 151-158.

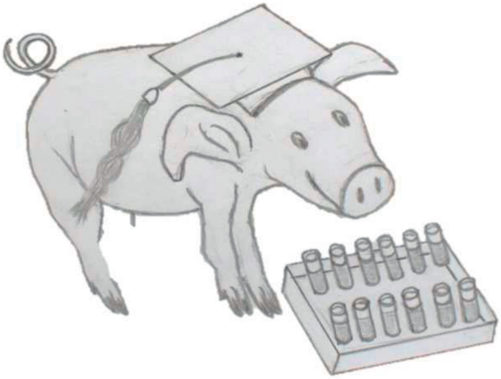
Zonderland, J.J., Kemp, B., Bracke, M.B.M., Den Hartog, L.A., and Spoolder, H.A.M. 2011a. Individual piglets contribution to the development of tail biting. Animal 5, 601-607.

Zonderland, J.J., Schepers, F., Bracke, M.B.M., Den Hartog, L.A., Kemp, B., and Spoolder, H.A.M. 2011b. Characteristics of biter and victim piglets apparent before a tail-biting outbreak. Animal 5, 767-775.

Zonderland, J.J., Van Riel, J.W., Bracke, M.B.M., Kemp, B., Den Hartog, L.A., and Spoolder, H.A.M. 2009. Tail posture predicts tail damage among weaned piglets. Appl. Anim. Behav. Sci. 121, 165-170.

Zonderland, J.J., Wolthuis-Fillerup, M., Van Reenen, C.G., Bracke, M.B.M., Kemp, B., Den Hartog, L.A., and Spoolder, H.A.M. 2008. Prevention and treatment of tail biting in weaned piglets. Appl. Anim. Behav. Sci. 110, 269-281.

Zupan, M., Janczak, A.M., Framstad, T., and Zanella, A.J. 2012. The effect of biting tails and having tails bitten in pigs. Physiol. Behav. 106, 638-644.



3

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 m^2) referred to conventional housing without enrichment substrate but with a chain; in addition to a chain, pigs housed in E pens (6.4 m^2) 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 ($HPClO_4$) 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 (10^9 cells/l) to obtain whole blood 5-HT. Platelet 5-HT level was expressed in nmol/10^9 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 ($CaCl_2$) (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* × 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.

Measures	N	Mean	SEM
Blood[a]			
Whole blood 5-HT	30	7.36	0.65
Platelet 5-HT level	31	22.74	1.32
Platelet 5-HT uptake	31	47.74	3.53
Brain[b]			
Hippocampus			
Left			
5-HT turnover	30	0.51	0.02
5-HT	30	1.41	0.11
5-HIAA	30	0.66	0.04
Right			
5-HT turnover	29	0.44	0.02
5-HT	29	1.87	0.20
5-HIAA	29	0.75	0.04
Frontal cortex			
Left			
5-HT turnover	31	0.46	0.05
5-HT	31	1.42	0.17
5-HIAA	31	0.57	0.08
Right			
5-HT turnover	31	0.47	0.04
5-HT	31	1.43	0.16
5-HIAA	31	0.62	0.07
Hypothalamus			
5-HT turnover	31	0.68	0.04
5-HT	31	3.24	0.32
5-HIAA	31	2.12	0.22

[a] 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.*

[b] 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.

Measures	**Blood**[c]		
Brain[b]	**Whole blood 5-HT**	**Platelet 5-HT level**	**Platelet 5-HT uptake**
Hippocampus			
Left			
5-HT turnover	0.15	**0.38***	-0.16
5-HT	-0.30	**-0.49****	-0.09
5-HIAA	-0.26	-0.24	-0.22
Right	0.24	**0.54****	-0.20
5-HT turnover			
5-HT	-0.27	**-0.40***	0.01
5-HIAA	-0.18	-0.02	-0.19
Frontal cortex			
Left			
5-HT turnover	-0.04	-0.14	-0.27
5-HT	-0.27	-0.08	-0.23
5-HIAA	-0.33+	-0.20	**-0.49****
Right	0.19	0.02	0.22
5-HT turnover			
5-HT	0.03	0.28	0.06
5-HIAA	0.17	0.31+	0.22
Hypothalamus			
5-HT turnover	0.02	0.27	-0.20
5-HT	-0.22	-0.30+	-0.25
5-HIAA	-0.23	-0.10	-0.34+

[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.

		NEe		NOe		
Behaviour[a]	**N**	**Mean**	**SEM**	**Mean**	**SEM**	**Test phase**[b]
Vocalizations	31	30.26	5.68	68.13	8.55	***
Excretions	31	1.61	0.25	2.71	0.23	**
Locomotion	31	57.43	1.65	46.58	1.95	**
Exploration	31	88.53	1.65	56.37	2.41	***
Standing alert	31	5.57	1.01	12.65	1.97	*
LAT approach NO	31			17.23	5.73	
LAT nosing NO	31			16.11	3.18	
Nosing NO	31			11.44	1.26	

[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[a] 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.

Measures	**Behaviour[d]**				
	Vocalizations	**Excretions**	**Locomotion**	**Exploration**	**Standing alert**
Blood[b]					
Whole blood 5-HT	0.14	0.10	0.22	-0.12	0.12
Platelet 5-HT level	0.22	0.20	0.30+	-0.06	0.07
Platelet 5-HT uptake	**0.39***	0.25	0.09	-0.06	0.20
Brain[c]					
Hippocampus					
Left					
5-HT turnover	-0.19	0.00	0.02	0.14	-0.29
5-HT	-0.23	-0.34+	-0.14	0.30	-0.15
5-HIAA	-0.28	-0.32+	-0.12	**0.38***	-0.25
Right					
5-HT turnover	-0.07	0.00	0.06	0.00	-0.18
5-HT	-0.06	0.16	-0.23	0.12	0.08
5-HIAA	-0.18	0.24	-0.26	0.21	-0.10
Frontal cortex					
Left					
5-HT turnover	-0.09	-0.22	0.17	0.08	-0.23
5-HT	-0.03	0.16	-0.33+	-0.24	0.08
5-HIAA	-0.11	-0.03	-0.19	-0.18	-0.12
Right					
5-HT turnover	0.14	0.30	-0.05	-0.04	0.05
5-HT	0.08	0.20	-0.03	-0.20	0.29
5-HIAA	0.19	**0.43***	-0.07	-0.24	0.34
Hypothalamus					
5-HT turnover	-0.16	0.19	0.29	0.29	-0.27
5-HT	0.34+	-0.11	0.13	-0.29	0.16
5-HIAA	0.21	-0.05	0.30+	-0.04	-0.05

[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[a] 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.

Measures	**Behaviour**[d]							
	Vocalizations	**Excretion**	**Locomotion**	**Exploration**	**Standing alert**	**LAT approach NO**	**LAT nosing NO**	**Nosing NO**
Blood[b]								
Whole blood 5-HT	-0.20	0.07	-0.04	**0.37***	0.00	-0.14	-0.07	0.08
Platelet 5-HT level	-0.06	0.01	0.21	**0.52****	-0.34+	-0.15	0.18	0.17
Platelet 5-HT uptake	0.11	0.08	-0.21	-0.01	0.28	-0.26	-0.12	0.16
Brain[c]								
Hippocampus								
Left								
5-HT turnover	-0.21	-0.34+	0.19	0.27	**-0.39**	0.03	0.22	0.08
5-HT	0.13	0.10	0.05	-0.13	0.21	0.27	-0.21	-0.07
5-HIAA	0.05	-0.03	0.29	0.04	-0.13	0.35+	-0.17	0.05
Right								
5-HT turnover	-0.02	-0.04	**0.44***	**0.52****	**-0.67****	0.25	0.23	0.34+
5-HT	-0.03	0.01	-0.20	**-0.37***	**0.40***	-0.02	-0.10	-0.23
5-HIAA	-0.07	0.01	0.17	-0.04	-0.08	0.20	0.05	0.03
Frontal cortex								
Left								
5-HT turnover	-0.05	0.20	-0.14	0.04	0.03	-0.01	0.20	0.04
5-HT	0.05	-0.19	0.13	-0.07	-0.24	0.13	0.12	-0.25
5-HIAA	0.00	-0.01	0.01	-0.04	-0.22	0.13	0.30+	-0.22
Right								
5-HT turnover	0.15	0.15	0.07	-0.07	0.05	0.03	-0.03	0.23
5-HT	-0.03	-0.18	0.12	0.07	-0.13	-0.14	0.09	-0.16
5-HIAA	0.08	-0.07	0.18	0.02	-0.09	-0.12	0.07	0.01
Hypothalamus								
5-HT TURN	-0.09	-0.07	0.18	0.16	-0.12	-0.15	-0.10	0.14
5-HT	0.29	0.11	0.26	-0.21	-0.04	0.19	0.14	0.09
5-HIAA	0.22	0.05	**0.39***	-0.03	-0.12	0.14	0.14	0.17

[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.*, 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-HT_2 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 focussing 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

Adeola, O., Ball, R.O., House, J.D., and O'Brien, P.J. 1993. Regional brain neurotransmitter concentrations in stress-susceptible pigs. J. Anim. Sci. 71, 968-974.

Andersen, I.L., Bøe, K.E., Foerevik, G., Janczak, A.M., and Bakken, M. 2000a. Behavioural evaluation of methods for assessing fear responses in weaned pigs. Appl. Anim. Behav. Sci. 69, 227-240.

Andersen, I.L., Foerevik, G., Bøe, K.E., Janczak, A.M., and Bakken, M. 2000b. Effects of diazepam on the behaviour of weaned pigs in three putative models of anxiety. Appl. Anim. Behav. Sci. 68, 121-130.

Andrade, R., and Beck, S.G. 2010. Cellular effects of serotonin in the CNS. In: Müller, C.R. and Jacobs, B. (Editors). Handbook of the behavioural neurobiology of serotonin. Academic Press, London, pp. 219-231.

Antoniadis, E.A. and McDonald, R.J. 2000. Amygdala, hippocampus and discriminative fear conditioning to context. Behav. Brain. Res. 108, 1-19.

Antoniadis, E.A. and McDonald, R.J. 2001. Amygdala, hippocampus, and unconditioned fear. Exp. Brain. Res. 138, 200-209.

Askenazy, F., Caci, H., Myquel, M., Darcourt, G., and Lecrubier, Y. 2000. Relationship between impulsivity and platelet serotonin content in adolescents. Psychiat. Res. 94, 19-28.

Badcock, N.R., Spence, J.G., and Stern, L.M. 1987. Blood serotonin levels in adults, autistic and non-autistic children - with a comparison of different methodologies. Ann. Clin. Biochem. 24, 625-634.

Bakish, D., Cavazzoni, P., Chudzik, J., Ravindran, A., and Hrdina, P.D. 1997. Effects of selective serotonin reuptake inhibitors on platelet serotonin parameters in major depressive disorder. Biol. Psychiat. 41, 184-190.

Barf, T., Korte, S.M, Korte-Bouws, G., Sonesson, C., Damsma, G., Bohus, B., and Wikström, H. 1996. Potential anxiolytic properties of R-(+)-8-OSO2CF3-PAT, a 5-HT1A receptor agonist. Eur. J. Pharmacol. 97, 205-211.

Barton, D.A., Esler, M.D., Dawood, T., Lambert, E.A., Haikerwal, D., Brenchley, C, Socratous, F., Hastings, J., Guo, L., Wiesner, G., Kaye, D.M., Bayles, R., Schlaich, M.P., and Lambert, G.W. 2008. Elevated brain serotonin turnover in patients with depression: Effect of genotype and therapy. Arch. Gen. Psychiat. 65, 38-46.

Beattie, V.E., Breuer, K., O'Connell, N.E., Sneddon, I.A., Mercer, J.T., Rance, K.A., Sutcliffe, M.E.M. and Edwards, S.A. 2005. Factors identifying pigs predisposed to tail biting. Animal Sci. 80, 307-312.

Benetti, F., De Araujo, P.A., Sanvitto, G.L., and Lucion, A.B. 2007. Effects of neonatal novelty exposure on sexual behavior, fear, and stress-response in adult rats. Dev. Psychobiol. 49, 258-264.

Berumen, L.C., Rodríguez, A., Miledi, R., and García-Alcocer, G. 2012. Serotonin receptors in hippocampus. TheScientificWorldJo. 2012, 823493.

Besiroglu, L., Çetinkaya, N., Selvi, Y., and Atli, A. 2011. Effects of selective serotonin reuptake inhibitors on thought-action fusion, metacognitions, and thought suppression in obsessive-compulsive disorder. Compr. Psychiat. 52, 556-561.

Bianchi, M., Moser, C., Lazzarini, C., Vecchiato, E., and Crespi, F. 2002. Forced swimming test and fluoxetine treatment: In vivo evidence that peripheral 5-HT in rat platelet-rich plasma mirrors cerebral extracellular 5-HT levels, whilst 5-HT in isolated platelets mirrors neuronal 5-HT changes. Exp. Brain Res. 143, 191-197.

Boissy, A. 1995. Fear and fearfulness in animals. Q. Rev. Biol. 70, 165-191.

Bolhuis, J.E., Ellen, E.D., Van Reenen, C.G., De Groot, J, Napel, J.T., Koopmanschap, R.E., De Vries Reilingh G., Uitdehaag K.A., Kemp B., and Rodenburg T.B. 2009. Effects of genetic group selection against mortality on behavior and peripheral serotonin in domestic laying hens with trimmed and intact beaks. Physiol. Behav. 97, 470-475.

Bolhuis, J.E., Schouten, W.G.P., Schrama, J.W., and Wiegant, V.M. 2006. Effects of rearing and housing environment on behaviour and performance of pigs with different coping characteristics. Appl. Anim. Behav. Sci. 101, 68-85.

Bracke, M.B.M. 2008. RICHPIG: A semantic model to assess enrichment materials for pigs. Anim. Welfare. 17, 289-304.

Bracke, M.B.M., Spruijt, B.M., and Metz, J.H.M. 1999. Overall animal welfare reviewed. Part 3: Welfare assessment based on needs and supported by expert opinion. Neth. J. Agr. Sci. 47, 307-322.

Brennan, M.J., Dallob, A., and Friedman, E. 1981. Involvement of hippocampal serotonergic activity in age-related changes in exploratory behaviour. Neurobiol. Aging. 2, 199-203.

Buckley, M.F., James, J.W., Brown, D.E, Whyte, G.S., Dean, M.G., Chesterman, C.N., and Donald, J.A. 2000. A novel approach to the assessment of variations in the human platelet count. Thromb Haemostasis. 83, 480-484.

Butler, J., O'Halloran, A., and Leonard, B.E. 1992. The Galway study of panic disorder II: Changes in some peripheral markers of noradrenergic and serotonergic function in DSM III-R panic disorder. J. Affect. Disorders. 26, 89-99.

Campbell, B.M. and Merchant, K.M. 2003. Serotonin 2C receptors within the basolateral amygdala induce acute fear-like responses in an open-field environment. Brain. Res. 993, 1-9.

Campler, M., Jöngren, M., and Jensen, P. 2009. Fearfulness in red junglefowl and domesticated White Leghorn chickens. Behav. Process. 81, 39-43.

Caramaschi, D., De Boer, S.F., and Koolhaas, J.M. 2007. Differential role of the 5-HT1A receptor in aggressive and non-aggressive mice: An across-strain comparison. Physiol. Behav. 90, 590-601.

Carlson, J.N. and Glick, S.D. 1989. Cerebral lateralization as a source of interindividual differences in behavior. Experientia. 45, 788-798.

Cavigelli, S.A., Michael, K.C., West, S.G., and Klein, L.C. 2011. Behavioral responses to physical vs. social novelty in male and female laboratory rats. Behav. Process. 88, 56-59.

Celada, P, Martín, F., and Artigas, F. 1994. Effects of chronic treatment with dexfenfluramine on serotonin in rat blood, brain and lung tissue. Life Sci. 55, 1237-1243.

Chou, DT, Cuzzone, H, and Hirsh, KR. 1983. Assessment of blood platelets as a model for CNS response: Comparative effects of caffeine on 5-HT uptake and release mechanisms in rat platelets and rat brain serotonin neurons. Life Sci. 33, 1149-1156.

Colpaert, F.C. 1975. The ventromedial hypothalamus and the control of avoidance behavior and aggression: fear hypothesis versus response suppression theory of limbic system function. Behav. Biol. 15, 27-44.

Curzon, G. and Green, A.R. 1970. Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. Brit. J. Pharmacol. 39, 653-655.

Da Prada, M. and Picotti, G.B. 1979. Content and subcellular localization of catecholamines and 5-hydroxytryptamine in human and animal blood platelets: monoamine distribution between platelets and plasma. Brit. J. Pharmacol. 65, 653-662.

Dayan, P. and Huys, Q.J.M. 2009. Serotonin in affective control. Annu. Rev. Neurosci. 32, 95–126.

Delrue, C., Deleplanque, B., Rougepont, F., Vitiello, S., and Neveu, P.J. 1994. Brain monoaminergic, neuroendocrine, and immune responses to an immune challenge in relation to brain and behavioral lateralization. Brain Behav. Immun. 8, 137-152.

Désiré, L., Veissier, I., Després, G., and Boissy, A. 2004. On the way to assess emotions in animals: Do lambs (Ovis aries) evaluate an event through its suddenness, novelty, or unpredictability? J. Comp. Psychol. 118, 363-374.

Dos Santos, L., De Andrade, T.G., and Graeff, F.G. 2010. Social separation and diazepam withdrawal increase anxiety in the elevated plus-maze and serotonin turnover in the median raphe and hippocampus. J. Psychopharmacol. 24, 725-731.

Edwards, S.A. 2006. Tail biting in pigs: Understanding the intractable problem. Vet. J. 171, 198-199.

Félix, B., Léger, M.E., Albe-Fessard, D., Marcilloux, J.C., Rampin, O., Laplace, J.P., Duclos, A., Fort, F., Gougis, S., Costa, M., and Duclos, N. 1999. Stereotaxic atlas of the pig brain. Brain Res. Bull. 49, 1-137.

Fitzgerald, P.J. 2012. Whose side are you on: Does serotonin preferentially activate the right hemisphere and norepinephrine the left? Med. Hypotheses. 79, 250-254.

Forkman, B., Boissy, A., Meunier-Salaün, M.C., Canali, E., and Jones, R.B. 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. Physiol. Behav. 92, 340-374.

Franke, L., Schewe, H.J., Müller, B., Campman, V., Kitzrow, W., Uebelhack, R., Berghöfer A, and Müller-Oerlinghausen B. 2000. Serotonergic platelet variables in unmedicated patients suffering from major depression and healthy subjects: Relationship between 5HT content and 5HT uptake. Life Sci. 67, 301-315.

Gentry, J.G., McGlone, J.J., Blanton Jr, J.R., and Miller, M.F. 2002. Alternative housing systems for pigs: Influences on growth, composition, and pork quality. J. Anim. Sci. 80, 1781-1790.

Hansenne, M., Pitchot, W., and Ansseau, M. 2002. Serotonin, personality and borderline personality disorder. Acta Neuropsychiatr. 14, 66-70.

Hashimoto, S., Inoue, T., and Koyama, T. 1999. Effects of conditioned fear stress on serotonin neurotransmission and freezing behavior in rats. Eur. J. Pharmacol. 378, 23-30.

Hecht, D. 2010. Depression and the hyperactive right-hemisphere. Neurosci. Res. 68, 77-87.

Hessing, M.J.C., Hagelsø, A.M., Van Beek, J.A.M., Wiepkema, R.P., Schouten, W.G.P., and Krukow, R. 1993. Individual behavioural characteristics in pigs. Appl. Anim. Behav. Sci. 37, 285-295.

Inderbitzen, H.M. and Hope, D.A. 1995. Relationship among adolescent reports of social anxiety, anxiety, and depressive symptoms. J. Anxiety Disord. 9, 385-396.

Jernej, B., Banović, M., Cicin-Šain, L., Hranilović, D., Balija, M., Orešković, D., and Folnegović-Smalc, V. 2000. Physiological characteristics of platelet/circulatory serotonin: Study on a large human population. Psychiat. Res. 94, 153-162.

Jernej, B. and Cicin-Šain, L. 1990. Platelet serotonin level in rats is under genetic control. Psychiat Res. 32, 167-174.

