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Stress measures in tail biters and bitten pigs in a matched case-control study

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Abstract

*This study aimed to identify differences in stress measures in pigs (*Sus scrofa*) with different roles during a tail-biting outbreak. Quartets (n = 16) of age- and gender-matched fattening pigs including a tail biter (TB; n = 16), a victim (V; n = 16), a control in the same pen (C_{tb}; n = 10), and one in a pen without tail biting (C_{no}; n = 14) were chosen by direct behavioural observation. Stress measures used were behaviour (dog-sitting, sniffing of pen-mates and aggression), thyroid hormone concentration, morphology of adrenal and thyroid glands and salivary cortisol concentration sampled at 0700, 1000, 1600 and 1900h. Category (TB, V, C_{tb}, C_{no}) effects were investigated using a mixed model with replicate as subject and category as repeated effect. Category had a significant effect on adrenal total (cortex + medulla) and cortical area, salivary cortisol at 1900h, serum triiodothyronine (T_3) and the behaviours performing and receiving sniffing. Victims suffered from a triad of chronic stress, pathology and suppressed T_3 secretion. Evidence for stress in tail biters, a possible cause of the behaviour, consisted of a slightly flattened day-time cortisol pattern and more performed sniffing than all other categories. Differences in evening cortisol concentration and T_3 levels between the categories in the pen with ongoing tail biting emphasise the qualities of the control animal. It supports the view that neutral pigs represent a phenotype that adopts a coping strategy leading to lower stress levels than in tail biters and victims, despite being housed in the same pen.*

Keywords: *adrenal, animal welfare, injurious behaviour, physiology, swine, thyroid*

Introduction

Tail biting is a widespread abnormal behaviour in pigs (*Sus scrofa*) with serious consequences for the victims' well-being, economics of production and microbiological hygiene of pig carcases. Although the behaviour is thought to be an expression of distress in the group (Broom 1996; Schrøder-Petersen & Simonsen 2001), attempts to measure stress in relation to tail-biting activity have been few. A number of environmental shortcomings have repeatedly been associated with tail biting on farm level. This knowledge has, however, proven not to be reliable for predicting outbreaks in practice or inducing the behaviour under experimental settings. Clearly, the underlying motivational basis is poorly understood (Edwards 2006).

Edwards (2006) emphasises the need to identify characteristics that differ between pigs initiating tail-biting behaviour and similar pigs under equal circumstances that do not express the activity. One of these factors may be stress susceptibility (Schrøder-Petersen & Simonsen 2001).

Stress is a physiological response to any stimulus perceived as a threat to internal homeostasis, evident as a number of changes in physiology and behaviour (for a review, see Salposky *et al* 2000). Different systems are altered in different types of challenging situations, and individual differences in stress susceptibility are striking (Ingram *et al* 1980). Thus, assessment of stress should involve monitoring of several response systems and secondary signs, including behaviour, immunity, endocrinology and pathology (Squires 2003).

Behaviour is considered a more sensitive stress measure than physiology, as it reflects an animal's first attempts to cope with a stressor (Dawkins 2004). Generally, acute stressors elicit a range of conflict behaviours (Salzen 1991). If the stressor persists, abnormal behaviours such as tail biting may emerge (Wiepkema & Koolhaas 1993).

The hypothalamic-pituitary-adrenocortical (HPA) axis is the main system responsible for maintaining internal homeostasis after the very first sympatho-adrenal stress response (Moberg 1985). The adrenal cortex secretes glucocorticoids (GCs), in pigs; mainly cortisol (Bottoms *et al* 1972). The salivary concentration of cortisol is a good indicator of the amount of the biologically active form in plasma (Cook *et al* 1996).

Increases in (relative) adrenal mass and/or cortical area are considered reliable indices of chronic adrenal hyperactivity

(Burchfield *et al* 1980; Herman *et al* 1995). Chronic stress may also be evident as a flattening of the normal circadian cortisol secretion pattern (Becker *et al* 1985; Janssens *et al* 1995). Increased GCs cause suppression of thyroid function, evident as decreased serum concentration of thyroid hormones (Nicoloff *et al* 1970; Bianco *et al* 1987).