Kluge, H., Bolle., M., Reuter, R., Werner, S., Zahlten, W., and Prudlo, J. 1999. Serotonin in platelets: Comparative analyses using new enzyme immunoassay and HPLC test kits and the traditional fluorimetric procedure. LaboratoriumsMedizin. 23, 360-364.

Koolhaas, J.M., De Boer, S.F., Coppens, C.M., and Buwalda, B. 2010. Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. Front. Neuroendocrin. 31, 307-321.

Korpi, E.R., Kleinman, J.E., and Goodman, S.I. 1986. Serotonin and 5-hydroxyindoleacetic acid in brains of suicide victims. Comparison in chronic schizophrenic patients with suicide as cause of death. Arch. Gen. Psychiat. 43, 594-600.

Korte, S.M. 2001. Corticosteroids in relation to fear, anxiety and psychopathology. Neurosci. Biobehav. R. 25, 117-142.

Korte, S.M. and De Boer, S.F. 2003. A robust animal model of state anxiety: Fear-potentiated behaviour in the elevated plus-maze. Eur. J. Pharmacol. 463, 163-175.

Korte-Bouws, G.A.H., Korte, S.M., De Kloet, E.R., and Bohus, B. 1996. Blockade of corticosterone synthesis reduces serotonin turnover in the dorsal hippocampus of the rat as measured by microdialysis. J. Neuroendocrinol. 8, 877-881.

Kruk, M.R., Westphal, K.G.C., Van Erp, A.M.M., Van Asperen, J, Cave, B.J., Slater, E., De Koning J., Haller J. 1998. The hypothalamus: Cross-roads of endocrine and behavioural regulation in grooming and aggression. Neurosci. Biobehav. R. 23, 163-177.

Lauber, M.C., Hemsworth, P.H., and Barnett, J.L. 2009. The effect of breed, time spent with dam and late pregnancy induction of parturition on behavioural development in dairy calves. Behav. Process. 82, 301-311.

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

Lucki, I. 1998. The spectrum of behaviors influenced by serotonin. Biol. Psychiat. 44:151-162.

Malyszko, J., Urano, T., Serizawa, K., Yan, D., Kozima, Y., Takada, Y., and Takada, A. 1993. Serotonergic measures in blood and brain and their correlations in rats treated with tranylcypromine, a monoamine oxidase inhibitor. Jpn. J. Physiol. 43, 613-626.

Melotti, L., Oostindjer, M., Bolhuis, J.E., Held, S., and Mendl, M. 2011. Coping personality type and environmental enrichment affect aggression at weaning in pigs. Appl. Anim. Behav. Sci. 133, 144-153.

Mück-Šeler, D. and Pivac, N. 2011. Serotonin. Period. Biol. 113, 29-41.

Munsterhjelm, C., Peltoniemi, O.A.T., Heinonen, M., Hälli, O., Karhapää, M., and Valros, A. 2009. Experience of moderate bedding affects behaviour of growing pigs. Appl. Anim. Behav. Sci. 118, 42-53.

Olton, D.S., Wenk, G.L., Church, R.M., and Meck, W.H. 1988. Attention and the frontal cortex as examined by simultaneous temporal processing. Neuropsychologia. 26, 307-318.

Oostindjer, M., Van den Brand, H., Kemp, B., and Bolhuis, J.E. 2011. Effects of environmental enrichment and loose housing of lactating sows on piglet behaviour before and after weaning. Appl. Anim. Behav. Sci. 134, 31-41.

Ostrowitzki, S., Rao, M.L., Redei, J., and Andres, A.H. 1993. Concurrence of cortex and platelet serotonin2 receptor binding characteristics in the individual and the putative regulation by serotonin. J. Neural Transm. - General Section. 93, 27-35.

Oquendo, M.A. and Mann, J.J. 2001. Neuroimaging findings in major depression, suicidal behavior and aggression. Clin. Neurosci. Res. 1, 377-380.

Parsey, R.V., Oquendo, M.A., Simpson, N.R., Ogden, R.T., Van Heertum, R., Arango, V., and Mann, J.J. 2002. Effects of sex, age, and aggressive traits in man on brain serotonin 5-HT 1A receptor binding potential measured by PET using [C-11]WAY-100635. Brain Res. 954, 173-182.

Pazos, A., Hoyer, D., and Palacios, J.M. 1985. Mesulergine, a selective serotonin-2 ligand in the rat cortex, does not label these receptors in porcine and human cortex: Evidence for species differences in brain serotonin-2 receptors. Eur. J. Pharmacol. 106, 531-538.

Pietraszek, M.H., Takada, Y., Yan, D., Urano, T., Serizawa, K., and Takada, A. 1992a. Relationship between serotonergic measures in periphery and the brain of mouse. Life Sci. 51, 75-82.

Pietraszek, M.H., Yan, D., Urano, T., Sakakibara, K., Serizawa, K., Takada, Y., and Takada A. 1992b. Correlations between serotonin and related substances simultaneously obtained from rat brain and blood. Biogenic Amines. 9, 123-129.

Prunier, A., Heinonen, M., and Quesnel, H. 2010. High physiological demands in intensively raised pigs: Impact on health and welfare. Animal. 4, 886-898.

Pussard, E., Guigueno, N., Adam, O., and Giudicelli, J.F. 1996. Validation of HPLC-amperometric detection to measure serotonin in plasma, platelets, whole blood, and urine. Clin. Chem. 42, 1086-1091.

Rausch, J.L., Johnson, M.E., Li, J., Hutcheson, J., Carr, B.M., Corley, K.M., Gowans A.B., and Smith J. 2005. Serotonin transport kinetics correlated between human platelets and brain synaptosomes. Psychopharmacology. 180, 391-398.

Reimert, I., Bolhuis, J.E., Kemp, B., and Rodenburg, T.B. 2013. Indicators of positive and negative emotions and emotional contagion in pigs. Physiol. Behav. 109, 42-50.

Ritvo, E., Yuwiler, A., Geller, E., Plotkin, S., Mason, A., and Saeger, K. 1971. Maturational changes in blood serotonin levels and platelet counts. Biochem. Med. 5, 90-96.

Rogers, L.J. 2010. Relevance of brain and behavioural lateralization to animal welfare. Appl. Anim. Behav. Sci. 127, 1-11.

Rosochacki, S.J., Piekarzewska, A.B., Połoszynowicz, J., and Sakowski, T. 2003. Genetic differences in brain monoamines level in Pietrain and Duroc pigs exposed to acute restraint stress. J. Anim. Breed. Genet. 120, 192-209.

Sandi, C., and Pinelo-Nava, M.T. 2007. Stress and memory: Behavioral effects and neurobiological mechanisms. Neural Plast. 2007, 78970.

Sánchez, C. 2003. R-citalopram attenuates anxiolytic effects of escitalopram in a rat ultrasonic vocalisation model. Eur J. Pharmacol. 464, 155-158.

Smith, L.T., Hanson, D.R., and Omenn, G.S. 1978. Comparisons of serotonin uptake by blood platelets and brain synaptosomes. Brain. Res. 146, 400-403.

Smith, D.F., Hansen, S.B., Østergaard, L., Gee, A.D., Danielsen, E., Ishizu, K., Bender D., Poulsen P.H., and Gjedde A. 2001. [14C]Serotonin uptake and [O-methyl-11C]venlafaxine kinetics in porcine brain. Nucl. Med. Biol. 28, 633-638.

Smulders, D., Hautekiet, V., Verbeke, G., and Geers, R. 2008. Tail and ear biting lesions in pigs: An epidemiological study. Anim. Welfare. 17, 61-69.

Song, G.H., Gwee, K.A., Moochhala, S.M., and Ho, K.Y. 2005. Melatonin attenuates stress-induced defecation: Lesson from a rat model of stress-induced gut dysfunction. Neurogastroent. Motil. 17, 744-750.

Stahl, S.M. 1977. The human platelet. A diagnostic and research tool for the study of biogenic amines in psychiatric and neurologic disorders. Arch. Gen. Psychiat. 34, 509-516.

Stanley, W.C. and Jaynes, J. 1949. The function of the frontal cortex. Psychol. Rev. 56, 18-32.

Thies-Flechtner, K., Weigel, I., and Muller-Oerlinghausen, B. 1994. 5-HT uptake in platelets of lithium-treated patients with affective disorders and of healthy controls. Pharmacopsychiatry, Supplement. 27, 4-6.

Thor, K.B. 2004. Targeting serotonin and norepinephrine receptors in stress urinary incontinence. Int. J. Gynecol. Obstet. 86, S38-S52.

Uitdehaag, K.A., Rodenburg, T.B., Van Hierden, Y.M., Bolhuis, J.E., Toscano, M.J., Nicol, C.J., and Komen J. 2008. Effects of mixed housing of birds from two genetic lines of laying hens on open field and manual restraint responses. Behav. Process. 79, 13-18.

Uitdehaag, K.A., Rodenburg, T.B., Van Reenen, C.G., Koopmanschap, R.E., De Vries, G.R., Engel, B., Buist W.G., Komen H., and Bolhuis, J.E. 2011. Effects of genetic origin and social environment on behavioral response to manual restraint and monoamine functioning in laying hens. Poultry Sci. 90, 1629-1636.

Vanderwolf, C.H. 1989. A general role for serotonin in the control of behavior: Studies with intracerebral 5,7-dihydroxytryptamine. Brain. Res. 504, 192-198.

Van der Staay, F.J., Schuurman, T., Van Reenen, C.G., and Korte, S.M. 2009. Emotional reactivity and cognitive performance in aversively motivated tasks: A comparison between four rat strains. Behav. Brain. Funct. 5, 50.

Van de Weerd, H.A., Docking, C.M., Day, J.E.L., Breuer, K., and Edwards, S.A. 2006. Effects of species-relevant environmental enrichment on the behaviour and productivity of finishing pigs. Appl. Anim. Behav. Sci. 99, 230-247.

Van Hierden, Y.M., Korte, S.M., Ruesink, E.W., Van Reenen, C.G., Engel, B., Korte-Bouws, G.A.H., Koolhaas J.M., Blokhuis, H.J. 2002. Adrenocortical reactivity and central serotonin and dopamine turnover in young chicks from a high and low feather-pecking line of laying hens. Physiol. Behav. 75, 653-659.

Van Reenen, C.G., Engel, B., Ruis-Heutinck, L.F.M., Van Der Werf, J.T.N., Buist, W.G., Jones, R.B., and Blokhuis, H.J. 2004. Behavioural reactivity of heifer calves in potentially alarming test situations: A multivariate and correlational analysis. Appl. Anim. Behav. Sci. 85, 11-30.

Varty, G.B., Morgan, C.A., Cohen-Williams, M.E., Coffin, V.L., and Carey, G.J. 2002. The gerbil elevated plus-maze I: Behavioral characterization and pharmacological validation. Neuropsychopharmacol. 27, 357-370.

Veenema, A.H., Koolhaas, J.M., and De Kloet, E.R. 2004. Basal and stress-induced differences in HPA axis, 5-HT responsiveness, and hippocampal cell proliferation in two mouse lines. Ann. NY. Acad. Sci. 1018, 255–265.

Vicens-Costa, E., Martínez-Membrives, E., López-Aumatell, R., Guitart-Masip, M., Cañete, T., Blázquez, G., Tobeña A., and Fernández-Teruel A. 2011. Two-way avoidance acquisition is negatively related to conditioned freezing and positively associated with startle reactions: A dissection of anxiety and fear in genetically heterogeneous rats. Physiol. Behav. 103, 148-156.

Wang, C.C., Billett, E., Borchert, A., Kuhn, H., and Ufer, C. 2013. Monoamine oxidases in development. Cell. Mol. Life. Sci. 70, 599-630.

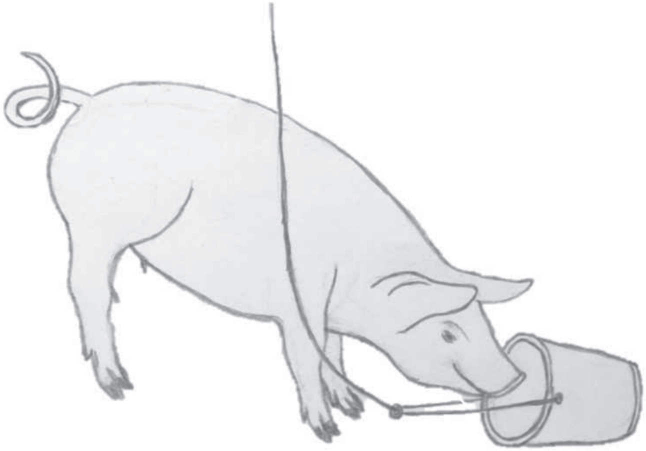
Weiss, J.M., Goodman, P.A., and Losito, B.G. 1981. Behavioral depression produced by an uncontrollable stressor: Relationship to norepinephrine, dopamine, and serotonin levels in various regions of rat brain. Brain. Res. Rev. 3, 167-205.

Yatham, L.N., Steiner, M., Liddle, P.F., Shiah, I.S., Lam, R.W., Zis, A.P., and Coote M. 2000. A PET study of brain 5-HT2 receptors and their correlation with platelet 5-HT2 receptors in healthy humans. Psychopharmacology. 151, 424-427.

Yuwiler, A., Brammer, G.L., Morley, J.E., Raleigh, M.J., Flannery, J.W., and Geller, E. 1981. Short-term and repetitive administration of oral tryptophan in normal men. Effects on blood tryptophan, serotonin, and kynurenine concentrations. Arch. Gen. Psychiat. 38, 619-626.

Zonderland, J.J., Wolthuis-Fillerup, M., Van Reenen, C.G., Bracke, M.B.M., Kemp, B., Den Hartog, L.A., and Spoolder, H.A.M. 2008. Prevention and treatment of tail biting in weaned piglets. Appl. Anim. Behav. Sci. 110, 269-281.

Zupan, M., Janczak, A.M., Framstad, T., and Zanella, A.J. 2012. The effect of biting tails and having tails bitten in pigs. Physiol. Behav. 106, 638-644.



4

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 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).

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.

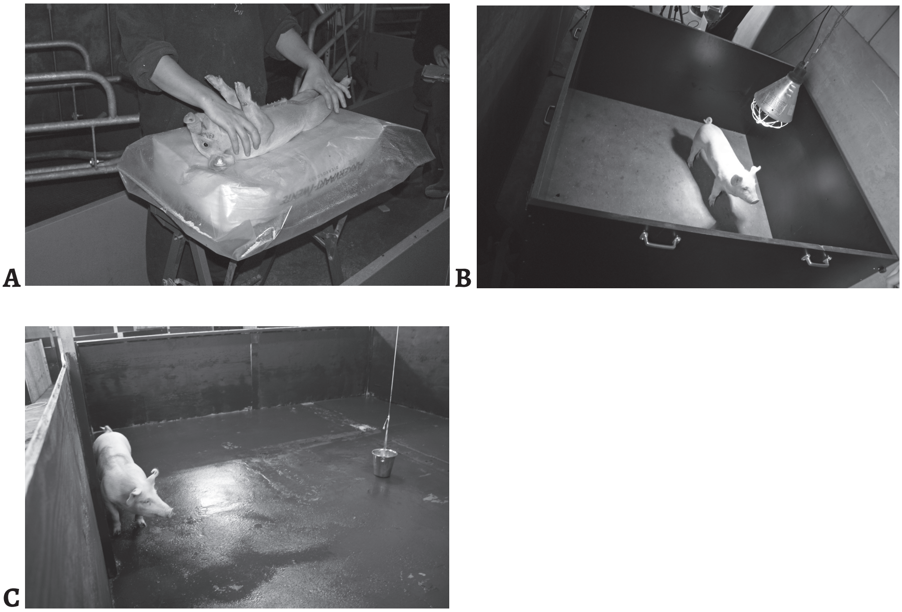

Figure 1. Pigs subjected to three behavioural tests. **A**: Back test. **B**: Pre-weaning novel environment exposure (pNEe). **C**: Novel object exposure (NOe).

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 (Δ t = 15-0) (i.e. cortisol rise from the first sample to the highest observed peak), and between the second and final sample (Δ 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[a].

Blood measures[b]	Barren	Enriched	P-value
Whole blood 5-HT T1	12.6 ± 0.52	11.4 ± 0.52	
Whole blood 5-HT T2	12.0 ± 0.60	11.9 ± 0.60	
Whole blood 5-HT T3	7.3 ± 0.48	7.6 ± 0.48	
Platelet 5-HT uptake T3	45.4 ± 1.43	40.1 ± 1.41	*
Platelet 5-HT level T1	22.9 ± 0.80	21.3 ± 0.80	+
Platelet 5-HT level T2	20.2 ± 1.00	20.7 ± 1.01	
Platelet 5-HT level T3	18.4 ± 0.91	19.5 ± 0.90	

[a] Untransformed LSmeans ± SEM.

[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*.

+ 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[a].

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

[a] Untransformed LSmeans ± SEM.

[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*.

+ $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

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[a].

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

[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* 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.

+ $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

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[a].

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

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

[a] 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[a] 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.

Measures	**Early life exploration**	**Near bucket**	**Cortisol**	**Vocalizations & standing alert**	**Back test activity**
Back test					
No. of struggles	-0.09	-0.07	0.08	-0.05	***0.72***
No. of vocalizations	-0.01	-0.04	0.12	-0.03	***0.80***
Pre-weaning novel environment exposure					
Exploration (%)	***0.75***	0.01	0.02	-0.08	-0.16
Walking (%)	***0.78***	0.02	0.03	-0.03	0.23
Standing alert (%)	***-0.78***	-0.03	-0.05	-0.01	-0.08
No. of vocalizations	0.24	0.05	-0.03	0.20	*0.42*
Novel object exposure					
No. of vocalizations	0.08	0.14	-0.16	*0.41*	*0.32*
Standing alert (%)	-0.19	*-0.33*	-0.01	***0.82***	-0.12
Exploration (%)	0.02	*-0.36*	0.05	***-0.82***	0.00
Exploring bucket (%)	0.07	***0.93***	-0.04	0.01	-0.02
Distance covered (m)	0.16	*0.36*	-0.19	-0.12	*0.41*
At door zone (%)	-0.01	*-0.33*	-0.01	-0.03	0.01
At wall zone (%)	-0.01	***-0.82***	0.09	-0.01	0.04
Salivary cortisol					
Basal cortisol	-0.01	-0.07	***0.85***	0.00	-0.02
Cortisol change (t=15 - t=0)	-0.14	0.19	0.07	0.08	0.09
Cortisol change (t=15 - t=60)	-0.06	0.02	-0.27	0.09	-0.05
Cortisol area under the curve	-0.04	0.06	***0.88***	0.04	-0.01
Variance explained (%)	**20.79**	**24.85**	**15.93**	**14.39**	**17.06**

[a] Loadings > 0.30 or < -0.30 are indicated in italics, and loadings > 0.50 or < -0.50 are also indicated in bold.

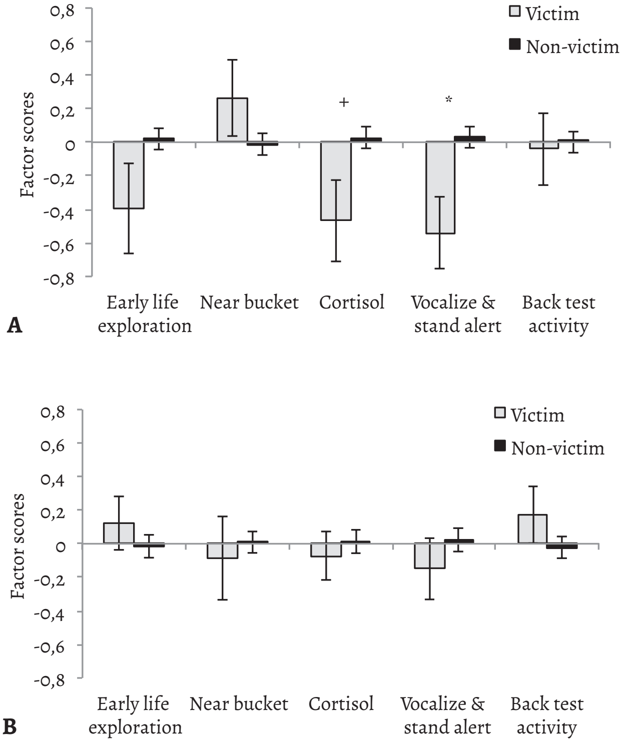

Figure 2. Factor scores of victims and non-victims of tail biting at time of weaning. Behavioural and physiological responses of pigs to novelty (pre- and post-weaning) were summarized in five factors using a PCA. Factor scores are presented per type of pig, i.e. victim (with tail wound) or non-victim (without tail wound) at time of weaning. **A:** Barren housing. **B:** Enriched housing. + $P < 0.10$, * $P < 0.05$.

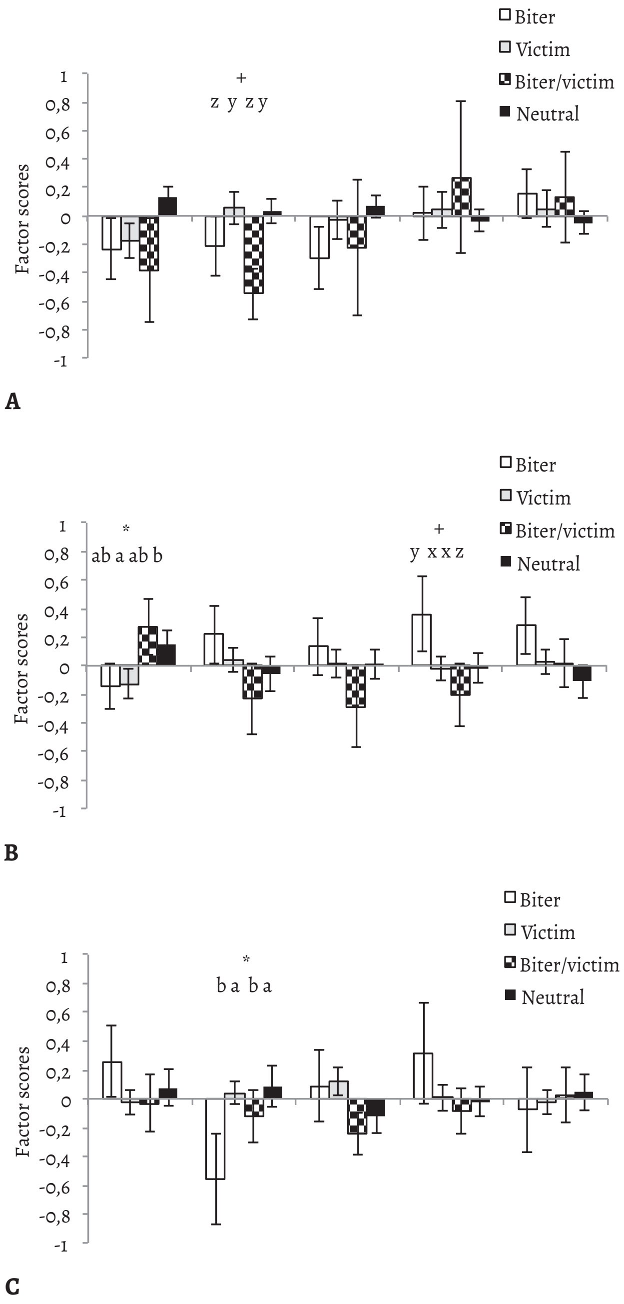

Figure 3. Factor scores of barren housed pigs classified as biters, victims, biter/victims or neutrals with respect to tail biting post-weaning. Behavioural and physiological responses of pigs to novelty (pre- and post-weaning) were summarized in five factors using a PCA. Factor scores are presented 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).

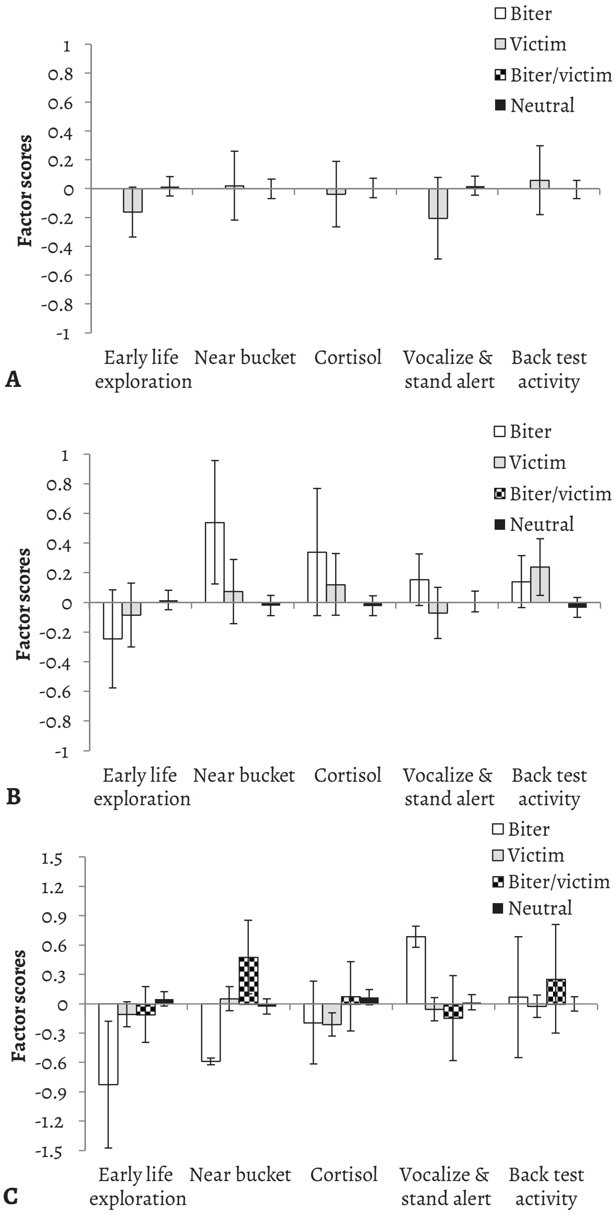

Figure 4. Factor scores of enriched housed pigs classified as biters, victims, biter/victims or neutrals with respect to tail biting post-weaning. Behavioural and physiological responses of pigs to novelty (pre- and post-weaning) were summarized in five factors using a PCA. Factor scores are presented 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 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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References

Ashwani, A., Tarun, K., Ajay, M., and Anil, H. 2011. Anxiety Disorders: A Review. IRJP 2, 18-23.

Azmitia, E.C. 2010. Evolution of serotonin: Sunlight to suicide. In: Müller, C.R. and Jacobs, B. (Editors). Handbook of the behavioural neurobiology of serotonin. Academic Press, London, pp. 3-22.

Beattie, V.E., Walker, N., and Sneddon, I.A. 1996. An investigation of the effect of environmental enrichment and space allowance on the behaviour and production of growing pigs. Appl. Anim. Behav. Sci. 48, 151-158.

Bell, A.M. and Sih, A. 2007. Exposure to predation generates personality in threespined sticklebacks (Gasterosteus aculeatus). Ecol. Lett. 10, 828-834.

Bolhuis, J.E., Ellen, E.D., Van Reenen, C.G., De Groot, J., Napel, J.T., Koopmanschap, R.E., De Vries Reilingh, G., Uitdehaag, K.A., Kemp, B., and Rodenburg, T.B. 2009. Effects of genetic group selection against mortality on behavior and peripheral serotonin in domestic laying hens with trimmed and intact beaks. Physiol. Behav. 97, 470-475.

Bolhuis, J.E., Parmentier, H.K., Schouten, W.G.P., Schrama, J.W., and Wiegant, V.M. 2003. Effects of housing and individual coping characteristics on immune responses of pigs. Physiol. Behav. 79, 289-296.

Bolhuis, J.E., Schouten, W.G.P., De Jong, I.C., Schrama, J.W., Cools, A.R., and Wiegant, V.M. 2000. Responses to apomorphine of pigs with different coping characteristics. Psychopharmacology. 152, 24-30.

Bolhuis, J.E., Schouten, W.G.P., Leeuw, J.A.D., Schrama, J.W., and Wiegant, V.M. 2004. Individual coping characteristics, rearing conditions and behavioural flexibility in pigs. Behav. Brain. Res. 152, 351-360.

Brunberg, E., Jensen, P., Isaksson, A., and Keeling, L.J. 2011. Feather pecking behavior in laying hens: Hypothalamic gene expression in birds performing and receiving pecks. Poultry Sci. 90, 1145-1152.

Brunberg, E., Jensen, P., Isaksson, A., and Keeling, L.J. 2013a. Behavioural and brain gene expression profiling in pigs during tail biting outbreaks - Evidence of a tail biting resistant phenotype. PLoS One 8: 6.

Brunberg, E., Jensen, P., Isaksson, A., and Keeling, L.J. 2013b. Brain gene expression differences are associated with abnormal tail biting behavior in pigs. Genes Brain. Behav. 12, 275-281.

Butler, J., O'Halloran, A., and Leonard, B.E. 1992. The Galway study of panic disorder II: Changes in some peripheral markers of noradrenergic and serotonergic function in DSM III-R panic disorder. J. Affect. Disorders. 26, 89-99.

Camerlink, I., Turner, S.P., Bijma, P., and Bolhuis, J.E. 2013. Indirect genetic effects and housing conditions in relation to aggressive behaviour in pigs. PLoS One 8: 6.

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.

Carver, C.S. and Miller, C.J. 2006. Relations of serotonin function to personality: Current views and a key methodological issue. Psychiat. Res. 144, 1-15.

Celada, P., Martín, F., and Artigas, F. 1994. Effects of chronic treatment with dexfenfluramine on serotonin in rat blood, brain and lung tissue. Life Sci. 55, 1237-1243.

Cleare, A.J. 1997. Reduced whole blood serotonin in major depression. Depress. Anxiety 5, 108-111.

Coppen, A., Turner, P., Rowsell, and A.R., Padgham, C. 1976. 5 Hydroxytryptamine (5 HT) in the whole blood of patients with depressive illness. Postgrad. Med. J. 52, 156-158.

Crespi, B.J. 2000. The evolution of maladaptation. Heredity 84, 623-629.

Curzon, G. and Green, A.R. 1970. Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. Brit. J. Pharmacol. 39, 653-655.

Da Prada, M., and Picotti, G.B. 1979. Content and subcellular localization of catecholamines and 5-hydroxytryptamine in human and animal blood platelets: monoamine distribution between platelets and plasma. Brit. J. Pharmacol. 65, 653-662.

De Haas, E.N., Bolhuis, J.E., Kemp, B., Groothuis, T.G.G., and Rodenburg, T.B. 2014. Parents and early life environment affect behavioral development of laying hen chickens. PLoS One 9: 3.

De Haas, E.N., Kemp, B., Bolhuis, J.E., Groothuis, T., and Rodenburg, T.B. 2013. Fear, stress, and feather pecking in commercial white and brown laying hen parent-stock flocks and their relationships with production parameters. Poultry Sci. 92, 2259-2269.

De Jong, I.C., Prelle, I.T., Van de Burgwal, J.A., Lambooij, E., Korte, S.M., Blokhuis, H.J., and Koolhaas, J.M. 2000. Effects of environmental enrichment on behavioral responses to novelty, learning, and memory, and the circadian rhythm in cortisol in growing pigs. Physiol. Behav. 68, 571-578.

Delorme, R., Betancur, C., Callebert, J., Chabane, N., Laplanche, J.L., Mouren-Simeoni, M.C. Launay, J.M., and Leboyer, M. 2005. Platelet serotonergic markers as endophenotypes for obsessive-compulsive disorder. Neuropsychopharmacol. 30, 1539-1547.

Dickerson, S.S. and Kemeny, M.E. 2004. Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. Psychol. Bull. 130, 355-391.

EFSA. 2007a. Scientific report on animal health and welfare aspects of different housing and husbandry systems for adult breeding boars, pregnant, farrowing sows and unweaned piglets. The EFSA J. 572, 1-107 (Question No EFSA-Q-2006-28).

EFSA. 2007b. Scientific report on the risks associated with tail biting in pigs and possible means to reduce the need for tail docking considering the different housing and husbandry systems. Annex to the EFSA J. 611, 1-13 (Question No EFSA-Q-2006-013, 1-98).

Fichtner, C.G., O'Connor, F.L., Yeoh, H.C., Arora, R.C., and Crayton, J.W. 1995. Hypodensity of platelet serotonin uptake sites in posttraumatic stress disorder: Associated clinical features. Life Sci, 57, PL37-44.

Forkman, B., Boissy, A., Meunier-Salaün, M.C., Canali, E., and Jones, R.B. 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. Physiol. Behav. 92, 340-374.

Friedman, B.H. and Thayer, J.F. 1998. Anxiety and autonomic flexibility: A cardiovascular approach. Biol. Psychol. 47, 243-263.

Gershenfeld, H.K. and Paul, S.M. 1997. Mapping quantitative trait loci for fear-like behaviors in mice. Genomics 46, 1-8.

Ghaffari, M.S. and Sabzevari, A. 2010. Successful management of psychogenic alopecia with buspirone in a crossbreed cat. Comp. Clin. Path. 19, 317-319.

Hashimoto, S., Inoue, T., and Koyama, T. 1999. Effects of conditioned fear stress on serotonin neurotransmission and freezing behavior in rats. Eur. J. Pharmacol. 378, 23-30.

Hyun, Y., Ellis, M., Riskowski, G., and Johnson, R.W. 1998. Growth performance of pigs subjected to multiple concurrent environmental stressors. J. Anim. Sci. 76, 721-727.

Jansen, J., Bolhuis, J.E., Schouten, W.G.P., Spruijt, B.M., and Wiegant, V.M. 2009. Spatial learning in pigs: Effects of environmental enrichment and individual characteristics on behaviour and performance. Anim. Cogn. 12, 303-315.

Jernej, B., Banović, M., Cicin-Šain, L., Hranilović, D., Balija, M., Orešković, D., and Folnegović-Šmalc, V. 2000. Physiological characteristics of platelet/circulatory serotonin: Study on a large human population. Psychiat. Res. 94, 153-162.

Jernej, B. and Cicin-Šain, L. 1990. Platelet serotonin level in rats is under genetic control. Psychiat. Res. 32, 167-174.

Jolliffe, I. 1986. Principal component analyses. New York, Springer-Verlag.

Kallai, J., Makany, T., Csatho, A., Karadi, K., Horvath, D., Kovacs-Labadi, B., Jarai, R., Nadel, L., and Jacobs, J. W. 2007. Cognitive and affective aspects of thigmotaxis strategy in humans. Behav. Neurosci. 121, 21-30.

Kemperman, R.F.J., Bruins, S., Te Lintelo, J.T.V., Van der Dijs, F.P.L., Erwich, J.J.H.M., Landman, H., Muskiet, F.D., Kema, I.P., and Muskiet, F.A.J. 2007. Relation between platelet serotonin and feeding mode in newborns suggests that gut motor activity is a determinant of platelet serotonin content. Biogenic Amines 21, 260-272.

Keszthelyi, D., Troost, F.J., Jonkers, D.M., Van Donkelaar, E.L., Dekker, J., Buurman, W.A., and Masclee, A.A., 2012. Does acute tryptophan depletion affect peripheral serotonin metabolism in the intestine? Am. J. Clin. Nutr. 95, 603-608.

Kluge, H., Bolle, M., Reuter, R., Werner, S., Zahlten, W., and Prudlo., J. 1999. Serotonin in platelets: Comparative analyses using new enzyme immunoassay and HPLC test kits and the traditional fluorimetric procedure. LaboratoriumsMedizin 23, 360-364.

Koe, B.K. and Weissman, A. 1966. p-Chlorophenylalanine: a specific depletor of brain serotonin. J. Pharmacol. Exp. Ther. 154: 499-516.

Koolhaas, J.M., De Boer, S.F., Coppens, C.M., and Buwalda, B. 2010. Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. Front. Neuroendocrin. 31, 307-321.

Koolhaas, J.M., Meerlo, P., De Boer, S.F., Strubbe, J.H., and Bohus, B. 1997. The temporal dynamics of the stress response. Neurosci. Biobehav. R. 21, 775-782.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van der Vegt, B.J., Van Reenen C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W., and Blokhuis, H.J. 1999. Coping styles in animals: Current status in behavior and stress-physiology. Neurosci. Biobehav. R. 23, 925-935.

Kops, M.S. 2014. Feather pecking and monoamines: A behavioral and neurobiological approach. Doctoral Thesis, Utrecht University, Utrecht, The Netherlands.

Kops, M.S., De Haas, E.N., Rodenburg, T.B., Ellen, E.D., Korte-Bouws, G.A.H., Olivier, B., Güntürkün, O., Bolhuis, J.E., and Korte, S.M. 2013. Effects of feather pecking phenotype (severe feather peckers, victims and non-peckers) on serotonergic and dopaminergic activity in four brain areas of laying hens (Gallus gallus domesticus). Physiol. Behav. 120, 77-82.

Korte, S.M. and De Boer, S.F. 2003. A robust animal model of state anxiety: Fear-potentiated behaviour in the elevated plus-maze. Eur. J. Pharmacol. 463, 163-175.

Korte, S.M., Prins, J., Vinkers, C.H., and Olivier, B. 2009. On the origin of allostasis and stress-induced pathology in farm animals: Celebrating Darwin's legacy. Vet. J. 182, 378-383.

Korte, S.M., Ruesink, W., and Blokhuis, H.J. 1998. Heart rate variability during manual restraint in chicks from high- and low-feather pecking lines of laying hens. Physiol. Behav. 65, 649-652.

León-Ponte, M., Ahern, G.P., and O'Connell, P.J. 2007. Serotonin provides an accessory signal to enhance T-cell activation by signaling through the 5-HT7 receptor. Blood 109, 3139-3146.

Martínez-Trejo, G., Ortega-Cerrilla, M.E., Rodarte-Covarrubias, L.F., Herrera-Haro, J.G., Figueroa-Velasco, J.L., Galindo-Maldonado, F., Sánchez-Martínez, O., and Lara-Bueno, A. 2009. Aggressiveness and productive performance of piglets supplemented with tryptophan. J. Anim. Vet. Adv. 8, 608-611.

Melotti, L., Oostindjer, M., Bolhuis, J.E., Held, S., and Mendl, M. 2011. Coping personality type and environmental enrichment affect aggression at weaning in pigs. Appl. Anim. Behav. Sci. 133, 144-153.

Mills, D.S. 2003. Medical paradigms for the study of problem behaviour: A critical review. Appl. Anim. Behav. Sci. 81, 265-277.

Morgan, K.N. and Tromborg, C.T. 2007. Sources of stress in captivity. Appl. Anim. Behav. Sci. 102, 262-302.

Morrison, R.S., Johnston, L.J., and Hilbrands, A.M. 2007. The behaviour, welfare, growth performance and meat quality of pigs housed in a deep-litter, large group housing system compared to a conventional confinement system. Appl. Anim. Behav. Sci. 103, 12-24.

Naesh, O., Hindberg, I., and Bruun, A.B. 2001, Decreased reuptake of serotonin in human platelets after surgery. Clin. Physiol. 21, 39-43.

Pearish, S., Hostert, L., and Bell, A.M. 2013. Behavioral type-environment correlations in the field: A study of three-spined stickleback. Behav. Ecol. Sociobiol. 67, 765-774.

Pond, W.G. 1987. Thoughts on fiber utilization in swine. J. Anim. Sci. 65, 497-499.

Reimert, I., Bolhuis, J.E., Kemp, B., and Rodenburg, T.B. 2013a. Indicators of positive and negative emotions and emotional contagion in pigs. Physiol. Behav. 109, 42-50.

Reimert, I., Rodenburg, T.B., Ursinus, W.W., Duijvesteijn, N., Camerlink, I., Kemp, B., and Bolhuis, J. E. 2013b. Backtest and novelty behavior of female and castrated male piglets with diverging social breeding values for growth. J. Anim. Sci. 91, 4589-4597.

Reimert, I., Rodenburg, T.B., Ursinus, W.W., Kemp, B., and Bolhuis, J.E. 2014. Responses to novel situations of female and castrated male pigs with divergent social breeding values and different backtest classifications in barren and straw-enriched housing. Appl. Anim. Behav. Sci. 151, 24-35.

Rodenburg, T.B., Bolhuis, J.E., Koopmanschap, R.E., Ellen, E.D., and Decuypere, E. 2009. Maternal care and selection for low mortality affect post-stress corticosterone and peripheral serotonin in laying hens. Physiol. Behav. 98, 519-523.

Rodenburg, T.B., De Haas, E.N., Nielsen, B.L., and Buitenhuis, A.J. 2010. Fearfulness and feather damage in laying hens divergently selected for high and low feather pecking. Appl. Anim. Behav. Sci. 128, 91-96.