Increased GCs are one important factor predisposing pigs for ulcers in the cutaneous part of the stomach. This condition belongs to the very small group of known psychosomatic diseases in animals (Radostits *et al* 1994). Other pathologies may also be valuable in the assessment of chronic stress, as GCs are very potent modulators of immune function (Salak-Johnson & McGlone 2007).

Evaluation of stress responses is an important part of animal welfare assessment, as high levels of welfare by definition require low levels of stress and *vice versa* (Broom & Johnson 1993). Conclusions do, however, require that a clear distinction is being made between physiologically identical unpleasant distress and positively perceived eustress. The latter, thought to increase the well-being of the individual, is present, for example, during play-fighting (Selye 1975). In the present experiment most measures are indicators of chronic activation of stress response systems and thus also compromised welfare. Only the interpretation of aggressive behaviour, as defined in this study, needs caution as play-fighting may be difficult to distinguish from aggression (Donaldson *et al* 2002).

This study aimed to identify differences in stress measures in tail biters, bitten pigs and controls during the actual tail-biting outbreak. The pigs were matched in order to minimise environmental influences.

Materials and methods

Study animals and husbandry

The study was conducted on an 800-pig fattening farm in south-western Finland with a history of tail-biting problems. EU and national animal protection legislation effective during the experiment was followed on the farm. Health of the study animals was cared for by a veterinarian or veterinary-supervised stock people. The study protocol was approved by the Ethical Committee at the University of Helsinki, Finland based on an application including the study plan, a list of the risks to the animals involved and the informed client consent. The main ethical concern was that no interventions were allowed in experimental pens with tail biting before the focal animals were euthanised, that is, for a period of 2–4 days. During the experiment it did, however, become clear that the presence of the researchers led to a long-term improvement of the welfare of the pigs on the farm.

Pens with severe tail lesions were withdrawn from the experiment using the same criteria as the caretakers normally used for interventions. These criteria were not suitable for successful control of tail biting as they led to actions being taken too late, and the situation on the farm improved as better management practices were taught. During the experiment, tail biting was controlled effectively and medical treatment given as needed in the non-experimental pens as well as in the experimental ones, once the focal animals had been removed. Control measures consisted of removal of the biter(s) and provision of extra enrichment material.

Only an overview of the animals and their housing is provided here, as details are given elsewhere (Brunberg *et al* 2011). The study farm raised mixed-breed pigs with intact tails from approximately 25–30 kg to slaughter according to an all-in all-out scheme. The animals (n = 56) were chosen from five rooms with different group size (6–20) and feeding methods (commercial dry feed manually or *ad libitum* from a feeder). All pens were part-slatted and unbedded with a small amount of peat provided daily for enrichment.

Behavioural observations and selection of case-control quartets

The study was designed as a case-control with quartets (n = 16) of gender- and age-matched animals (ten quartets with gilts and six with barrows) forming the experimental unit. The animals originated from two consecutive batches raised on the farm between May and October 2009.

The animals were chosen according to a three-stage process of direct observation of tail-biting behaviour, described in detail by Brunberg and others (2011). After scanning the whole farm for any signs of ongoing tail biting, which were based on observations of the behaviour of the pigs as well as on the appearance on their tails, pen-level observations of tail-biting behaviour were carried out for 2 × 30-min periods. Finally, individual animals in the most promising pens were observed for 8 × 15 min distributed over mornings and afternoons. The goal was to identify gender-matched quartets including an active tail biter (TB), a frequently bitten individual or victim (V), a control individual in the same pen not involved in tail biting (C_{tb}), and a control from another pen in the same room without any tail-biting activity (C_{no}). Behavioural observations were conducted according to all occurrence sampling by one person standing in front of the pen. The ethogram (Table 1) was designed not only to identify study animals, but also to quantify behaviours thought to indicate stress. For each quartet of animals the three-stage process was completed in 2–4 days.

Tail-biting behaviour spread rapidly in the affected pens making identification of control animals difficult and leaving many of the 16 matched quartets incomplete. The study included 16 TB, 16 V, 10 C_{tb} (missing in quartets 4, 6, 11, 13, 14 and 15) and 14 C_{no} (missing in quartets 1 and 3).