Ruis, M.A.W, Te Brake, J.H.A., Engel, B., Ekkel, E.D., Buist, W.G., Blokhuis, H.J., and Koolhaas, J.M. 1997. The circadian rhythm of salivary cortisol in growing pigs: Effects of age, gender, and stress. Physiol. Behav. 62, 623-630.

Souza da Silva, C., Haenen D., Koopmans, S.J., Hooiveld, G.J.E.J., Bosch, G., Bolhuis, J.E., Kemp, B., Müller, M., and Gerrits, W.J.J. 2014. Effects of resistant starch on behaviour, satiety-related hormones and metabolites in growing pigs. Animal. doi:10.1017/S1751731114001116.

Spiller, R. 2008. Serotonin and GI clinical disorders. Neuropharmacology 55: 1072-1080.83.

Seksel, K. and Lindeman, M.J. 2001. Use of clomipramine in treatment of obsessive-compulsive disorder, separation anxiety and noise phobia in dogs: A preliminary, clinical study. Aust. Vet. J. 79, 252-256.

Studnitz, M., Jensen, M.B., and Pedersen, L.J. 2007. Why do pigs root and in what will they root? A review on the exploratory behaviour of pigs in relation to environmental enrichment. Appl. Anim. Behav. Sci. 107, 183-197.

Taylor, N.R., Main, D.C.J., Mendl, M., and Edwards, S.A. 2010. Tail-biting: A new perspective. Vet. J. 186, 137-147.

Thies-Flechtner, K., Weigel, I., and Muller-Oerlinghausen, B. 1994. 5-HT uptake in platelets of lithium-treated patients with affective disorders and of healthy controls. Pharmacopsychiatry, Supplement 27: 4-6.

Torres, A.R., Prince, M.J., Bebbington, P.E., Bhugra, D., Brugha, T.S., Farrell, M., Jenkins, R., Lewis, G., Meltzer, H., and Singleton, N. 2006. Obsessive-compulsive disorder: Prevalence, Comorbidity, impact, and help-seeking in the British National Psychiatric Morbidity Survey of 2000. Am. J. Psychiat. 163, 1978-1985.

Uitdehaag, K.A., Rodenburg, T.B., Van Reenen, C.G., Koopmanschap, R.E., De Vries G.R., Engel, B., Buist, W.G., Komen, H., and Bolhuis, J.E. 2011. Effects of genetic origin and social environment on behavioral response to manual restraint and monoamine functioning in laying hens. Poultry. Sci. 90, 1629-1636.

Ursinus, W.W., Bolhuis, J.E., Zonderland, J.J., Rodenburg, T.B., De Souza, A.S., Koopmanschap, R.E., Kemp, B., Korte-Bouws, G.A.H., Korte, S.M., and Van Reenen, C.G. 2013. Relations between peripheral and brain serotonin measures and behavioural responses in a novelty test in pigs. Physiol. Behav. 118, 88-96.

Ursinus, W.W., Van Reenen, C.G., Kemp, B., and Bolhuis, J.E. 2014. Tail biting behaviour and tail damage in pigs and the relationship with general behaviour: Predicting the inevitable? Appl. Anim. Behav. Sci. 156, 22-36.

Valros, A., Palander, P.A., Heinonen, M., Brunberg, E., Keeling, L., and Piepponen, T.P. 2013. Preliminary evidence of an altered serotonin metabolism in the prefrontal cortex of tail biting pigs. In: Hötzel, M.J. and Pinheiro Machado Filho, L.C. (Editors). Proceedings of the 47th Congress of the International Society for Applied Ethology (ISAE), Florianopolis, Brazil, p. 105.

Van de Weerd, H.A., Docking, C.M., Day, J.E.L., and Edwards, S.A. 2005. The development of harmful social behaviour in pigs with intact tails and different enrichment backgrounds in two housing systems. Anim. Sci. 80, 289-298.

Van Reenen, C.G., Engel, B., Ruis-Heutinck, L.F.M., Van der Werf, J.T.N., Buist, W.G., Jones, R.B., and Blokhuis, H.J. 2004. Behavioural reactivity of heifer calves in potentially alarming test situations: A multivariate and correlational analysis. Appl. Anim. Behav. Sci. 85: 11-30.

Van Reenen, C.G., Hopster, H., Van der Werf, J.T.N., Engel, B., Buist, W.G., Jones, R.B., Blokhuis, H.J., and Korte, S.M. 2009. The benzodiazepine brotizolam reduces fear in calves exposed to a novel object test. Physiol. Behav. 96, 307-314.

Van Zeeland, Y.R.A, Van der Aa, M.M.J.A., Vinke, C.M., Lumeij, J.T., and Schoemaker, N.J. 2013. Behavioural testing to determine differences between coping styles in Grey parrots (Psittacus erithacus erithacus) with and without feather damaging behaviour. Appl. Anim. Behav. Sci. 148, 218-231.

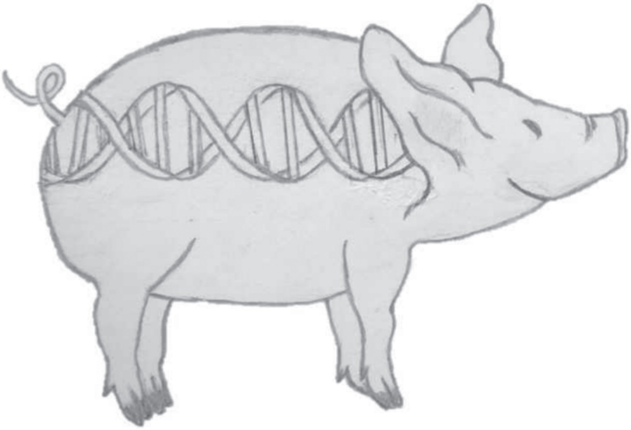
Van Zeeland, Y.R.A., Spruit, B.M., Rodenburg, T.B., Riedstra, B., Van Hierden, Y.M., Buitenhuis, B., Korte, S.M., and Lumeij, J.T. 2009. Feather damaging behaviour in parrots: A review with consideration of comparative aspects. Appl. Anim. Behav. Sci. 121, 75-95.

Vicens-Costa, E., Martínez-Membrives, E., López-Aumatell, R., Guitart-Masip, M., Cañete, T., Blázquez, G., Tobeña, A., and Fernández-Teruel, A. 2011. Two-way avoidance acquisition is negatively related to conditioned freezing and positively associated with startle reactions: A dissection of anxiety and fear in genetically heterogeneous rats. Physiol. Behav. 103, 148-156.

Yuwiler, A., Brammer, G.L., Morley, J.E., Raleigh, M.J., Flannery, J.W., and Geller, E. 1981. Short-term and repetitive administration of oral tryptophan in normal men. Effects on blood tryptophan, serotonin, and kynurenine concentrations. Arch. Gen. Psychiat. 38, 619-626.

Zonderland, J.J., Wolthuis-Fillerup, M., Van Reenen, C.G., Bracke, M.B.M., Kemp, B., Den Hartog, L.A., and Spoolder, H.A.M. 2008. Prevention and treatment of tail biting in weaned piglets. Appl. Anim. Behav. Sci. 110, 269-281.

Zupan, M., Janczak, A.M., Framstad, T., and Zanella, A.J. 2012. The effect of biting tails and having tails bitten in pigs. Physiol. Behav. 106, 638-644.



5

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. Schrøder-Petersen and Simonsen, 2001). Selection on IGEg might contribute to a solution to 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 × 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 m × 3.20 m or 2.25 × 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 cm^2. 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 cm^2 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 cm^2, results are presented in number of jute sacks consumed (total cm^2 / (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.

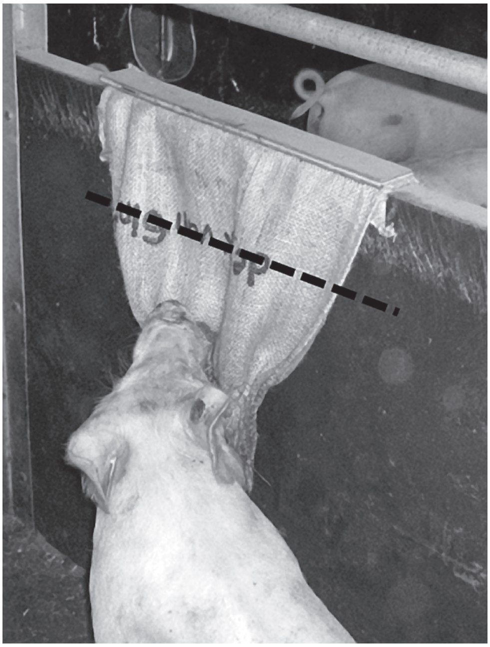

Figure 1. Jute sack attached to pen wall as distraction material to limit tail biting. The sack was replaced when the sack was 'consumed' till the dashed line or further.

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 spent 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[1]. Values are LSmeans of untransformed data with standard error (SEM).

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

[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).

Behav. nursery	High E	High B	Low E	Low B	SEM	*P*-IGE	*P*-HC	IGE×HC
Sleeping	38.0	47.0	39.0	46.0	1.1	0.94	<0.001	0.30
Lying inactive	9.6	12.8	10.6	13.2	0.39	0.08	<0.001	0.44
Standing	2.2	2.9	2.0	2.7	0.2	0.31	<0.001	0.91
Locomotion	2.5	2.5	2.6	2.7	0.13	0.24	0.89	0.72
Sitting	0.82	0.90	0.78	1.0	0.06	0.60	0.01	0.25
Comfort behav.	0.31	0.24	0.30	0.24	0.03	0.92	0.02	0.87
Eating	7.9	8.2	8.7	8.3	0.2	0.89	0.09	0.56
Drinking	1.5	1.8	1.6	1.5	0.09	0.26	0.22	0.02
Urinate/defecate	0.29	0.45	0.36	0.5	0.03	0.09	<0.001	0.75
Playing	1.1	0.63	0.96	0.66	0.1	0.66	<0.001	0.46
Exploration floor	16.0	11.0	14.0	11.0	0.5	0.14	<0.001	0.43
Nosing object	1.8	2.5	1.9	2.9	0.14	0.13	<0.001	0.37
Rooting	5.8	1.6	5.4	2.2	0.4	0.49	<0.001	0.10
Rooting object	0.07	0.26	0.09	0.31	0.03	0.23	<0.001	0.59
Chewing	10.0	3.0	10.0	3.0	0.5	0.73	<0.001	0.69
Chewing toy	0.28	0.33	0.15	0.37	0.04	0.26	0.0002	0.13
Nosing body	0.60	1.0	0.63	0.96	0.05	0.87	<0.001	0.55
Nose contact	0.26	0.23	0.27	0.34	0.03	0.03	0.47	0.06
Belly nosing	0.05	0.53	0.11	0.25	0.09	0.20	<0.001	0.02
Mounting	0.26	0.23	0.19	0.26	0.03	0.53	0.53	0.12
Fighting	0.19	0.18	0.15	0.25	0.03	0.18	0.15	0.15
Head knock	0.16	0.11	0.09	0.13	0.02	0.17	0.07	0.07
Biting	0.08	0.10	0.09	0.14	0.01	0.06	0.005	0.23
Fighting at feeder	0.09	0.11	0.08	0.10	0.01	0.54	0.23	0.99
Tail biting	0.02	0.15	0.02	0.17	0.02	0.80	<0.001	0.46
Ear biting	0.10	0.40	0.09	0.37	0.03	0.63	<0.001	0.92
Manip. other	0.11	0.50	0.15	0.50	0.04	0.40	<0.001	0.30

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).

Behav. finishing	High E	High B	Low E	Low B	SEM	*P*-IGE	*P*-HC	IGE×HC
Sleeping	51	55	50	53	1	0.14	0.004	0.54
Lying inactive	14	17	16	17	0.4	0.12	0.002	0.03
Standing	1.1	0.88	0.96	0.95	0.1	0.65	0.12	0.15
Locomotion	0.97	0.79	0.76	0.82	0.1	0.11	0.33	0.04
Sitting	1.9	1.3	2.0	1.4	0.1	0.50	<0.001	0.80
Comfort behav.	0.13	0.07	0.08	0.05	0.01	0.005	<0.001	0.06
Eating	7.2	8.0	7.2	8.1	0.2	0.72	<0.001	0.91
Drinking	2.8	1.9	2.8	2.3	0.1	0.13	<0.001	0.08
Urinate/defecate	0.26	0.34	0.32	0.36	0.03	0.09	0.02	0.41
Playing	0.08	0.05	0.13	0.11	0.03	0.14	0.28	0.69
Exploration floor	8.0	6.0	7.5	6.8	0.4	0.73	0.004	0.09
Nosing object	1.9	1.4	1.8	1.7	0.1	0.37	0.004	0.08
Rooting	1.8	0.4	1.6	0.45	0.1	0.82	<0.001	0.40
Rooting object	0.08	0.06	0.08	0.07	0.01	0.99	0.74	0.85
Chewing	5.8	3.5	5.6	3.4	0.2	0.41	<0.001	0.86
Chewing toy	0.82	1.1	1.1	1.8	0.2	0.005	0.03	0.22
Nosing body	0.75	0.87	0.79	1.0	0.1	0.21	0.02	0.52
Nose contact	0.17	0.17	0.15	0.15	0.02	0.34	0.76	0.95
Belly nosing	0.03	0.13	0.02	0.09	0.03	0.37	0.002	0.40
Mounting	0.03	0.00	0.01	0.01	0.01	0.45	0.18	0.23
Fighting	0.05	0.03	0.03	0.02	0.01	0.25	0.07	0.39
Head knock	0.03	0.04	0.04	0.03	0.01	0.82	0.80	0.92
Biting	0.01	0.04	0.04	0.05	0.01	0.006	0.03	0.30
Fighting at feeder	0.03	0.05	0.05	0.06	0.01	0.30	0.19	0.97
Tail biting	0.05	0.18	0.07	0.17	0.02	0.70	<0.001	0.51
Ear biting	0.08	0.14	0.11	0.18	0.02	0.03	0.004	0.86
Manip. other	0.17	0.40	0.20	0.40	0.04	0.70	<0.001	0.73

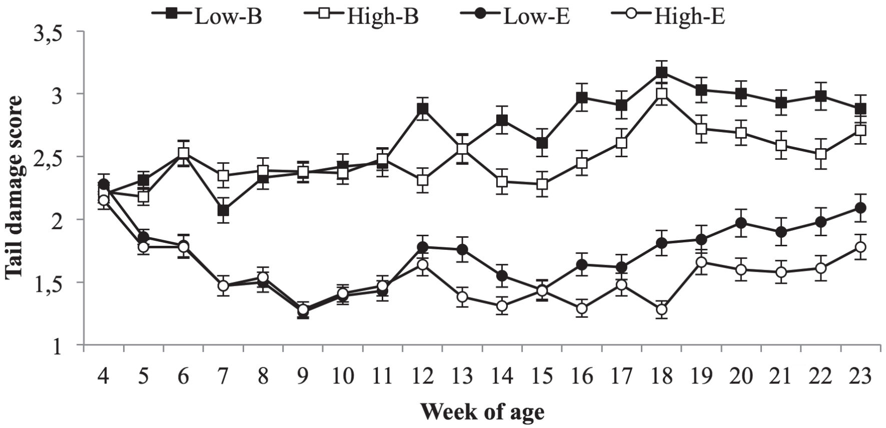

Figure 2. Tail damage score for high IGEg pigs in barren pens, high IGEg pigs in enriched pens, low IGEg pigs in barren pens, and low IGEg pigs in enriched pens. Note that the y-axis ranges from 1 – 3.5 while tail damage scores from individual pigs may range from 1 – 4.

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 ± 0.04; enriched 1.8 ± 0.04; $P < 0.001$). During the finishing phase (wk 8 – 23) high IGEg pigs had a lower tail damage score (high 2.0 ± 0.05; low 2.2 ± 0.05; $P = 0.004$), and the positive effect of enrichment remained (mean tail damage score finishing: barren 2.6 ± 0.05; enriched 1.6 ± 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 with low IGEg pigs. Over a period of 15 weeks, high IGEg pigs consumed 2.9 ± 0.3 jute sacks per pen, whereas low IGEg pigs consumed 4.2 ± 0.3 sacks per pen ($P = 0.002$). Pigs in barren pens consumed 4.3 ± 0.3 jute sacks whereas in enriched pens on average 2.8 ± 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; Schrøder-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 spent 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.

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Appendix

Table A1. Ethogram

Behaviour	Description
General individual	
Sleeping	Lying without performing any other described behaviour, eyes closed
Lying inactive	Lying without performing any other described behaviour, eyes opened
Standing	Standing without performing any other described behaviour
Locomotion	Walking or running without performing any other described behaviour
Sitting	Sitting or kneeling without performing any other described behaviour
Comfort behaviour	Rubbing body against objects or pen mate, scratching body with hind leg or stretching (part of) body
Eating feeder	Eating at feeder
Drinking	Drinking from drinking nipple
Urinate/defecate	Urinating or defecating
Exploration	
Exploration floor	Sniffing, touching or scraping floor
Nosing object	Nosing above floor level
Rooting	Rooting pen floor or in wood shavings or straw
Rooting object	Rooting above floor level or object
Chewing	Non-feed chewing or chewing straw
Chewing toy	Chewing toy: chain with ball or jute sack
Social	
Nosing head or body	Touching/sniffing any part of a pen mate except nose
Nose contact	Mutual nose contact
Playing	Individual or group wise gamboling, pivoting: running around the pen, sometimes 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

Agrawal, A.F., Brodie III, E.D., and Wade, M.J. 2001. On indirect genetic effects in structured populations. Am. Nat. 158, 308-323.

Alemu, S.W., Bijma, P., Møller, S.H., Janss, L., and Berg, P. 2014. Indirect genetic effects contribute substantially to heritable variation in aggression-related traits in group-housed mink (*Neovison vison*). Genet. Sel. Evol. 46: 30.

Arango, J., Misztal, I., Tsuruta, S., Culbertson, M., and Herring, W. 2005. Estimation of variance components including competitive effects of Large White growing gilts. J. Anim. Sci. 83, 1241-1246.

Barr, C.S., Newman, T.K., Becker, M.L., Parker, C.C., Champoux, M., Lesch, K.P., Goldman, D., Suomi, S.J., and Higley, J.D. 2003. The utility of the non-human primate model for studying gene by environment interactions in behavioral research. Genes. Brain. Behav. 2, 336-340.

Bergsma, R., Mathur, P.K., Kanis, E., Verstegen, M.W.A., Knol, E.F., and Van Arendonk, J.A.M. 2013. Genetic correlations between lactation performance and growing-finishing traits in pigs. J. Anim. Sci. 91, 3601-3611.

Bijma, P., Muir, W.M., and Van Arendonk, J.A.M. 2007. Multilevel selection 1: quantitative genetics of inheritance and response to selection. Genetics. 175, 277-288.

Bijma, P. and Wade, M.J. 2008. The joint effects of kin, multilevel selection and indirect genetic effects on response to genetic selection. J. Evol. Biol. 21, 1175-1188.

Bijma, P. 2011. Breeding for social interaction, for animal welfare. In Meyers, R.A. (Editor) Encyclopedia of sustainability science and technology. Press: New York. Springer Science and Business Media LLC.

Camerlink, I., Turner, S.P., Bijma, P., and Bolhuis, J.E. 2013. Indirect genetic effects and housing conditions in relation to aggressive behaviour in pigs. Plos One. 8, 6.

Camerlink, I., Bolhuis, J.E., Duijvesteijn, N., Van Arendonk, J.A.M., and Bijma, P. 2014. Growth performance and carcass traits in pigs selected for indirect genetic effects on growth rate in two environments. J. Anim. Sci. 92, 2612-2619.

Canario, L., Turner, S.P., Roehe, R., Lundeheim, N., D'Eath, R.B., Lawrence, A.B., Knol, E.F., Bergsma, R., and Rydhmer, L. 2012. Genetic associations between behavioral traits and direct-social effects of growth rate in pigs. J. Anim. Sci. 90, 4706-4715.

Danielson-François, A.M., Zhou, Y., and Greenfield, M.D. 2009. Indirect genetic effects and the lek paradox: inter-genotypic competition may strengthen genotype × environment interactions and conserve genetic variance. Genetica. 136, 27-36.

Frank, S.A. 2007. All of life is social. Curr. Biol. 17, R648-R650.

Fraser, D., Phillips, P.A., Thompson, B.K., and Tennessen, T. 1991. Effect of straw on the behaviour of growing pigs. Appl. Anim. Behav. Sci. 30, 307-318.

Griffing, B. 1967. Selection in reference to biological groups. I. Individual and group selection applied to populations of unordered groups. Austr. J. Biol. Sci. 10, 127-139.

Gunsett, F.C. 2005. Group selection in swine – a case study. Proceedings National Swine Improvement Federation Conference and Annual Meeting, December 1-2, 2005, Ottawa, Canada. Retrieved from http://www.nsif.com/Conferences/2005/contents.html.

Guy, J.H., Rowlinson, P., Chadwick, J.P., and Ellis, M. 2002. Behaviour of two genotypes of growing–finishing pig in three different housing systems. Appl. Anim. Behav. Sci. 75, 193-206.

Hessing, M.J., Hagelsø, A.M., Van Beek, J.A.M., Wiepkema, R.P., Schouten, W.G.P., and Krukow, R. 1993. Individual behavioural characteristics in pigs. Appl. Anim. Behav. Sci. 37, 285-295.

Marler, P. 1976. On animal aggression: The roles of strangeness and familiarity. Am. Psychol. 31, 239.

McGlothlin, J.W., Moore, A.J., Wolf, J.B., and Brodie III, E.D. 2010. Interacting phenotypes and the evolutionary process. III. Social evolution. Evolution. 64, 2558-2574.

Moore, A.J., Brodie III, E.D., and Wolf, J.B. 1997. Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. Evolution. 51, 1352-1362.

Muir, W.M. 1996. Group selection for adaptation to multiple-hen cages: selection program and direct responses. Poult. Sci. 75, 447-458.

Muir, W.M., Bijma, P., and Schinckel, A. 2013. Multilevel selection with kin and non-kin groups, experimental results with Japanese quail (*Coturnix japonica*). Evolution. 67, 1598-1606.

Mutic, J.J. and Wolf, J.B. 2007. Indirect genetic effects from ecological interactions in Arabidopsis thaliana. Mol. Ecol. 16, 2371-2381.

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

Reimert, I., Rodenburg, T.B., Ursinus, W.W., Duijvesteijn, N., Camerlink, I., Kemp, B., and Bolhuis, J.E. 2013. Backtest and novelty behavior of female and castrated male piglets, with diverging social breeding values for growth. J. Anim. Sci. 91, 4589-4597.

Reimert, I., Rodenburg, T.B., Ursinus, W.W., Kemp, B., and Bolhuis, J.E. 2014. Behavioral and cortisol responses to novel situations of female and castrated male pigs with divergent social breeding values and different backtest classifications in barren and straw-enriched housing. Appl. Anim. Behav. Sci. 151, 24-35.

Rodenburg, T.B., Bijma, P., Ellen, E.D., Bergsma, R., De Vries, S., Bolhuis, J.E., Kemp, B., and Van Arendonk, J.A.M. 2010. Breeding amiable animals? Improving farm animal welfare by including social effects in breeding programmes. Anim. Welfare. 19, 77-82.

Rodenburg, T.B. and Turner, S.P. 2012. The role of breeding and genetics in the welfare of farm animals. Anim. Front. 2, 16-21.

Schinckel, A.P., Richert, B.T., Frank, J.W., and Kendall, D.C. 1999. Genetic by environmental interactions for pig growth. Purdue University Swine Day Reports, Purdue University, West Lafayette, US, 93-104.

Schrøder-Petersen, D.L. and Simonsen, H.B. 2001. Tail biting in pigs. Vet. J. 162, 196-210.

Scott, J.P. 1948. Dominance and the frustration-aggression hypothesis. Physiol. Zool. 21, 31-39.

Statham, P., Green, L., Bichard, M., and Mendl, M. 2009. Predicting tail-biting from behaviour of pigs prior to outbreaks. Appl. Anim. Behav. Sci. 121, 157-164.

Taylor, N.R., Main, D.C., Mendl, M., and Edwards, S.A. 2010. Tail-biting: a new perspective. Vet. J. 186, 137-147.

Van de Weerd, H.A. and Day, J.E. 2009. A review of environmental enrichment for pigs housed in intensive housing systems. Appl. Anim. Behav. Sci. 116, 1-20.

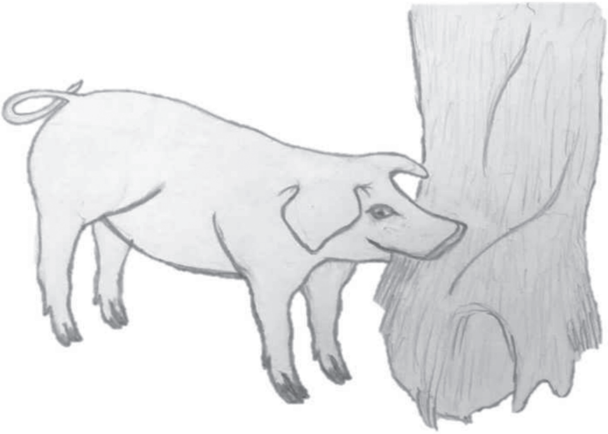
Wilson, A.J., Gelin, U., Perron, M.C., and Réale, D. 2009. Indirect genetic effects and the evolution of aggression in a vertebrate system. P. R. Soc. B. 276, 533-541.

Wilson, A.J. 2013. Competition as a source of constraint on life history evolution in natural populations. Heredity. 112, 70-78.

Wolf, J.B., Brodie III, E.D., Cheverud, J.M,, Moore, A.J., and Wade, M.J. 1998. Evolutionary consequences of indirect genetic effects. TREE. 13, 64-69.

Zonderland, J.J., Wolthuis-Fillerup, M., Van Reenen, C.G., Bracke, M., Kemp, B., Den Hartog, L.A, and Spoolder, H.A.M. 2008. Prevention and treatment of tail biting in weaned piglets. Appl. Anim. Behav. Sci. 110, 269-281.

Zupan, M., Janczak, A.M., Framstad, T., and Zanella, A.J. 2012. The effect of biting tails and having tails bitten in pigs. Physiol. Behav. 106, 638-644.



6

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.

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 al 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 (Tuyttens, 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.

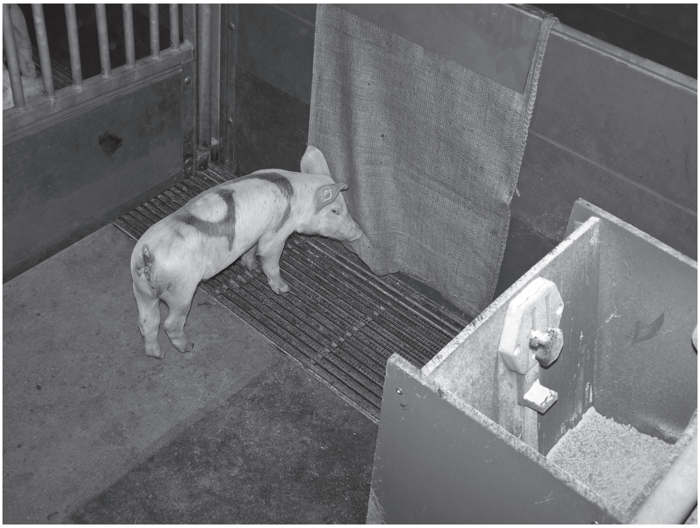

Figure 2. Jute sack attached to the front wall of a weaner pen.

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[1] and ear[2] damage scores of pigs kept in pens with or without jute sacks, recorded at time of weaning and during the weaner and rearing phase.

	Jute sack	**Control**	
Damage score	**Mean**	**Mean**	**P-Value**
Weaning (week 4)	*36 pens*	*36 pens*	
Tail (gilts)	1.4 ± 0.05	1.5 ± 0.04	0.38
Ear (gilts)	1.7 ± 0.06	1.8 ± 0.05	0.36
Tail (all piglets)	1.4 ± 0.03	1.5 ± 0.03	***0.01***
Ear (all piglets)	1.7 ± 0.04	1.8 ± 0.04	***0.01***
Weaner (week 9)	*17 pens*	*19 pens*	
Tail	1.6 ± 0.05	1.8 ± 0.04	***0.03***
Ear	1.7 ± 0.08	1.9 ± 0.09	*0.08*
Rearing (week 11, 13)	*17 pens*	*19 pens*	
Tail	1.5 ± 0.04	1.8 ± 0.06	***< 0.001***
Ear	1.7 ± 0.04	1.7 ± 0.05	0.96

[1] Tail damage scores: 1 (no damage) - 5 (severe damage).

[2] Ear damage scores: 1 (no damage) - 4 (severe damage).

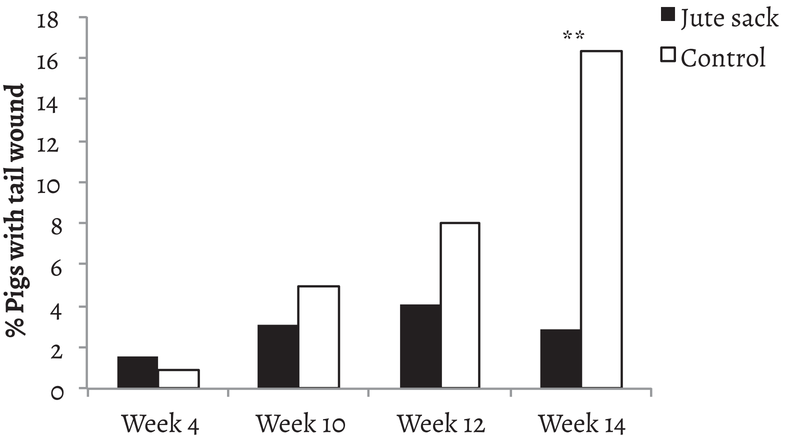

Figure 3. Percentage of gilts with a (small) tail wound at time of weaning (week 4), during the weaner phase (week 9) and the rearing phase (week 11 and 13) for gilts kept in pens with or without jute sacks. ** P = 0.01.

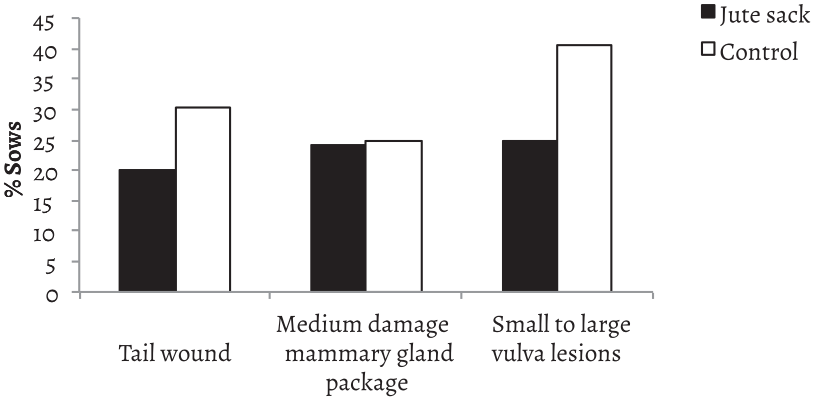

Figure 4. Percentage of sows with a tail wound, medium damage to the mammary gland package, and with small to large vulva lesions at time of weaning in pens with or without jute sacks available for the piglets.

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 (ß = 0.002, P = 0.04) and EBV for litter birth weight (ß = 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 (ß = 0.003, P = 0.09). Moreover, the higher EBV for TDG (ß = 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 (ß = 0.51, P = 0.06). The higher EBV for litter birth weight (ß = 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 (ß = 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 (ß = 0.31, P = 0.01) and litter size CS (ß = 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 (ß = 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.

	Jute sack	Control	
Behavior[1]	**Mean**	**Mean**	**P-Value**
Weaner (week 6, 7, 8)	*17 pens*	*19 pens*	
Tail biting	0.7 ± 0.08	1.1 ± 0.07	**< 0.001**
Ear biting	1.8 ± 0.11	3.4 ± 0.22	**< 0.001**
Other biting	1.6 ± 0.11	3.0 ± 0.24	**< 0.001**
Jute sack biting	9.6 ± 0.79	-	-
Jute sack snout contact	2.4 ± 0.11	-	-
Rearing (week 11)	*17 pens*	*19 pens*	
Tail biting	0.5 ± 0.09	1.0 ± 0.19	**0.04**
Ear biting	1.7 ± 0.15	2.9 ± 0.22	**< 0.001**
Other biting	1.8 ± 0.17	3.5 ± 0.28	**< 0.001**
Jute sack biting	9.8 ± 1.46	-	-
Jute sack snout contact	1.2 ± 0.13	-	-

[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 '+'.

	Jute sack		Control	
EBV	**Mean**	**Range**	**Mean**	**Range**
EBV litter birth weight (g)	-22.3 ± 15.77	-156.6 - 152.0	-24.8 ± 12.66	-170.3 - 151.9
EBV litter size (n)	0.6 ± 0.13	-0.8 - 2.1	0.3 ± 0.11	-1.0 - 1.6
EBV TDG[1] (g)	8.7 ± 4.57	-62.6 - 52.4	10.2 ± 4.05	-52.0 - 52.4
EBV LDG[2] (g)	3.0 ± 3.22	-50.7 - 24.6	3.9 ± 2.54	-35.4 - 26.8

[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) ($P \leq 0.05$, 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[1].

	Non tail biter	Medium tail biter	High tail biter	P-value
***Weaner phase*[2]**				
Production				
Birth weight (kg)	1.2 ± 0.03	1.2 ± 0.03	1.3 ± 0.06	0.35
Weaning weight (kg)	7.0 ± 0.15^{a}	7.4 ± 0.12^{ab}	7.8 ± 0.33^{b}	**0.03**
Weight at 9 weeks (kg)	22.7 ± 0.43^{a}	23.4 ± 0.39^{a}	25.3 ± 0.97^{b}	**0.04**
ADG Birth-weaning (g)	211.3 ± 4.57^{a}	223.7 ± 3.69^{ab}	234.9 ± 8.91^{b}	**0.05**
ADG Weaning-9 weeks (g)	463.4 ± 9.91^{a}	470.5 ± 9.49^{a}	513.4 ± 20.13^{b}	0.08
***Behavior*[3]**				
Tail biting rearing	0.3 ± 0.13	0.6 ± 0.12	0.7 ± 0.25	0.17
Ear biting weaner	1.5 ± 0.16^{a}	1.8 ± 0.13^{ab}	2.3 ± 0.30^{b}	0.06
Ear biting rearing	1.5 ± 0.26	1.6 ± 0.22	2.4 ± 0.70	0.28
Other biting weaner	1.3 ± 0.13^{a}	1.6 ± 0.13^{ab}	2.5 ± 0.56^{b}	0.08
Other biting rearing	1.5 ± 0.24	1.9 ± 0.25	2.3 ± 0.57	0.29
Jute sack biting weaner	9.6 ± 0.85^{a}	9.2 ± 0.56^{a}	11.8 ± 1.67^{b}	0.07
Jute sack biting rearing	8.9 ± 1.45^{a}	9.9 ± 0.98^{ab}	12.7 ± 3.26^{b}	0.10
Jute sack nosing weaner	2.3 ± 0.21	2.4 ± 0.17	2.6 ± 0.38	0.79
Jute sack nosing rearing	1.1 ± 0.22	1.3 ± 0.14	0.9 ± 0.30	0.20
***Rearing phase*[4]**				
Birth weight (kg)	1.2 ± 0.02	1.3 ± 0.04	1.3 ± 0.09	0.75
Weaning weight (kg)	7.3 ± 0.12	7.6 ± 0.18	7.6 ± 0.32	0.71
Weight at 9 weeks (kg)	23.6 ± 0.33	24.7 ± 0.57	24.6 ± 1.01	0.59
ADG Birth-weaning (g)	220.6 ± 3.64	228.5 ± 5.46	223.8 ± 9.04	0.77
ADG Weaning-9 weeks (g)	480.9 ± 7.30	501.5 ± 12.80	500.3 ± 23.72	0.65
***Behavior*[3]**				
Tail biting weaner	0.6 ± 0.09	0.9 ± 0.15	0.6 ± 0.25	0.19
Ear biting weaner	1.7 ± 0.11	2.0 ± 0.28	1.7 ± 0.47	0.37
Ear biting rearing	1.6 ± 0.19	1.8 ± 0.40	1.9 ± 0.61	0.82
Other biting weaner	1.6 ± 0.14	1.7 ± 0.21	1.2 ± 0.33	0.61
Other biting rearing	1.6 ± 0.18	2.4 ± 0.48	1.4 ± 0.38	0.23
Jute sack biting weaner	9.7 ± 0.57	9.2 ± 1.17	10.7 ± 1.10	0.70
Jute sack biting rearing	8.9 ± 0.87	12.3 ± 1.76	12.4 ± 5.12	0.19
Jute sack nosing weaner	2.4 ± 0.16	2.5 ± 0.26	3.0 ± 0.56	0.50
Jute sack nosing rearing	1.0 ± 0.11^{y}	1.6 ± 0.32^{z}	1.8 ± 0.52^{z}	0.08

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[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].

	Non tail biter	Medium tail biter	High tail biter	P-value
***Weaner phase*[2]**				
Production				
Birth weight (kg)	1.2 ± 0.05	1.2 ± 0.03	1.2 ± 0.06	0.88
Weaning weight (kg)	7.5 ± 0.23	7.5 ± 0.12	7.9 ± 0.33	0.83
Weight at 9 weeks (kg)	22.3 ± 0.75	23.0 ± 0.30	23.5 ± 0.83	0.82
ADG Birth-weaning (g)	229.9 ± 7.25	227.2 ± 3.69	242.7 ± 10.19	0.71
ADG Weaning-9 weeks (g)	435.2 ± 17.03	456.2 ± 6.52	458.0 ± 17.66	0.67
***Behavior*[3]**				
Tail biting rearing	1.0 ± 0.59	0.9 ± 0.15	1.7 ± 0.61	0.14
Ear biting weaner	2.0 ± 0.26^{a}	3.6 ± 0.20^{b}	5.1 ± 0.63^{c}	**<0.001**
Ear biting rearing	3.5 ± 0.62	2.8 ± 0.23	3.4 ± 0.78	0.38
Other biting weaner	1.8 ± 0.28^{a}	3.1 ± 0.33^{b}	3.9 ± 0.44^{b}	**0.002**
Other biting rearing	3.0 ± 0.60	3.4 ± 0.32	5.0 ± 1.11	0.43
***Rearing phase*[4]**				
Production				
Birth weight (kg)	1.2 ± 0.03	1.3 ± 0.04	1.1 ± 0.06	0.68
Weaning weight (kg)	7.6 ± 0.13	7.7 ± 0.20	7.0 ± 0.40	0.65
Weight at 9 weeks (kg)	23.5 ± 0.32	23.6 ± 0.47	21.8 ± 0.81	0.54
ADG Birth-weaning (g)	232.2 ± 4.27	233.2 ± 5.66	218.2 ± 13.36	0.65
ADG Weaning-9 weeks (g)	466.6 ± 7.12	468.8 ± 9.91	433.3 ± 15.94	0.68
***Behavior*[3]**				
Tail biting weaner	1.1 ± 0.11^{a}	1.2 ± 0.14^{a}	2.1 ± 0.79^{b}	**0.02**
Ear biting weaner	3.3 ± 0.24^{a}	3.5 ± 0.28^{a}	5.6 ± 1.41^{b}	**0.04**
Ear biting rearing	2.6 ± 0.27	3.3 ± 0.36	3.9 ± 1.39	0.20
Other biting weaner	3.0 ± 0.24	3.2 ± 0.68	3.3 ± 0.86	0.76
Other biting rearing	2.9 ± 0.33^{a}	4.5 ± 0.53^{b}	3.4 ± 1.03^{ab}	**0.04**

[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 worthwile 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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References

Anonymous. 2010. Doe de zeugenuiercheck, Netwerk 'Vitaal uier', The Netherlands.

Azmitia, E.C. 2010. Evolution of serotonin: Sunlight to suicide. In: Müller, C.R. and Jacobs, B. (Editors). Handbook of the behavioural neurobiology of serotonin. Academic Press, London, pp. 3-22.

Bell, A.M. and Sih, A. 2007. Exposure to predation generates personality in threespined sticklebacks (Gasterosteus aculeatus). Ecol. Lett. 10, 828-834.

Beattie, V.E., Walker, N., and Sneddon, I.A. 1996. An investigation of the effect of environmental enrichment and space allowance on the behaviour and production of growing pigs. Appl. Anim. Behav. Sci. 48, 151-158.