Saliva and blood sampling, euthanasia and pathological examinations

On the day of individual behaviour observations (third stage of the selection process), saliva samples for cortisol assessment were obtained at 0700, 1000, 1600 and 1900h. Each animal was allowed to chew on a cotton bud (Salivette®, Sarstedt, Nümbrecht, Germany) until thoroughly wet (30–60 s). Saliva was extracted by centrifugation for 10 min at 3,000 rpm and immediately frozen for storage at –18°C until analysis. The animals were not trained prior to sampling.

Table 1 Ethogram used for identification of tail biters, victims and controls as well as assessment of stress.

Behaviour	Description
1 Performing tail biting[†]	Chewing or biting on pen-mate's tail
2 Receiving tail biting [†]	Being the recipient of 1, irrespective of behavioural reaction
3 Performing aggression[‡]	Mutual pushing parallel or perpendicular, ramming or pushing pen-mate with the head accompanied by biting, lifting by pushing the snout under the pen-mate's body
4 Receiving aggression[‡]	Being the recipient of 3
5 Performing sniffing[‡]	Nosing or sniffing any body part of pen-mate, distance from snout to skin less than 5 cm
6 Receiving sniffing[‡]	Being the recipient of 5
7 Sitting inactive[‡]	Dog-sitting on tail with both forelegs stretched underneath doing nothing

[†] For behaviours 1 and 2 a new occurrence was counted if the biter let go of the tail and started again, even immediately.
[‡] For behaviours 3–7 a new occurrence was counted after a pause of at least 5 s.

Two to 12 h after completion of the behavioural observations, between 0740 and 1215h, 1–2 matched quartets (three to eight pigs) were sedated in the home pen with intramuscular injections of midazolam (0.5 mg kg^{-1}, Midazolam Hameln R®, Hameln Pharmaceuticals GmbH, Hameln, Germany), butorphanol (0.2 mg kg^{-1}, Butordol®, 10 mg ml^{-1}, Intervet International, BV Boxmeer, The Netherlands) and ketamine (50 mg ml^{-1}, Ketalar® [Pfizer Inc, New York, USA] or 50 mg ml^{-1} Ketaminol® vet [Intervet International, BV Boxmeer, The Netherlands]). The sedated animals were subjected to jugular venipuncture using 18-G needles for drawing blood into 10-ml polypropylene vacuum serum tubes. When signs of deep sedation were fulfilled, euthanasia was immediately carried out using an intra-cardial injection of pentobarbital (20 mg kg^{-1}, Mebunat® vet 60 mg ml^{-1}, Orion Corporation, Espoo, Finland). The procedures are described in more detail by Munsterhjelm and others (2013).

An autopsy was performed the morning following euthanasia, including histological examination of the tail and several other tissues. The adrenal and thyroid glands were weighed and processed routinely for histopathology, taking care that the adrenals were cut to yield the largest possible cross-sectional area. After staining with haematoxylin-eosin, the samples were observed using standard techniques. Inflammatory changes present in the adrenals were classified according to Kumar *et al* (2010) as: 0 = no lesion; 1 = acute adrenalitis; and 2 = chronic adrenalitis.

In order to determine adrenal total and medullary cross-sectional areas, the free hand tool and the Oberon CurveWorks macro package for CorelDraw Graphics Suite X3 (Corel Inc, CA, USA) were used after saving the slices as digital pictures using Cell^o software.

Cortisol analysis and characterisation of day-time cortisol rhythm

Salivary cortisol concentration was analysed in duplicate by radioimmunoassay with a kit validated for use with pig saliva (Coat-A-Count Cortisol, Orion-Diagnostica, Turku, Finland; Oliviero *et al* 2008). Samples were rerun if duplicates differed more than 9%. Average inter- and intra-assay coefficient of variation (CV) was 9.2 and 7.5%, respectively.