Beattie, V.E., Sneddon, I.A., Walker, N., and Weatherup R.N. 2001. Environmental enrichment of intensive pig housing using spent mushroom compost. Anim. Sci. 72, 35-42.

Beattie, V.E., Breuer, K., O'Connell, N.E., Sneddon, I.A., Mercer, J.T., Rance, K.A., Sutcliffe, M.E.M. and Edwards, S.A. 2005. Factors identifying pigs predisposed to tail biting. Anim. Sci. 80, 307-312.

Blackshaw, J. K. 1981. Some behavioural deviations in weaned domestic pigs: persistent inguinal nose thrusting, and tail and ear biting. Anim. Prod. 33, 325-332.

Bracke, M.B.M., Zonderland, J.J., Lenskens, P., Schouten, W.G.P., Vermeer, H., Spoolder, H.A.M., Hendriks, H.J.M., and Hopster, H. 2006. Formalised review of environmental enrichment for pigs in relation to political decision making. Appl. Anim. Behav. Sci. 98, 165-182.

Bracke, M.B.M., Zonderland, J.J., and Bleumer, E.J.B. 2007. Expert judgement on enrichment materials for pigs validates preliminary RICHPIG model. Appl. Anim. Behav. Sci. 104, 1-13.

Bracke, M.B.M., De Lauwere, C.C., Wind S.M.M., and Zonderland, J. J. 2013. Attitudes of Dutch pig farmers towards tail biting and tail docking. J. Agr. Environ. Ethic. 26, 847-868.

Breuer, K., Sutcliffe, M.E.M., Mercer, J.T., Rance, K.A., Beattie, V.E., Sneddon, I.A., and Edwards, S.A. 2003. The effect of breed on the development of adverse social behaviours in pigs. Appl. Anim. Behav. Sci. 84, 59-74.

Breuer, K., Sutcliffe, M.E.M., Mercer, J.T., Rance, K.A., O'Connell, N.E., Sneddon I.A., and Edwards, S.A. 2005. Heritability of clinical tail-biting and its relation to performance traits. Livest. Prod. Sci. 93, 87-94.

Brunberg, E., Wallenbeck, A., and Keeling, L.J. 2011. Tail biting in fattening pigs: Associations between frequency of tail biting and other abnormal behaviours. Appl. Anim. Behav. Sci. 133, 18-25.

Day, J.E.L., Kyriazakis, I., and Lawrence, A.B. 1995. The effect of food deprivation on the expression of foraging and exploratory behaviour in the growing pig. Appl. Anim. Behav. Sci. 42, 193-206.

D'Eath, R.B. 2002. Individual aggressiveness measured in a resident-intruder test predicts the persistence of aggressive behaviour and weight gain of young pigs after mixing. Appl. Anim. Behav. Sci. 77, 267-283.

EFSA. 2007. Scientific report on the risks associated with tail biting in pigs and possible means to reduce the need for tail docking considering the different housing and husbandry systems. Annex to the EFSA J. 611, 1-13 (Question No EFSA-Q-2006-013, 1-98).

Fraser, D. 1987a. Attraction to blood as a factor in tail-biting by pigs. Appl. Anim. Behav. Sci. 17, 61-68.

Fraser, D. 1987b. Mineral-deficient diets and the pig's attraction to blood: implications for tail-biting. Can. J. Anim. Sci. 67, 909-918.

Kritas, S.K., and Morrison, R.B. 2007. Relationships between tail biting in pigs and disease lesions and condemnations at slaughter. Vet. Rec. 160, 149-152.

Martínez-Trejo, G., Ortega-Cerrilla, M.E., Rodarte-Covarrubias, L.F., Herrera-Haro, J.G., Figueroa-Velasco, J.L., Galindo-Maldonado, F., Sánchez-Martínez, O., and Lara-Bueno, A. 2009. Aggressiveness and productive performance of piglets supplemented with tryptophan. J. Anim. Vet. Adv. 8, 608-611.

McGlone, J.J. 1985. A quantitative ethogram of aggressive and submissive behaviors in recently regrouped pigs. J. Anim. Sci. 61, 559-565.

Mulder, H.A., Lidauer, M., Stranden, I., Mantysaari, E.A., Pool, M.H., and Veerkamp, R.F. 2010. MiXBLUP manual. Animal Breeding and Genomics Centre, Wageningen UR Livestock Research, Lelystad, The Netherlands.

Pearish, S., Hostert, L., and Bell, A.M. 2013. Behavioral type-environment correlations in the field: A study of three-spined stickleback. Behav. Ecol. Sociobiol. 67, 765-774.

Penny, R.H., Walters, J.R., and Tredget, S.J. 1981. Tail-biting in pigs: a sex frequency between boars and gilts. Vet. Rec. 108, 35.

Reimert, I., Rodenburg, T.B., Ursinus, W.W., Duijvesteijn, N., Camerlink, I., Kemp, B., and Bolhuis, J. E. 2013. Backtest and novelty behavior of female and castrated male piglets with diverging social breeding values for growth. J. Anim. Sci. 91, 4589-4597.

Reimert, I., Rodenburg, T.B., Ursinus, W.W., Kemp, B., and Bolhuis, J.E. 2014. Behavioral and cortisol responses to novel situations of female and castrated male pigs with divergent social breeding values and different backtest classifications in barren and straw-enriched housing. Appl. Anim. Behav. Sci. 151, 24-35.

Studnitz, M., Jensen, M.B., and Pedersen, L.J. 2007. Why do pigs root and in what will they root? A review on the exploratory behaviour of pigs in relation to environmental enrichment. Appl. Anim. Behav. Sci. 107, 183-197.

Taylor, N.R., Main, D.C., Mendl, M., and Edwards, S.A. 2010. Tail-biting: a new perspective. Vet. J. 186, 137-147.

Tuyttens, F.A.M. 2005. The importance of straw for pig and cattle welfare: A review. Appl. Anim. Behav. Sci. 92, 261-282.

Ursinus, W.W., Van Reenen, C.G., Kemp, B., and Bolhuis, J.E. 2014. Tail biting behaviour and tail damage in pigs and the relationship with general behaviour: Predicting the inevitable? Appl. Anim. Behav. Sci. 156, 22-36.

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.

Van de Perre V., Driessen, B., Van Thielen, J., Verbeke, G., Geers, R. 2011. Comparison of pig behaviour when given a sequence of enrichment objects or a chain continuously. Anim. Welfare 20, 641-649.

Van de Weerd, H.A., Docking, C.M., Day, J.E.L., Avery, P.J., and Edwards, S.A. 2003. A systematic approach towards developing environmental enrichment for pigs. Appl. Anim. Behav. Sci. 84, 101-118.

Van de Weerd, H.A., Docking, C.M., Day, J.E.L., Breuer, K., and Edwards, S.A. 2006. Effects of species-relevant environmental enrichment on the behaviour and productivity of finishing pigs. Appl. Anim. Behav. Sci. 99, 230-247.

Van de Weerd, H.A., Docking, C.M., Day, J.E.L., and Edwards, S.A. 2005. The development of harmful social behaviour in pigs with intact tails and different enrichment backgrounds in two housing systems. Anim. Sci. 80, 289-298.

Van Putten, G. 1969. An investigation into tail-biting among fattening pigs. Brit. Vet. Jl. 125, 511-517.

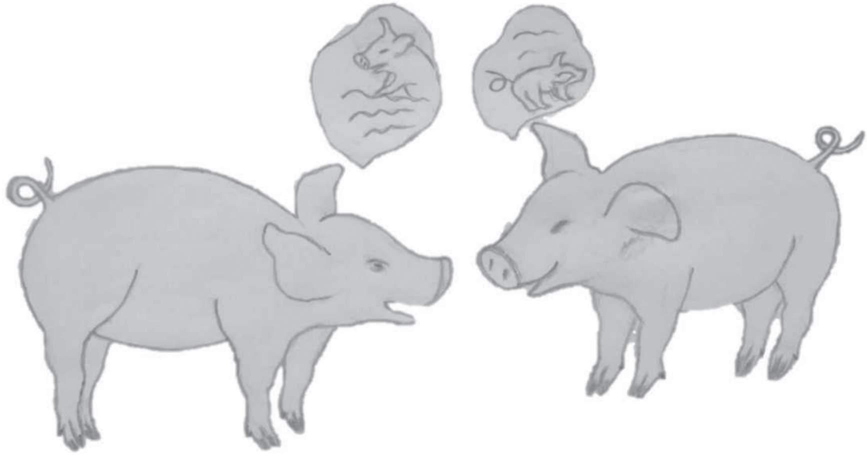
Van Putten, G. and Van de Burgwal, J.A. 1990. Vulva biting in group-housed sows: Preliminary report. Appl. Anim. Behav. Sci. 26, 181-186.

Wallgren, P. and Lindahl, E. 1996. The influence of tail biting on performance of fattening pigs. Acta Vet. Scand. 37, 453-460.

Welfare Quality. 2009. Welfare Quality® assessment protocol for pigs (sows and piglets, growing and finishing pigs). Welfare Quality® consortium, Lelystad, The Netherlands.

Zonderland, J.J., Wolthuis-Fillerup, M., Van Reenen, C.G., Bracke, M.B.M., Kemp, B., Den Hartog, L.A., and Spoolder, H.A.M. 2008. Prevention and treatment of tail biting in weaned piglets. Appl. Anim. Behav. Sci. 110, 269-281.

Zonderland, J.J., Bracke, M.B.M., Den Hartog, L.A., Kemp, B., and Spoolder, H.A.M. 2010. Gender effects on tail damage development in single- or mixed-sex groups of weaned piglets. Livest. Sci. 129, 151-158.



7

General discussion

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 (Schrøder-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 (Schrøder-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;

Zonderland *et al.*, 2011b). 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 (Schrøder-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*

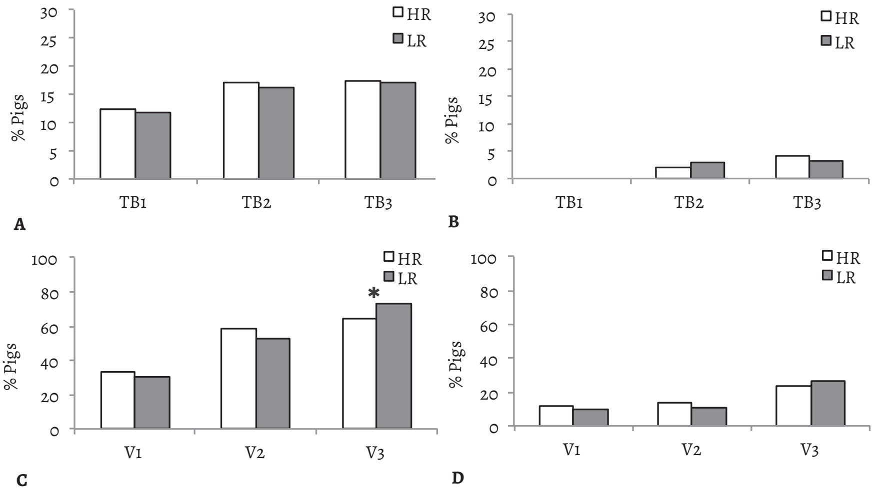


Figure 1. Percentage of low resisters (LR) or high resisters (HR) as determined by a back test at two weeks of age, classified as tail biters or victims of tail biting. Three consecutive phases post-weaning were considered, here indicated with 1-3. TB = Tail biter, V = Victim. **A**: Barren housed tail 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 (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.

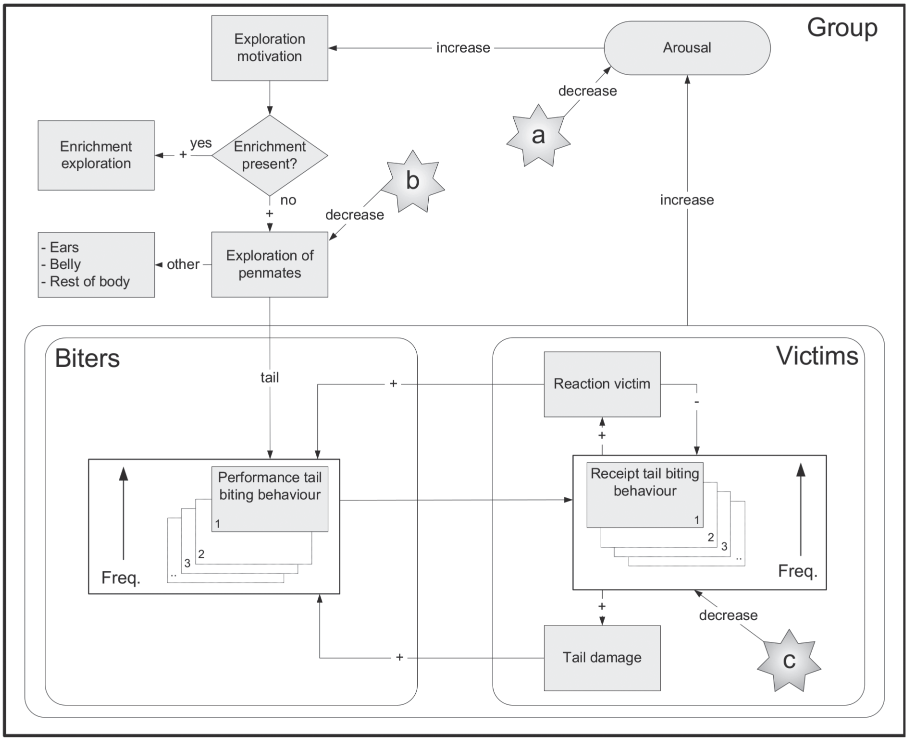


Figure 2. Aetiology model of a tail biting outbreak. The exploration motivation can lead to tail biting behaviour in the tail biter, and tail damage in the victim of tail biting. Group arousal may affect the level of exploration motivation. An increased chance is indicated with '+' and a decreased chance with '-'. Key points where preventive measures can be undertaken were indicated with 'a', 'b' and 'c' (Zonderland, 2010).

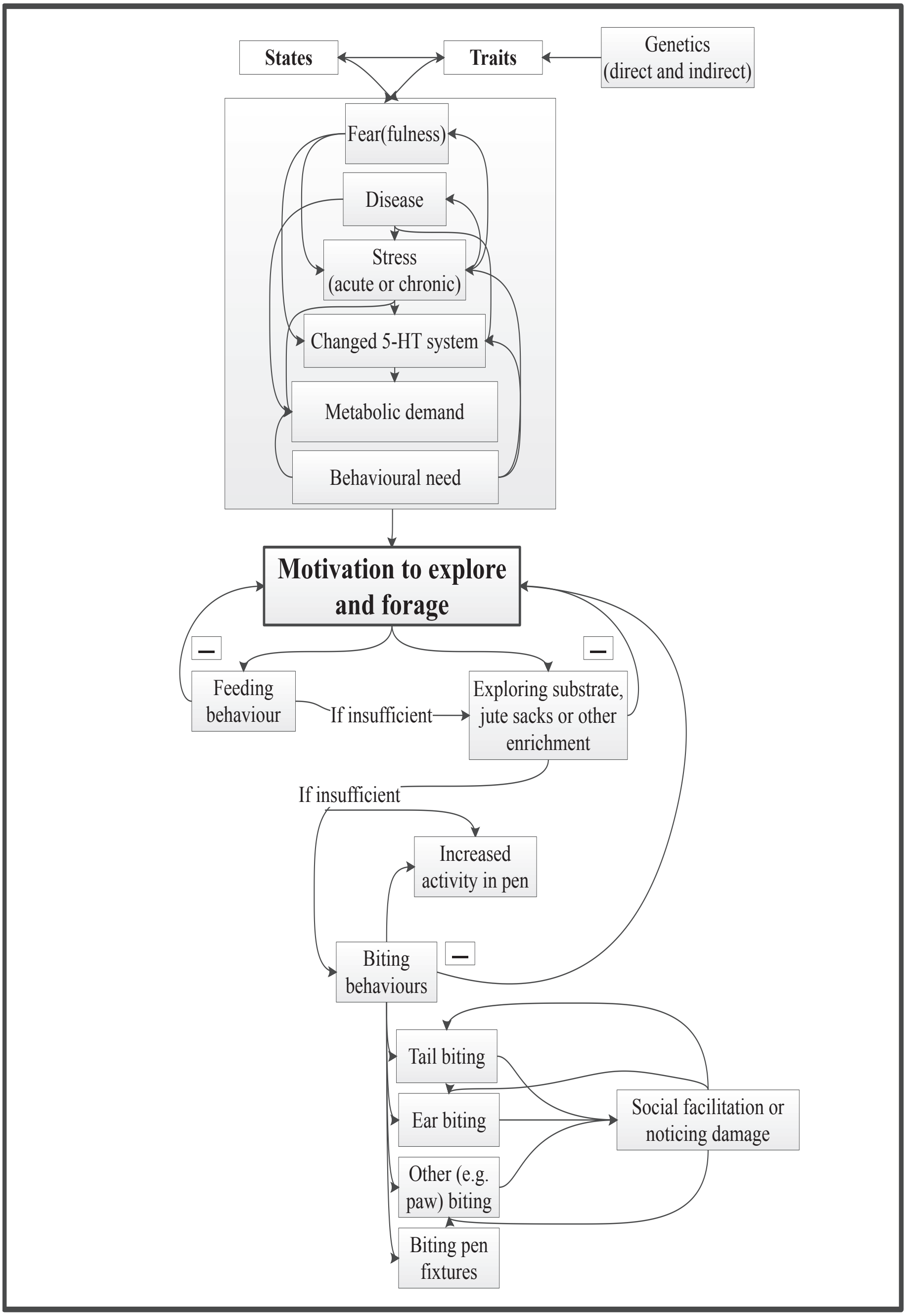


Figure 3. Conceptual framework of the motivation to explore and forage affected by different (combinations of) states and traits in pigs. Biting behaviours may follow when an increased motivation to explore and forage has developed and if feeding and/or exploring enrichment proves to be insufficient. 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.

References

Azmitia, E.C. 2010. Evolution of serotonin: Sunlight to suicide. In: Müller, C.R. and Jacobs, B. (Editors). Handbook of the behavioural neurobiology of serotonin. Academic Press, London, pp. 3-22.

Ball, R.O. and Bayley, H.S. 1984. Tryptophan requirement of the 2.5-kg piglet determined by the oxidation of an indicator amino acid. J. Nutr. 114, 1741-1746.

Barton, D.A., Esler, M.D., Dawood, T., Lambert, E.A., Haikerwal, D., Brenchley, C, Socratous, F., Hastings, J., Guo, L., Wiesner, G., Kaye, D.M., Bayles, R., Schlaich, M.P., and Lambert, G.W. 2008. Elevated brain serotonin turnover in patients with depression: Effect of genotype and therapy. Arch. Gen. Psychiat. 65, 38-46.

Beattie, V.E., Breuer, K., O'Connell, N.E., Sneddon, I.A., Mercer, J.T., Rance, K.A., Sutcliffe, M.E.M. and Edwards, S.A. 2005. Factors identifying pigs predisposed to tail biting. Animal Sci. 80, 307-312.

Bell, A.M. and Sih, A. 2007. Exposure to predation generates personality in threespined sticklebacks (Gasterosteus aculeatus). Ecol. Lett. 10, 828-834.

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

Boissy, A. 1995. Fear and fearfulness in animals. Q. Rev. Biol. 70, 165-191.

Bolhuis, J.E., Ellen, E.D., Van Reenen, C.G., De Groot, J, Napel, J.T., Koopmanschap, R.E., De Vries Reilingh G., Uitdehaag K.A., Kemp B., and Rodenburg T.B. 2009. Effects of genetic group selection against mortality on behavior and peripheral serotonin in domestic laying hens with trimmed and intact beaks. Physiol. Behav. 97, 470-475.

Bolhuis, J.E., Schouten, W.G.P., De Jong, I.C., Schrama, J.W., Cools, A.R., and Wiegant, V.M. 2000. Responses to apomorphine of pigs with different coping characteristics. Psychopharmacology. 152, 24-30.

Bracke, M.B.M. 2007. Multifactorial testing of enrichment criteria: Pigs 'demand' hygiene and destructibility more than sound. Appl. Anim. Behav. Sci. 107, 218-232.

Breuer, K., Sutcliffe, M.E.M., Mercer, J.T., Rance, K.A., O'Connell, N.E., Sneddon I.A., and Edwards, S.A. 2005. Heritability of clinical tail-biting and its relation to performance traits. Livest. Prod. Sci. 93, 87-94.