In order to characterise the secretion rhythm of cortisol, the percentage of change in the concentration from morning (average of the 0700 and 1000h samples) to the evening values (average of the 1600 and 1900h samples) was calculated for each animal. This approach was chosen instead of attempts to classify the individual secretion patterns as normal or abnormal, as the methodology to do this has yet to be established (de Weerth *et al* 2003).

Thyroid hormone analysis

Serum for free triiodothyronine (T_3) and thyroxine (T_4) analyses was collected after centrifugation (1,300 rpm for 10 min) of serum tubes kept at room temperature for a maximum of 3 h and stored in –80°C until analysis. Samples were analysed in singular with an Immulite® 2000 immunoassay system (Siemens Healthcare Diagnostics, Deerfield, IL, USA) with standard software and reagents for human serum provided by the manufacturer.

Statistical analysis

PASW software (SPSS Inc, Chicago, USA), version 18, was used for statistical analyses. Adrenal characteristics for each individual are expressed as the average of both glands. According to the distribution of variables, Pearson's product-moment (r) or Spearman's rank correlation coefficient (r_s) was used to determine correlations.

The distributions of continuous variables were investigated using Q-Q plots, Kolmogorov-Smirnov and Shapiro-Wilk tests. Consequently, the variable 'performing sniffing' was subjected to a square-root transformation, salivary cortisol concentration at 1900h raised to the power of 0.25 and 'sitting inactive' was normalised by taking the square root of e raised in the power of the variable.

In order to test the null hypothesis that the observations in the different categories forming the matched quartets came from the same distribution, linear mixed models with quartet as subject and category as repeated effect were fitted. The non-constant variability in the data was

Table 2 Stress-related behaviours in tail biters, victims and control pigs categorised based on tail-biting activity.

Behaviour	Tail biter (n = 16)	Victim (n = 16)	Control, biting pen (n = 10)	Control, non-biting pen (n = 14)	*P*-value, LMM[†]
Performing aggression	1.4 (0.9–2.3)[‡]	1.3 (0.5–1.5)	1.1 (1.0–1.8)	0.9 (0.5–1.7)	*P* > 0.1
Receiving aggression	1.5 (0.9–2.0)	1.2 (0.6–1.4)	0.8 (0.6–1.0)	1.1 (0.8–1.6)	*P* > 0.1
Performing sniffing	7.0 (4.6–11.8)[ab§]	3.0 (2.5–4.5)[a]	3.0 (2.5–4.3)[b]	2.9 (2.4–3.9)[§]	*P* = 0.01
Receiving sniffing	2.9 (2.4–4.0)[a§]	3.5 (2.6–5.7)[a]	3.1 (2.4–3.4)	2.1 (1.9–2.6)[§]	*P* = 0.02
Sitting inactive	0.3 (0.1–0.6)	0.1 (0.0–0.3)	0.4 (0.0–0.4)	0.3 (0.1–0.3)	*P* > 0.1

[†] Linear mixed model.
[‡] Values are given as the median (25–75% percentiles) of the number of bouts initiated by an individual during 15-min observation.
[§] Indicates a trend (0.05 < *P* < 0.1).
Common superscripts indicate a significant (*P* < 0.05) difference in pair-wise comparisons.

controlled for by including pen nested within batch and farm section as random effects. The models were built using backward elimination starting with category, gender, and bodyweight as fixed effects and a *P*-value > 0.1 as removal criterion. A *P*-value of < 0.05 was used to reject the null hypothesis and allow for pair-wise testing with a Bonferroni correction for multiple comparisons.

Results

Characteristics of the matched quartets and categories of animals

The estimated age of the animals was between 10 and 21 weeks, and the mean (± SEM) weight at autopsy within the matched quartets between 29.0 (± 2.0) and 75.0 (± 2.7) kg. Weight did not differ between the categories of pigs (Munsterhjelm *et al* 2013).

During the individual observations of 8 × 15 min, TB performed a median of 36 bites (range 8–65) on their pen-mates' tails while the victims received eleven bites (1–31). A minority of V or controls were observed occasionally to perform tail biting, and a minority of TB or controls observed occasionally to receive bites on the tail. Tail biting had, however, occurred quite frequently in the study pens as indicated by a large number of lesions detected at autopsy also in non-victims: upon histological examination of the sagittally cut tail, acute lesions were present in 57% of TB, 55% of C_{tb} and 14% of C_{no}. Additionally, evidence of healed trauma was seen in 1–2 individuals per category (11–14%). For details on tail-biting behaviour and tail pathology, see Munsterhjelm and others (2013).

Stress-related behaviours

Category had a significant effect on the behaviours performing and receiving sniffing (Table 2). In pair-wise comparisons, TB performed more sniffing than all the other groups (P = 0.01 compared to V; P = 0.03 to C_{tb} and P = 0.05 to C_{no}). Victims received more sniffing than C_{no} (P = 0.01) and TB (P = 0.02).

Adrenal size and morphology

Forty-five percent of the animals had histological signs of mild adrenal inflammation. The effect of the adrenalitis score on adrenal characteristics was tested for by regressing it and bodyweight on adrenal gland weight and on the combined area as well as on the separate cortical and medullary areas. Adrenalitis was not found to predict any of these characteristics (P > 0.5), thus, all animals were kept in the data set for further analyses.

The average total cross-sectional adrenal area was 37 (± 1.7) mm^2, the cortex 29 (± 1.5) mm^2 and the medulla 7.7 (± 0.4) mm^2. The ratio between medulla and cortex was 27 (± 1.1)%. It did not correlate with bodyweight. The results from linear mixed modelling are summarised in Table 3. Controlling for bodyweight, category was found to affect total and cortical area significantly, but not medullary area or adrenal weight. In pair-wise comparisons, the total area was significantly larger in V than C_{no} (P = 0.001) with a similar trend between V and TB (P = 0.07). The cortical area was larger in V than C_{no} (P = 0.02).

Thyroid size and hormones

Histological examinations revealed a moderate focal thyroiditis in one victim. In order to test if the inflammation affected the results, the data were analysed with and without this individual, but no difference occurred, and the animal with thyroiditis was kept in the data.

The weight of the thyroid gland (4.0 [± 0.2] g) was highly correlated with bodyweight (r = 0.8, P < 0.001, n = 54) and unaffected by category (P > 0.1). The serum concentration of T_3, in contrast to T_4, differed between categories (P = 0.02 and P > 0.1, respectively). In pair-wise comparisons, T_3 was found to be higher in TB than V (P = 0.04), with a similar trend between TB and C_{tb} (P = 0.06; Table 3). Bodyweight or gender did not affect thyroid hormone concentrations.

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Table 3 Mean (± SEM) adrenal and thyroid characteristics and thyroid hormone concentrations in tail biters, victims and controls categorised based on tail-biting activity.

Factor	Tail biter (n = 16)	Victim (n = 16)	Control, biting pen (n = 10)	Control, non-biting pen (n = 14)	*P*-value, LMM[†]
Adrenal weight (g)	1.8 (± 0.1)	2.0 (± 0.1)	1.7 (± 0.2)	1.7 (± 0.1)	$P = 0.09$
Adrenal total area (mm^2)	34.7(± 2.3)[#]	39.8 (± 1.5)[a#]	34.9 (± 3.5)	29.9 (± 1.9)[a]	$P = 0.01$
Adrenal cortical area (mm^2)	27.5 (± 1.8)	31.5 (± 1.4)[a]	27.7 (± 2.8)	23.3 (± 1.7)[a]	$P = 0.01$
Adrenal medullary area (mm^2)	7.2 (± 0.7)	8.3 (± 0.6)	7.2 (± 0.8)	6.6 (± 0.5)	$P = 0.07$
Thyroid weight (g)	4.1 (± 0.4)	4.2 (± 0.4)	3.6 (± 0.4)	3.9 (± 0.4)	$P > 0.1$
Serum T3 (nmol L^{-1})[‡]	1.8 (± 0.1)[a#]	1.4 (± 0.2)[a]	1.3 (± 0.1)[#]	1.7 (± 0.1)	$P = 0.02$
Serum T4 (nmol L^{-1})[§]	45.1 (± 1.8)	44.5 (± 3.3)	39.3 (± 2.9)	43.0 (± 1.7)	$P = 0.1$

[†] Linear mixed model.
[‡] T3 = triiodothyronine; [§]T4 = thyroxine.
[#] Indicates a trend ($0.05 < P < 0.1$).
Common superscripts indicate a significant ($P < 0.05$) difference in pair-wise comparisons.