Brunberg, E., Wallenbeck, A., and Keeling, L.J. 2011. Tail biting in fattening pigs: Associations between frequency of tail biting and other abnormal behaviours. Appl. Anim. Behav. Sci. 133, 18-25.

Camerlink, I., Bijma, P., Kemp, B., and Bolhuis, J.E. 2012. Relationship between growth rate and oral manipulation, social nosing, and aggression in finishing pigs. Appl. Anim. Behav. Sci. 142, 11-17.

Chou, DT, Cuzzone, H, and Hirsh, KR. 1983. Assessment of blood platelets as a model for CNS response: Comparative effects of caffeine on 5-HT uptake and release mechanisms in rat platelets and rat brain serotonin neurons. Life Sci. 33, 1149-1156.

Coppen, A., Turner, P., Rowsell, and A.R., Padgham, C. 1976. 5 Hydroxytryptamine (5 HT) in the whole blood of patients with depressive illness. Postgrad. Med. J. 52, 156-158.

Day, J.E.L., Kyriazakis, I., and Lawrence, A.B. 1996. An investigation into the causation of chewing behaviour in growing pigs: The role of exploration and feeding motivation. Appl. Anim. Behav. Sci. 48, 47-59.

De Haas, E.N., Kemp, B., Bolhuis, J.E., Groothuis, T., and Rodenburg, T.B. 2013. Fear, stress, and feather pecking in commercial white and brown laying hen parent-stock flocks and their relationships with production parameters. Poultry Sci. 92, 2259-2269.

Delorme, R., Betancur, C., Callebert, J., Chabane, N., Laplanche, J.L., Mouren-Simeoni, M.C. Launay, J.M., and Leboyer, M. 2005. Platelet serotonergic markers as endophenotypes for obsessive-compulsive disorder. Neuropsychopharmacol. 30, 1539-1547.

De Ridder, K., Levesque, C.L., Htoo, J.K., and De Lange, C.F.M., 2012. Immune system stimulation reduces the efficiency of tryptophan utilization for body protein deposition in growing pigs. J. Anim. Sci. 90, 3485-3491.

Dingerkus, V.L.S., Gaber, T.J., Helmbold, K., Bubenzer, S., Eisert, A., Sánchez, C.L., and Zepf, F.D. 2012. Acute tryptophan depletion in accordance with body weight: Influx of amino acids across the blood-brain barrier. J. Neural. Transm. 119, 1037-1045.

Docking, C.M., Van de Weerd, H.A., Day, J.E.L., and Edwards, S.A., 2008. The influence of age on the use of potential enrichment objects and synchronisation of behaviour of pigs. Appl. Anim. Behav. Sci. 110, 244-257.

D'Souza, U.M. and Craig, I.W. 2010. Genetic Organization of the Serotonergic System. In: Müller, C.R. and Jacobs, B. (Editors). Handbook of the behavioural neurobiology of serotonin. Academic Press, London, 23-50.

Edwards, S.A. 2006. Tail biting in pigs: understanding the intractable problem. Vet. J. 171, 198-199.

EFSA. 2007. Scientific report on the risks associated with tail biting in pigs and possible means to reduce the need for tail docking considering the different housing and husbandry systems. Annex to the EFSA J. 611, 1-13 (Question No EFSA-Q-2006-013, 1-98).

Fraser, D. 1987a. Attraction to blood as a factor in tail-biting by pigs. Appl. Anim. Behav. Sci. 17, 61-68.

Fraser, D. 1987b. Mineral-deficient diets and the pig's attraction to blood: implications for tail-biting. Can. J. Anim. Sci. 67, 909-918.

Fraser, D., Phillips, P.A., Thompson, B.K., and Tennessen, T. 1991. Effect of straw on the behaviour of growing pigs. Appl. Anim. Behav. Sci. 30, 307-318.

Fritschen, R. and Hogg, A. 1983. Preventing tail biting in swine (anti-comfort syndrome). Nebguide G 75-246 (Revised). Institute of Agriculture and Natural Resources, University of Nebraska, Lincoln, NE.

Gifford, A.K., Cloutier, S., and Newberry, R.C., 2007. Objects as enrichment: Effects of object exposure time and delay interval on object recognition memory of the domestic pig. Appl. Anim. Behav. Sci. 107, 206-217.

Griffing, B. 1967. Selection in reference to biological groups. I. Individual and group selection applied to populations of unordered groups. Austr. J. Biol. Sci. 10, 127-139.

Guzik, A.C., Matthews, J.O., Kerr, B.J., Bidner, T.D., and Southern, L.L. 2006. Dietary tryptophan effects on plasma and salivary cortisol and meat quality in pigs. J. Anim. Sci. 84, 2251-2259.

Heinonen, M., Orro, T., Kokkonen, T., Munsterhjelm, C., Peltoniemi, O., and Valros, A. 2010. Tail biting induces a strong acute phase response and tail-end inflammation in finishing pigs. Vet. J. 184, 303-307.

Hessing, M.J.C., Hagelso, A.M., Schouten, W.G.P., Wiepkema, P.R., and Van Beek, J.A.M. 1994. Individual behavioral and physiological strategies in pigs. Physiol. Behav. 55, 39-46.

Hughes, B.O. and Duncan, I.J.H. 1988. The notion of ethological 'need', models of motivation and animal welfare. Anim. Behav. 36, 1696-1707.

Joseph, M.H. and Kennett, G.A. 1983. Corticosteroid response to stress depends upon increased tryptophan availability. Psychopharmacology. 79, 79-81.

Kampman-Van de Hoek, E., Gerrits, W.J.J., Van der Peet-Schwering, C.M.C., Jansman, A.J.M., and Van den Borne, J.J.G.C. 2013. A simple amino acid dose-response method to quantify amino acid requirements of individual meal-fed pigs. J. Anim. Sci. 91, 4788-4796.

Keszthelyi, D., Troost, F.J., and Masclee, A.A.M. 2009. Understanding the role of tryptophan and serotonin metabolism in gastrointestinal function. Neurogastroenterol. Motil. 21, 1239-1249.

Koolhaas, J.M., De Boer, S.F., Buwalda, B., and Van Reenen, K. 2007. Individual variation in coping with stress: A multidimensional approach of ultimate and proximate mechanisms. Brain Behav. Evolut. 70, 218-226.

Korte, S.M., Prins, J., Vinkers, C.H., and Olivier, B. 2009. On the origin of allostasis and stress-induced pathology in farm animals: Celebrating Darwin's legacy. Vet. J. 182, 378-383.

La Guardia, J.G., Ryan, R.M., 2007. Why identities fluctuate: Variability in traits as a function of situational variations in autonomy support. J. Pers. 75, 1205-1228.

Le Floc'h, N., Melchior, D., and Obled, C. 2004. Modifications of protein and amino acid metabolism during inflammation and immune system activation. Livest. Prod. Sci. 87, 37-45.

Le Floc'h, N., Otten, W., and Merlot, E. 2011. Tryptophan metabolism, from nutrition to potential therapeutic applications. Amino Acids 41, 1195-1205.

Leplow, B., Murphy, R., and Nutzinger, D.O. 2002. Specificity of conditional associative-learning deficits in obsessive-compulsive disorder (OCD) and non-OCD anxiety disorders. J. Clin. Exp. Neuropsyc. 24, 792-805.

MacDonald, S.W.S., Nyberg, L., and Bäckman, L. 2006. Intra-individual variability in behavior: links to brain structure, neurotransmission and neuronal activity. Trends Neurosci. 29, 474-480.

Maes, M. 1995. Effects of serotonin precursors on the negative feedback effects of glucocorticoids on hypothalamic-pituitary-adrenal axis function in depression. Psychoneuroendocrino. 20, 149-167.

Martínez-Trejo, G., Ortega-Cerrilla, M.E., Rodarte-Covarrubias, L.F., Herrera-Haro, J.G., Figueroa-Velasco, J.L., Galindo-Maldonado, F., Sánchez-Martínez, O., and Lara-Bueno, A. 2009. Aggressiveness and productive performance of piglets supplemented with tryptophan. J. Anim. Vet. Adv. 8, 608-611.

Meeusen, R. and Watson, P. 2007. Amino acids and the brain: do they play a role in "central fatigue"? Int. J. Sport Nutr. Exe. 17, Suppl: S37-46.

Melotti, L., Oostindjer, M., Bolhuis, J.E., Held, S., and Mendl, M. 2011. Coping personality type and environmental enrichment affect aggression at weaning in pigs. Appl. Anim. Behav. Sci. 133, 144-153.

Meunier-Salaün, M.C., Monnier, M., Colléaux, Y., Sève, B., and Henry, Y. 1991. Impact of dietary tryptophan and behavioral type on behavior, plasma cortisol, and brain metabolites of young pigs. J. Anim. Sci. 69, 3689-3698.

Moore, A.J., Brodie III, E.D., and Wolf, J.B. 1997. Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. Evolution. 51, 1352-1362.

Mück-Šeler, D. and Pivac, N. 2011. Serotonin. Period. Biol. 113, 29-41.

Munsterhjelm, C., Brunberg, E., Heinonen, M., Keeling, L., and Valros, A. 2013. Stress measures in tail biters and bitten pigs in a matched case-control study. Anim. Welfare. 22, 331-338.

Munsterhjelm, C., Peltoniemi, O.A.T., Heinonen, M., Hälli, O., Karhapää, M., and Valros, A. 2009. Experience of moderate bedding affects behaviour of growing pigs. Appl. Anim. Behav. Sci. 118, 42-53.

O'Connell, N.E., Beattie, V.E., Sneddon, I.A., Breuer, K., Mercer, J.T., Rance, K.A., Sutcliffe, M.E.M., and Edwards, S.A. 2005. Influence of individual predisposition, maternal experience and lactation environment on the responses of pigs to weaning at two different ages. Appl. Anim. Behav. Sci. 90, 219-232.

Palander, P.A., Heinonen, M., Simpura, I., Edwards, S.A., and Valros, A.E. 2013. Jejunal morphology and blood metabolites in tail biting, victim and control pigs. Animal 7, 1523-1531.

Pearish, S., Hostert, L., and Bell, A.M. 2013. Behavioral type-environment correlations in the field: A study of three-spined stickleback. Behav. Ecol. Sociobiol. 67, 765-774.

Reimert, I., Rodenburg, T.B., Ursinus, W.W., Kemp, B., and Bolhuis, J.E. 2014. Responses to novel situations of female and castrated male pigs with divergent social breeding values and different backtest classifications in barren and straw-enriched housing. Appl. Anim. Behav. Sci. 151, 24-35.

Rodenburg, T.B., De Haas, E.N., Nielsen, B.L., and Buitenhuis, A.J. 2010. Fearfulness and feather damage in laying hens divergently selected for high and low feather pecking. Appl. Anim. Behav. Sci. 128, 91-96.

Sällvik, K. and Walberg, K. 1984. The effects of air velocity and temperature on the behaviour and growth of pigs. J. Agr. Eng. Res. 30, 305-312.

Sanger, G.J. 2008. 5-Hydroxytryptamine and the gastrointestinal tract: Where next? Trends Pharmacol. Sci. 29, 465-471.

Scheepens, C.J.M., Hessing, M.J.C., Laarakker, E., Schouten, W.G.P., and Tielen, M.J.M. 1991. Influences of intermittent daily draught on the behaviour of weaned pigs. Appl. Anim. Behav. Sci. 31, 69-82.

Schrøder-Petersen, D.L. and Simonsen, H.B. 2001. Tail biting in pigs. Vet. J. 162, 196-210.

Schrøder-Petersen D.L., Simonsen H.B., and Lawson L.G. 2003. Tail-in-mouth behaviour among weaner pigs in relation to age, gender and group composition regarding gender. Acta Agric. Scand. A-AN 53: 29-34.

Segall, P.E. and Timiras, P.S. 1976. Pathophysiologic findings after chronic tryptophan deficiency in rats: a model for delayed growth and aging. Mech. Ageing Dev. 5, 109-124.

Sève, B., Meunier-Salaün, M.C., Monnier, M., Colléaux, Y., and Henry, Y. 1991. Impact of dietary tryptophan and behavioral type on growth performance and plasma amino acids of young pigs. J. Anim. Sci. 69, 3679-3688.

Shen, Y.B., Voilqué, G., Kim, J.D., Odle, J., and Kim, S.W. 2012. Effects of increasing tryptophan intake on growth and physiological changes in nursery pigs. J. Anim. Sci. 90, 2264-2275.

Siegford, J.M., Rucker, G., and Zanella, A.J. 2008. Effects of pre-weaning exposure to a maze on stress responses in pigs at weaning and on subsequent performance in spatial and fear-related tests. Appl. Anim. Behav. Sci. 110, 189-202.

Souza da Silva, C., Haenen D., Koopmans, S.J., Hooiveld, G.J.E.J., Bosch, G., Bolhuis, J.E., Kemp, B., Müller, M., and Gerrits, W.J.J. 2014. Effects of resistant starch on behaviour, satiety-related hormones and metabolites in growing pigs. Animal. doi:10.1017/S1751731114001116.

Smulders, D., Hautekiet, V., Verbeke, and G., Geers, R. 2008. Tail and ear biting lesions in pigs: An epidemiological study. Anim. Welfare 17, 61-69.

Smulders, D., Verbeke, G., Mormède, P., and Geers, R. 2006. Validation of a behavioral observation tool to assess pig welfare. Physiol. Behav. 89, 438-447.

Stamps, J.A., Briffa, M., and Biro, P.A. 2012. Unpredictable animals: Individual differences in intraindividual variability (IIV). Anim. Behav. 83, 1325-1334.

Statham, P., Green, L., Bichard, M., and Mendl, M. 2009. Predicting tail-biting from behaviour of pigs prior to outbreaks. Appl. Anim. Behav. Sci. 121, 157-164.

Studnitz, M., Jensen, M.B., and Pedersen, L.J. 2007. Why do pigs root and in what will they root? A review on the exploratory behaviour of pigs in relation to environmental enrichment. Appl. Anim. Behav. Sci. 107, 183-197.

Taylor, N.R., Main, D.C., Mendl, M., and Edwards, S.A. 2010. Tail-biting: a new perspective. Vet. J. 186, 137-147.

Taylor, N.R., Parker, R.M.A., Mendl, M., Edwards, S.A., and Main, D.C.J. 2012. Prevalence of risk factors for tail biting on commercial farms and intervention strategies. Vet. J. 194, 77-83.

Telkänranta, H., Swan, K., Hirvonen, H., and Valros, A. 2014. Chewable materials before weaning reduce tail biting in growing pigs. Appl. Anim. Behav. Sci. doi: 10.1016/j.applanim.2014.01.004.

Turner, S.P., 2011. Breeding against harmful social behaviours in pigs and chickens: State of the art and the way forward. Appl. Anim. Behav. Sci. 134, 1-9.

Valros, A., Keeling, L., Thodberg, K., and Zanella, A.J. 2012. Tail biting and tail docking in the pig: Biological mechanisms, prevention, treatment and economic aspects. Slutrapport. Nordic Joint Committee for Agricultural and Food Research (NKJ), Oslo, Norway.

Valros, A., Palander, P.A., Heinonen, M., Brunberg, E., Keeling, L., and Piepponen, T.P. 2013. Preliminary evidence of an altered serotonin metabolism in the prefrontal cortex of tail biting pigs. In: Hötzel, M.J. and Pinheiro Machado Filho, L.C. (Editors). Proceedings of the 47th Congress of the International Society for Applied Ethology (ISAE), Florianopolis, Brazil, pp 105.

Van der Mast, R.C. and Fekkes, D. 2000. Serotonin and amino acids: partners in delirium pathophysiology? Sem. Clin. Neuropsy. 5: 125-131.

Van de Perre, V., Driessen, B., Van Thielen, J., Verbeke, G., and Geers, R. 2011. Comparison of pig behaviour when given a sequence of enrichment objects or a chain continuously. Anim. Welfare 20, 641-649.

Van de Weerd, H.A., Docking, C.M., Day, J.E.L., Avery, P.J., and Edwards, S.A. 2003. A systematic approach towards developing environmental enrichment for pigs. Appl. Anim. Behav. Sci. 84, 101-118.

Van de Weerd, H.A., Docking, C.M., Day, J.E.L., and Edwards, S.A. 2005. The development of harmful social behaviour in pigs with intact tails and different enrichment backgrounds in two housing systems. Anim. Sci. 80, 289-298.

Van Hierden, Y.M., De Boer, S.F., Koolhaas, J.M., and Korte, S.M. 2004. The control of feather pecking by serotonin. Behav. Neurosci. 118, 575-583.

Van Reenen, C.G., O'Connell, N.E., Van Der Werf, J.T.N., Korte, S.M., Hopster, H., Jones, R.B., and Blokhuis, H.J. 2005. Responses of calves to acute stress: Individual consistency and relations between behavioral and physiological measures. Physiol. Behav. 85, 557-570.

Van Putten, G. 1969. An investigation into tail-biting among fattening pigs. Brit. Vet. Jl. 125, 511-517.

Velie, B.D., Maltecca, C., and Cassady, J.P. 2009. Genetic relationships among pig behavior, growth, backfat, and loin muscle area. J. Anim. Sci. 87, 2767–2773.

Walker, P.K. and Bilkei, G. 2006. Tail-biting in outdoor pig production. Vet. J. 171, 367-369.

Wallenbeck, A. and Keeling, L.J. 2013. Using data from electronic feeders on visit frequency and feed consumption to indicate tail biting outbreaks in commercial pig production. J. Anim. Sci. 91, 2879-2884.

Wallenbeck, A., Larsen A., Holmgren N., and Keeling L.J. 2010. Predicting tail biting outbreaks among growing-finishing pigs under commercial conditions. In: Lidfors, L., Blokhuis, H.J., Keeling, L.J. (Editors). Proceedings of the 44th Congress of the International Society for Applied Ethology (ISAE), Uppsala, Sweden. p. 76.

Wallgren, P. and Lindahl, E. 1996. The influence of tail biting on performance of fattening pigs. Acta Vet. Scand. 37, 453-460.

Wilson, K., Zanella, R., Ventura, C., Johansen, H.L., Framstad, T., Janczak, A., Zanella, A.J., and Neibergs, H.L. 2012. Identification of chromosomal locations associated with tail biting and being a victim of tail-biting behaviour in the domestic pig (Sus scrofa domesticus). J. Appl. Genet. 53: 449-456.

Wood-Gush, D.G.M. and Vestergaard, K., 1989. Exploratory behavior and the welfare of intensively kept animals. J. Agric. Ethics. 2, 161-169.

Yatham, L.N., Steiner, M., Liddle, P.F., Shiah, I.S., Lam, R.W., Zis, A.P., and Coote, M. 2000. A PET study of brain 5-HT2 receptors and their correlation with platelet 5-HT2 receptors in healthy humans. Psychopharmacology. 151, 424-427.

Zitterl, W., Aigner, M., Stompe, T., Zitterl-Eglseer, K., Gutierrez-Lobos, K., Wenzel, T., Zettinig, G., Hornik, K., Pirker, W., and Thau, K. 2008. Changes in thalamus-hypothalamus serotonin transporter availability during clomipramine administration in patients with obsessive-compulsive disorder. Neuropsychopharmacol. 33, 3126-3134.

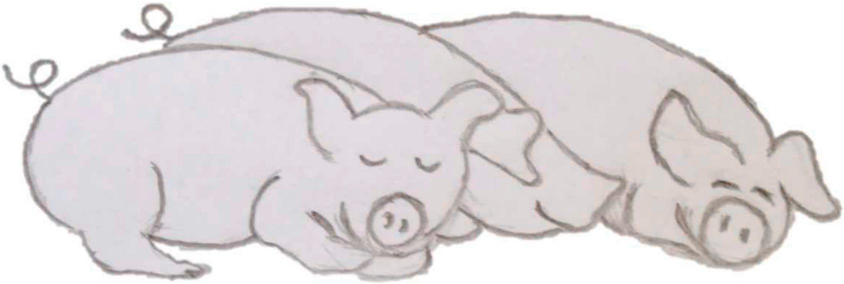
Zonderland, J.J. 2010. Talking Tails: Quantifying the development of tail biting in pigs. Doctoral Thesis, Wageningen University, Wageningen, The Netherlands.