Table 4 Correlations (Spearman's r_s) between stress measures and tail lesion severity. Only associations with $r_s \geq 0.30$ and $r_s \leq -0.30$ are shown.

Factor	Performing aggression	Performing sniffing	Thyroid weight	Adrenal weight	Adrenal area (total)	Adrenal cortical area	Adrenal medullary area
Tail lesion severity score[†]				0.33* (n = 50)	0.39** (n = 49)	0.40** (n = 49)	
Receiving aggression	0.30* (n = 49)	0.58*** (n = 49)					
Adrenal weight			0.32* (n = 49)		0.66*** (n = 50)	0.66*** (n = 50)	0.33* (n = 50)
Adrenal area (total)			0.39** (n = 49)	0.66*** (n = 50)		0.96*** (n = 51)	0.69*** (n = 51)
Adrenal cortical area			0.40** (n = 49)	0.66*** (n = 50)	0.96*** (n = 51)		0.50*** (n = 51)
Adrenal medullary area			0.34* (n = 49)	0.33* (n = 50)	0.69*** (n = 51)	0.50*** (n = 51)	
Serum T3[§]						–0.40* (n = 54)	
Cortisol 0700h[‡]			0.65*** (n = 51)	–0.40* (n = 32)			
Cortisol 1000h[‡]			–0.37* (n = 31)				
Cortisol 1600h[‡]			–0.35* (n = 35)				
Coerisol 1900h[‡]					–0.30[¶] (n = 33)		–0.38* (n = 33)
Relative change, cortisol[#]			0.36* (n = 33)				

[†] Histologically classified as no, mild, moderate or severe, see Munsterhjelm *et al* (2013).
[‡] Salivary cortisol concentration.
[§] Triiodothyronine.
[#] Relative change in salivary cortisol concentration from morning to evening.
Superscripts indicate significance: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$, [¶] $0.05 < P < 0.1$.

Salivary cortisol

Cortisol data were acquired from only 36 animals due to technical issues and difficulties getting some individuals to chew on the cotton bud, apparently due to fear when approaching the person. The average salivary cortisol concentration pooled over all subjects was 8.3 (± 0.5) ng ml^{-1} at 0700h, 6.8 (± 0.4) ng ml^{-1} at 1000h, 6.2 (± 0.5) ng ml^{-1} at 1600h and 4.6 (± 0.4) ng ml^{-1} at 1900h. In statistical analyses, category affected the concentration only at 1900h ($P = 0.01$), when C_{tb} had a lower concentration than both TB ($P = 0.01$) and V ($P = 0.01$). Bodyweight and gender were significant predictors as well ($P < 0.001$ and $P = 0.03$, respectively), with an increasing size and male as compared to female sex predicting a decreasing concentration at 1900h. The relative change in salivary cortisol concentration from morning to evening was unaffected by category ($P = 0.10$), although the numerical differences seemed substantial: –40 (± 12)% in C_{tb} (n = 9), –30 (± 10)% in C_{no} (n = 12), –20 (± 10)% in TB (n = 16) and –0.9 (± 15)% in V (n = 16).

Correlations between stress measures

Correlations between stress measures and tail lesion severity are given in Table 4. Serum T4 concentration, cardial lesion score (results given in Munsterhjelm *et al* 2013) and the behaviours sitting inactive and receiving sniffing were not correlated to any other measures (–0.30 < Spearman's r_s < 0.30).

Discussion

This research aimed to identify differences in stress measures in pigs in relation to tail-biting activity during the actual outbreak. The indicators chosen provide information on both acute (behaviour) and chronic (adrenal and thyroid morphology and hormones, stomach lesions) changes in stress systems. Several measures, not surprisingly, indicated that the victims suffered from chronic activation of the HPA axis. The results provided only scarce evidence of stress in tail biters, although stress has been suggested to cause the behaviour (Wiepkema & Koolhaas 1993; Schrøder-Petersen & Simonsen 2001). Certain differences between the categories within the biting pen contribute to the discussion on the possible existence of a tail-biting resistant phenotype of pigs recently opened by Brunberg (2011).