Zonderland, J.J., Kemp, B., Bracke, M.B.M., Den Hartog, L.A., and Spoolder, H.A.M. 2011a. Individual piglets contribution to the development of tail biting. Animal 5, 601-607.

Zonderland, J.J., Schepers, F., Bracke, M.B.M., Den Hartog, L.A., Kemp, B., and Spoolder, H.A.M. 2011b. Characteristics of biter and victim piglets apparent before a tail-biting outbreak. Animal 5, 767-775.

Zonderland, J.J., Van Riel, J.W., Bracke, M.B.M., Kemp, B., Den Hartog, L.A., and Spoolder, H.A.M. 2009. Tail posture predicts tail damage among weaned piglets. Appl. Anim. Behav. Sci. 121, 165-170.

Zonderland, J.J., Wolthuis-Fillerup, M., Van Reenen, C.G., Bracke, M.B.M., Kemp, B., Den Hartog, L.A., and Spoolder, H.A.M. 2008. Prevention and treatment of tail biting in weaned piglets. Appl. Anim. Behav. Sci. 110, 269-281.



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.

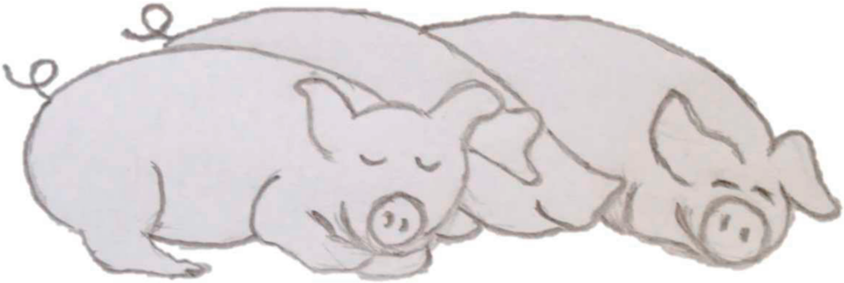
Pigs may affect each other's production level through behavioural interactions. The effect of one pig on another pig may be heritable and is then called an indirect genetic effect. In Chapter 5, it was aimed to assess whether selection for indirect genetic effects on growth of pen mates may alter the 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

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 geïndentificeerd 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 staarbijten 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 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 metaboliet 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 beangstigender 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. Serotonine 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 beangstigender 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 onbekenheid 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 gelten 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 fysiologische 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 optimaliseren van de fokkerij van het varken, het management systeem op het varkensbedrijf, de voeding en de huisvestingscondities waarin de varkens leven.

Dankwoord

Dankwoord

'Bedankt', het is zo'n woordje dat zo gemakkelijk gezegd is, maar zo ontzettend veel kan betekenen. In dit dankwoord zal ik me tot lang niet iedereen kunnen richten die het eigenlijk wel verdiend heeft. Dat ik dit proefschrift heb kunnen schrijven ligt aan het hele levenspad dat ik heb mogen bewandelen en waarin ik ontzettend veel inspirerende mensen ben tegengekomen. Daarom wil ik graag als eerste eenieder bedanken die mij heeft geaccepteerd en gewaardeerd zoals ik ben, die op enigerwijze interesse heeft getoond in de dingen die ik deed en waar ik leerzame en vooral ook leuke momenten mee heb gekend. *To say thanks is easily said, but can mean a lot. I am not able to thank everybody here, although a personal 'thanks' would be appropriate. Writing this PhD thesis was only possible due to the path of life I followed and the many inspiring people I met along the way. Therefore, I first would like to thank everbody who appreciated me for who I am, who was interested in (at least a few) things I did, and with whom I had moments of wisdom, and especially joy. "Gratiam vobis ago!!"*

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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., and Bolhuis, J.E. 2014. Struggling to survive: early life challenges in relation to the backtest in pigs. J. Anim. Sci. 92: 3088-3095.

Ursinus, W.W., Van Reenen, C.G., Kemp, B., and Bolhuis, J.E. 2014. Tail biting behaviour and tail damage in pigs and the relationship with general behaviour: Predicting the inevitable? Appl. Anim. Behav. Sci. 156, 22-36.

Reimert, I., Rodenburg, T.B., Ursinus, W.W., Kemp, B., and Bolhuis, J.E. 2014. Responses to novel situations of female and castrated male pigs with divergent social breeding values and different backtest classifications in barren and straw-enriched housing. Appl. Anim. Behav. Sci. 151, 24-35.

Reimert, I., Rodenburg, T.B., Ursinus, W.W., Duijvesteijn, N., Camerlink, I., Kemp, B., and Bolhuis, J. E. 2013. Backtest and novelty behavior of female and castrated male piglets with diverging social breeding values for growth. J. Anim. Sci. 91, 4589-4597.

Ursinus, W.W., Bolhuis, J.E., Zonderland, J.J., Rodenburg, T.B., De Souza, A.S., Koopmanschap, R.E., Kemp, B., Korte-Bouws, G.A.H., Korte, S.M., and Van Reenen, C.G. 2013. Relations between peripheral and brain serotonin measures and behavioural responses in a novelty test in pigs. Physiol. Behav. 118, 88-96.

Ursinus, W.W., Schepers, F., De Mol, R.M., Bracke, M.B.M., Metz, J.H.M., and Groot Koerkamp, P.W.G. 2009. COWEL: a decision support system to assess welfare of husbandry systems for dairy cattle. Anim. Welfare 18, 545-552.

Mollenhorst, H., Kettenis, D.L., Koene, P., Ursinus, W.W., and Metz, J.H.M. 2008. Behaviour-based simulation of facility demand of laying hens. Biosyst. Eng. 100, 581-590.

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.

Ursinus, W.W., Wijnen, H.J., Bartels, A.C., Duijvesteijn, N., Van Reenen, C.G., and Bolhuis, J.E. Damaging biting behaviors in intensively kept rearing gilts: the effect of jute sacks, and relations with production characteristics. Under revision in J. Anim. Sci.

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.

Camerlink, I., Turner, S.P., Ursinus, W.W., Reimert, I., and Bolhuis, J.E. Aggression and affiliation during social conflict in 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

Ursinus, W.W., Van Reenen, C.G., Reimert, I., Kemp, B., and Bolhuis, J.E. 2014. Tail biting in pigs: (In)consistency, blood serotonin, and responses to novelty. In: Proceedings of the 48th Congress of the International Society for Applied Ethology (ISAE), July 29-August 2, Vitoria-Gasteiz, Spain. p. 136.

Bolhuis, J.E., Reimert, I., Ursinus, W.W., Camerlink, I., Rodenburg, T.B., and Kemp, B. Changing the animal or the environment: changes in breeding strategy and housing conditions to improve the welfare of pigs. In: Proceedings of the 48th Congress of the International Society for Applied Ethology (ISAE), July 29-August 2, Vitoria-Gasteiz, Spain. p. 163.

Ursinus, W.W., Van Reenen, C.G., Reimert, I., Kemp, B., and Bolhuis, J.E. 2014. Inevitable or not, tail biting in pigs is associated with activity, fearfulness and blood serotonin (poster). WIAS Science Day, April 30, Wageningen, The Netherlands.

Ursinus, W.W., Van Reenen, C.G., Reimert, I., Kemp, B., and Bolhuis, J.E. 2013. Pigs make up their mind early in life: behaviour in novelty tests and blood serotonin. In: Proceedings of the 47th Congress of the International Society for Applied Ethology (ISAE), June 2-6, Florianopolis, Brazil. p. 61.

Reimert, I., Rodenburg, T.B., Ursinus, W.W., Kemp, B., and Bolhuis, J.E. 2013. Differences between female and castrated male pigs in their response to novelty. In: Proceedings of the 47th Congress of the International Society for Applied Ethology (ISAE), June 2-6, Florianopolis, Brazil. p. 60.

Ursinus, W.W., Bolhuis, J.E., Zonderland, J.J., Rodenburg, T.B., Souza, A.S., Koopmanschap, R.E., Kemp, B., Korte-Bouws, G.A.H., Korte, S.M., and Van Reenen, C.G. 2013. Secrets about the inner pig may be revealed by the challenges of a Novelty test. In: Proceedings of the WIAS Science Day 2013, February 28, Wageningen, the Netherlands. p. 10.

Reimert, I., Rodenburg, T.B., Ursinus, W.W., and Bolhuis, J.E. 2012. Differences in novelty behaviour for pigs with different coping styles (poster). In: Proceedings of the 20th Annual Meeting of the Netherlands Society for Behavioural Biology, November 28–30, Soesterberg, The Netherlands. p. 14.

Ursinus, W.W., Bolhuis, J.E., Zonderland, J.J., Kemp, B., Korte-Bouws, G.A.H., Korte, S.M., and Van Reenen, C.G. 2012. Relationship between blood platelet serotonin parameters, brain serotonin turnover and behavioural responses of pigs. In: Proceedings of the 46th Congress of the International Society for Applied Ethology (ISAE), July 31–August 4, Vienna, Austria. p. 81.

Camerlink, I., Bijma, P., Ursinus, W.W., and Bolhuis, J.E. 2012. Behaviour of finishing pigs divergently selected for social genetic effects in barren and straw-enriched pens. In: Proceedings of the 46th Congress of the International Society for Applied Ethology (ISAE), July 31–August 4, Vienna, Austria. p. 35.

Ursinus, W.W., Bolhuis, J.E., Zonderland, J.J., Korte, S.M., and Van Reenen, C.G. 2012. Pig behaviour in novelty test relates to serotonergic brain-blood parameters. In: Minding Animals Conference, July 4-6, Utrecht, the Netherlands.

Ursinus, W.W., Bolhuis, J.E., Zonderland, J.J., Kemp, B., Korte-Bouws, G.A.H., Korte, S.M., and Van Reenen, C.G. 2012. Correlations between pig behaviour and serotonergic blood & brain parameters (poster). WIAS Science Day, February 2, Wageningen, The Netherlands.

Camerlink, I., Reimert, I., Duijvesteijn, N., Benard, M., Ursinus, W.W., Bijma, P., Bolhuis, J.E., Kemp, B., and Van Arendonk, J.A.M. 2011. Improving pig welfare and production through social genetic effects (poster). NWO symposium 'Waardering van dierenwelzijn', November 30, Utrecht, The Netherlands.

Reimert, I., Benard, M., Camerlink, I., Duijvensteijn, N., Ursinus, W.W., Bolhuis, J.E., Rodenburg, T.B., Koolhaas, J.M., and Kemp, B. 2011. Sociable Swine: effects of social interactions on welfare (poster). NWO symposium 'Waardering van dierenwelzijn', November 30, Utrecht, The Netherlands.

De Greef, K.H., Averos, X., Brossard, L., Dourmad, J.Y., Edge, H.L., Edwards, S.A., Meunier-Salaun, M., and Ursinus, W.W. 2009. Pig welfare model development: consumers and citizens. In: Book of Abstracts of the 60th Annual Meeting of the European Association for Animal Production (EAAP), August 24-27, Barcelona, Spain. p. 624.

Ursinus, W.W. and De Greef, K.H. 2009. What matters to a sow in a farrowing system: the needs of lactating sows. In: Book of Abstracts of the 60th Annual Meeting of the European Association for Animal Production (EAAP), August 24-27, Barcelona, Spain. p. 480.

Groot Koerkamp, P.W.G., Ursinus, W.W., Schepers, F., De Mol, R.M., Bracke, M.B.M., Metz, J.H.M., and Houwers, H.W.J. 2009. COWEL: Semantic modelling of the relation between husbandry characteristics and animal welfare performance to evaluate existing and design new husbandry systems for dairy cows. In: Proceedings of Knowing Animals: a cross-fertilisation between natural and social sciences for understanding the quality of life of animals, March 5-6, Florence, Italy. p. 49.

Ursinus, W.W., Schepers, F., De Mol, R.M., Metz, J.H.M., Bracke, M.B.M., Houwers, H.W.J., and Groot Koerkamp, P.W.G. 2008. COWEL: A decision support system for welfare scoring of new husbandry systems for dairy cattle. In: Book of Abstracts of the 4th International Workshop on the Assessment of Animal Welfare at Farm and Group Level (WAFL), September 10-13, Ghent, Belgium. p. 53.

Reports and brochures

Houwers, H.W.J. and Ursinus, W.W. 2010. An inventory of loose housing systems for farrowing and lactating sows in the Netherlands. Wageningen UR Livestock Research, Lelystad, Report no. 551.

Ursinus, W.W. and De Greef, K.H. 2010. Towards animal friendly sow housing: The needs of farrowing sows and examples of loose housing systems. Wageningen UR Livestock Research, Lelystad, Internal report no.: 201202.

Van Eijk, O.N.M., De Lauwere, C.C., Van Weeghel, H.J.E., Lansbergen, L.M.T.E., Miedema, A.M.M.A., Ursinus, W.W., Janssen, A.P.H.M., Cornelissen, J.M.R., and Zonderland, J.J. 2010. Varkansen: De Pagode, De Pijler en De Parel (brochure). Wageningen UR Livestock Research, Lelystad.

Van Eijk, O.N.M., De Lauwere, C.C., Van Weeghel, H.J.E., Lansbergen, L.M.T.E., Miedema, A.M.M.A., Ursinus, W.W., Janssen, A.P.H.M., Cornelissen, J.M.R., and Zonderland, J.J. 2010. Pork Opportunities: Three design directions for economically, ecologically and socially sustainable pig production and steps towards realisation (brochure). Wageningen UR Livestock Research, Lelystad.

Van Eijk, O.N.M., De Lauwere, C.C., Van Weeghel, H.J.E., Lansbergen, L.M.T.E., Miedema, A.M.M.A., Ursinus, W.W., Janssen, A.P.H.M., Cornelissen, J.M.R., and Zonderland, J.J. 2010. Varkansen, springplank naar een duurzame veehouderij: varkenshouderij met neus voor dier, ondernemer, milieu en burgerconsument (brochure). Wageningen UR Livestock Research, Lelystad.

Van Weeghel, H.J.E., Cornelissen, J.M.R., De Greef, K.H., Lansbergen, L.M.T.E., De Lauwere, C.C., Ursinus, W.W., Vermeer, H.M., and Zonderland, J.J. 2009. Wat wil het varken? Van behoeften naar stalontwerpen (brochure). Wageningen UR Livestock Research, Lelystad.

Cornelissen, J.M.R., Ursinus, W.W., Schepers, F., Groot Koerkamp, P.W.G., and Van Dixhoorn, I.D.E. 2009. Brief of requirements of the dairy cow. Wageningen UR Livestock Research, Lelystad, Report no. 264.

Van Eijk, O.N.M., Janssen, A.P.H.M., De Lauwere, C.C., Lokhorst, C., Miedema, A.M.M.A., Ursinus, W.W., Van Weeghel, H.J.E., and Zonderland, J.J. 2009. Varkansen: de eerste ontwerpronde. Animal Sciences Group van Wageningen UR, Lelystad, Internal report no. 200906.

Ursinus, W.W., Schepers, F., Bokkers, E.A.M., Bracke, M.B.M., and Spoolder, H.A.M. 2009. General overview of animal welfare in a global perspective. In: Bracke, M.B.M. (Editor). Animal Welfare in Global Perspective. Wageningen UR Livestock Research, Lelystad, Report no. 240: 4 - 53.

Bokma-Bakker, M.H., Munnichs, G., Bracke, M.B.M., Visser, E.K., Schepers, F., Ursinus, W.W., Blokhuis, H.J., Gerritzen, M.A., Ter Gast, E., Evers, A.G., De Haan, M.H.A., Van Mil, E.M., Van Reenen, C.G., and Brom, F.W.A. 2009. Animal-based welfare monitoring: final report. Part 1. Scientific and technological state-of-the-art. Part 2. Impact of animal-based welfare assessment. Rathenau Institute, The Hague.

Miscellaneous

Telkänranta, H. 2014. Juuttisäkin pureskelu vähentää hännänpurentaa. KMVET 3, 38-39.

Lesscher, I. 2014. Staartbijten komt vaak al voor het spenen voor. Nieuwe Oogst, editie LTO Noord. June 7. p. 22.

Smit, G. 2014. Why social pigs do better. Noldus.com, January 28.

Ter Beek, V. 2013. A revolution in thinking about pig tails. Pig Progress 29:6, 13-15.

Ursinus, W.W. 2013. Bloed vertelt over persoonlijkheid varken: zoeken naar voorspeller(s) voor staartbijten. V-focus 10:4, 28-29.

Anonymous. 2013. Zes tips voor langere staarten. Varkens.nl. May 17.

Anonymous. 2013. Lange weg naar lange staarten. Varkens.nl. May 16.

Ten Hooven, M. 2013. Varkenshouders zien snel kans op staartbijten. Boerderij.nl, March 15.

Lamers, J. 2012. Knabbelen al vroeg aangeleerd. Varkens 76, 35.

Lamers, J. 2012. Schade door onopvallend bijten. Varkens 76, 34.

Lesscher, I. 2012. Staartbijten en genetica. Varkensnet.nl. November 12.

Lamers, J. 2012. Asociale bijters. Varkens.nl. March 14.

Ursinus, W.W. and Van Weeghel, H.J.E. 2009. De behoeften van varkens: uitleg. V-focus 6:5, 34-35.

Groot Koerkamp, P.W.G., Bos, A.P., Ursinus, W.W., Schepers, F., Van Dixhoorn, I.D.E., and Cornelissen, J.M.R. 2009. Hoge score voor welzijn en praktisch haalbaar. V-focus 6:5, 8-9.

Ursinus, W.W., Groot Koerkamp, P.W.G., and Bos, A.P. 2009. Verantwoording van de welzijnsscore in Cowel van de ontwerpen van Kracht van Koeien. Wageningen UR Livestock Research, Lelystad.

Cornelissen, J.M.R., Ursinus, W.W., Schepers, F., Groot Koerkamp, P.W.G., and Van Dixhoorn, I.D.E. 2009. Als je het de koe vraagt… : de 14 behoeften van de lacterende melkkoe (poster). System Innovations, Wageningen UR Livestock Research, Lelystad.

Cornelissen, J.M.R., Ursinus, W.W., Schepers, F., Groot Koerkamp, P.W.G., and Van Dixhoorn, I.D.E. 2009. De belangrijkste eisen die de melkkoe aan haar omgeving stelt (poster). System Innovations, Wageningen UR Livestock Research, Lelystad.

Schepers, F., Ursinus, W.W., De Mol, R.M., Metz, J.H.M., Bracke, M.B.M., Houwers, H.W.J., Groot Koerkamp, P.W.G., and Van Dixhoorn, I.D.E. 2009. COWEL: A Decision Support System for Welfare Scoring of New Husbandry Systems for Dairy Cattle (poster). Farm Technology Group, Wageningen University, Wageningen.

Bracke, M.B.M., Visser, E.K., Ursinus, W.W., Schepers, F., Bokma-Bakker, M.H., Enting, J., Leenstra, F.R., Blokhuis, H.J., and Ter Gast, E. 2008. Animal-based parameters for welfare assessment (poster). LNV Kennisdag Dierenwelzijn, November 6, Baarn Nederland.

Groot Koerkamp, P.W.G., Schepers, F., Ursinus, W.W., De Mol, R.M., Bracke, M.B.M., Metz, J.H.M., Houwers, H.W.J., and Van Dixhoorn, I.D.E. 2008. COWEL: een welzijnsmodel voor melkkoeien (poster). LNV Kennisdag Dierenwelzijn, November 6, Baarn Nederland.

Groot Koerkamp, P.W.G., Ursinus, W.W., Schepers, F., De Mol, R.M., Bracke, M.B.M., Metz, J.H.M., Bos, A.P., Houwers, H.W.J., and Van Dixhoorn, I.D.E. 2008. Cowel-model geeft bedrijven een welzijnsscore V-focus 5:6, 34-35.

Groot Koerkamp, P.W.G., Ursinus, W.W., Schepers, F., Cornelissen, J.M.R., Bos, A.P., and Van Dixhoorn, I.D.E. 2008. Welzijn melkkoe bepaald door rust, keuzes, voer en vloer. V-focus 5:5, 28-29.

Education Certificate

WIAS Training and Supervision Plan

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

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
Trends in stress biology: Interpretation of animal stress responses, Hobro, Denmark	2013
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

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): organizing start-up meeting and posting news & announcements, Wageningen, The Netherlands	2012-2013
Assisting at 'Open Innovatiedagen Varkens Innovatie Centrum', Sterksel, The Netherlands	2013

Education and Training Total	**62.65 ECTS**

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

Colophon

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