The evidence for a higher degree of chronic HPA axis activation in V as compared to all other categories seems strong, including both significantly higher salivary cortisol in the evening than C_{tb}, lower serum T3 than TB and a larger cross-sectional area of the adrenal cortex than C_{no}. Looking at the matched pairs, every single V had a larger cortical area than its C_{no}-counterpart, with a seemingly substantial within-pair difference of 19%. In this study, with limited sample size and missing subjects, it is also worth noting that although non-significant, V showed the largest deviation from normal or 'non-stressed' in all other non-behavioural measures with the heaviest adrenals, largest adrenal medullary area, smallest change in salivary cortisol concentration during the day and most severe cardial lesions. The reason for V to stand out seems clear in repeated painful bites and more severe pathology in both the tail and respiratory organs (Munsterhjelm *et al* 2013).

Interpretation of adrenal hypertrophy as a sign of long-term HPA axis activation is straightforward (Burchfield *et al* 1980; Herman *et al* 1995) in contrast to interpretation of the changes in cortisol secretion patterns, especially in this study where tail-biting activity was found to affect only the evening value. Cortisol secretion follows a circadian rhythm, where plasma concentrations peak in the morning and decline to reach a nadir in the evening, sometimes with an additional afternoon peak (Becker *et al* 1985). Chronic stress has the potential to flatten this rhythm in several different ways. The most frequently described change is a diminishing morning surge, which has been demonstrated in pigs in response to tethering (Janssens *et al* 1995) or barren housing (de Jong *et al* 2000; Munsterhjelm *et al* 2010), and in certain types of depression and stress-related disorders in man (for a review, see Gunnar & Vazquez 2001). Flattening due to failure to reach the evening nadir, as seems to have happened in the present TB and V as compared to C_{tb}, has been reported in pigs by Barnett *et al* (1981) in response to individual housing.

In V, the causes for a stress-related cortisol pattern are obvious and discussed above. In TB, the deviation may be a reflection of stress underlying the development of the injurious behaviour or even some kind of mental disorder, as reported in man (see Gunnar & Vazquez 2001). The finding that the 0700h concentration of salivary cortisol was (negatively) correlated with adrenal weight supports the assumption that chronic stress was indeed the phenomenon measured in the experiment.

Category affected salivary cortisol concentration only within the biting pen. This means that the effects are truly associated with tail-biting activity, not with the physical or social environment. Neutral pigs in pens with tail biting (C_{tb} in this experiment) have been suggested to represent a resistant phenotype, not resorting to tail biting in situations when other pigs do. Brunberg (2011) formulated this hypothesis upon investigations of the animals in the present study showing that C_{tb} were less pig-directed in behaviours classified as abnormal, than were C_{no}, and that they differed more in brain gene expression from TB, V and C_{no} than they differed from each other (Brunberg *et al* 2011, 2013). Zupan and others (2012) emphasised the qualities of these neutral animals as well when measuring autonomous nervous system function and responses to a novel object in triplets of pigs selected on commercial farms. They concluded that as opposed to biters and victims, the neutral pigs (C_{tb}) had a normally functioning emotional regulation.

In the present study, category effects within the biting pen were found not only for evening cortisol, but also for the thyroid hormone T3. TB had a higher serum concentration of T3 than both V and C_{tb}, although the latter contrast only approached significance. Looking at the matched pairs, the difference between TB and C_{tb} was substantial, with higher concentration in TB in 7 out of 8 pairs and a median within-pair difference in concentration of 40%.

Many causes for T3 suppression have been identified. The thyroid ones, including hypoplasia, congenital hypothyroidism, thyroiditis, neoplasia and hyperplastic goiter, were ruled out in pathological analyses (Munsterhjelm *et al* 2013), leaving illness, decreased carbohydrate intake and increased GC secretion as possible aetiologies (Nicoloff *et al* 1970; Bianco *et al* 1987). In the present study, the cause for the lowered concentration in T3 is most probably different in V and C_{tb}. V had more (severe) tail and respiratory organ pathology than C_{tb} and TB, whereas no difference in health status was discovered between C_{tb} and TB (Munsterhjelm *et al* 2013). V seems to have suffered from a condition referred to as 'euthyroid sick syndrome' or 'non-thyroidal illness syndrome' (NTIS) in human medicine, caused by pro-inflammatory cytokines released at sites of inflammation (for a review, see De Groot 1999). The syndrome is in its mild form characterised by T3 but not T4 decrease, as was the case in the present animals (Harris *et al* 1978).

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The decrease in T3 concentration in C_{tb} as compared to TB was not caused by a difference in health status or cortisol levels, but it may have been related to differences in feed intake between the different categories of pigs, as has been shown recently by Wallenbeck and Keeling (2013). No category effect on bodyweight existed to support a theory of longer term deviations, but liver-specific enzyme activity (Munsterhjelm *et al* 2013) and differences in brain gene expression (Brunberg 2011) indicated decreased eating in C_{tb} as compared to TB. Comparison of jejunal villus morphology and serum amino acid and mineral concentrations between C_{tb} and C_{no} yielded the same conclusion (Palander *et al*, submitted; no comparison to TB). It can be speculated that the decrease in feed intake in C_{tb} may be a by-product of the less pig-directed type of behaviour shown by Brunberg (2011), perhaps leading to avoidance of the crowd at the feeding trough or feeder during the tail-biting outbreak when overall activity in the group will increase.

Behaviour was assessed as a measure of acute stress. Pen-mate manipulation and aggression can be categorised as conflict behaviours, expected to occur in acute stress situations (reviewed by Salzen 1991). Tail biting, on the other hand, is a disturbed behaviour indicative of quite serious and/or long-term stress (Wiepkema & Koolhaas 1993). Category effects were present only for performing sniffing (higher frequency in TB as compared to all other categories) and receiving sniffing (higher frequency in V as compared to TB and C_{no}). Although manipulation of pen-mates is a sign of stress, the presence of a potentially interesting injured tail in the biting pen complicates the interpretation of these results. C_{tb} was also shown to avoid other pigs as targets for abnormal manipulation (Brunberg 2011) as compared to C_{no}, and direct that behaviour towards pen fittings, leaving performing sniffing of pen-mates not useful for acute stress assessment in C_{tb}. In TB, many bouts of sniffing were probably unsuccessful biting attempts with the target moving away.

The non-longitudinal design of the present study does not provide information on causalities. Looking at the victims, sickness, T3-deficiency and changes in HPA-activity all have the potential to change the behaviour (Hart 1988), perhaps increasing the risk to be tail bitten. Unfortunately, the changes observed may also be a consequence of the development of tail biting in the pen as these factors may be interrelated in a number of ways with each other and with being tail bitten.

The social environment in the tail-biting pen seemed to have no effect on stress mechanisms, as indicated by no differences between C_{no} and C_{tb}. This conclusion has, however, to be drawn with caution for two reasons. Firstly, rapidly spreading tail biting left many C_{tb} missing from the matched quartets. Secondly, according to Brunberg (2011), investigating gene expression and abnormal behaviours in the same animals, C_{no} were probably not optimal controls as they were a mixture of possible future performers (of tail biting), receivers and neutral pigs.

Animal welfare implications and conclusion

This study provides new information on the stress levels of different categories of pigs involved in an outbreak of tail biting. The welfare of the victims was seriously compromised as indicated by a triad of chronic stress, inflammations and T3-depression, emphasising the need for rapid interventions in pens with signs of tail biting. Given that there was also evidence that the individuals who perform tail biting were stressed, the results support other findings that neutral pigs in pens with tail biting may adopt a coping strategy leading to less overall stress. It is proposed that focusing attention on these individuals and their coping strategy may contribute to improving our understanding of the role of stress in causing tail biting as well as how to minimise the consequences.

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