



Behaviour, heart rate, and heart rate variability in pigs exposed to novelty

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ABSTRACT - In the present study, we investigated behavioural responses and determined parameters of heart rate variability (HRV) to elucidate a relative activation of autonomic nervous system (ANS) during baseline (10 min) and in response to potentially stressful situations (10 min) in two pig breeds and sexes. Gilts ($n = 21$) and barrows ($n = 9$) of the Landrace \times Yorkshire (LY; $n = 15$) and Landrace/Yorkshire \times Landrace/Duroc (LYLD; $n = 15$) breeds were subjected to a novel object test (NOT) and a novel arena test (NAT). Basal ANS state differed in pigs across breeds but not sexes. Landrace \times Yorkshire pigs had a significantly lower basal heart rate (HR) and low-frequency band (LF) with a higher root mean square of successive interbeat intervals (RMSSD) and high-frequency band (HF) than LYLD pigs. In the NOT, despite having similar cardiac responses, gilts had a longer duration of contact with a novel object, higher lying and standing duration, and a lower duration of walking compared with barrows. In the NAT, we found similar behaviour across sexes but a different degree of ANS state, with barrows having a significantly higher increase in LF/HF (power of the low frequency component divided by the power of the high-frequency band) compared with gilts. Landrace/Yorkshire \times Landrace/Duroc pigs showed longer duration of contact with a novel object in the NOT accompanied by less lying and standing than LY pigs in both tests. No difference in ANS activation between breeds was found in the NOT. In the NAT, HR increased more from baseline to testing in LY pigs than in LYLD pigs. There is a complex and often contradictory nature of relationships between behaviour and cardiac responses to novelty in pigs of different breeds and sexes.

Key Words: breed, *Sus scrofa*

Introduction

Heart rate variability (HRV) is a valid, non-invasive method to assess autonomic nervous system (ANS) regulation of cardiac activity in relation to stress responses and welfare (von Borell et al., 2007). It reflects an ever changing psycho-physiological state of the animal that is regulated by the parasympathetic (vagal) and sympathetic branches of the ANS (Mohr et al., 2002).

The basal autonomic state, i.e., during non-challenging conditions, can be considered an index of the susceptibility of an individual to stress, since individuals exposed to stressful situations show lower vagal tone, potentially being more vulnerable to stress (Porges, 1995). Recently, HRV was used as a method to assess the impact of the ANS on

cardiac activity in pigs in relation to tail biting (Zupan et al., 2012). It was found that both victims of tail biting and biters might have a dysfunctional autonomic regulation.

In an intensive as well as in an outdoor pig production unit, several animal welfare problems such as tail-biting and belly nosing have been recognised (Hansson et al., 2000; Walker and Bilkei, 2006), which points out that at least some animals cannot cope with the environment. To the awareness of the authors, there is a lack of data concerning the measurement of the relationship between behavioural and physiological characteristics under fear-induced situations in pigs. The aim of our study was to investigate changes in HRV for the better understanding of sex and breed effects on pig behaviour and cardiac activity in novel situations.

We focused on sources of variation in behavioural and cardiac responses by means of heart rate (HR) and of HRV related to sex and breed in the two fear tests: a novel object (NOT) and a novel arena test (NAT). The same animals have been analysed before in relation to tail biting (Zupan et al., 2012). We tested gilts and barrows of two crossbreeds. In our previous paper (Zupan et al., 2012), it was demonstrated that tail biters, victims of tail biting, and control pigs respond

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differently to a novel positive or negative environment due to having different emotional processing and autonomic regulation of HRV. To fully assess the validity of HRV measures to identify susceptible phenotypes, knowledge of the relationship between behavioural and physiological characteristics and specific external factors, such as responses of the ANS to different stressful challenges, should be further investigated to better understand what consequences tail biting has on pigs. It has been documented that pigs have a great individual different behaviour as well as physiology (Jensen et al., 1995; Geverink et al., 2002; Taylor et al., 2010). In our study, we hypothesise to find an individual variation in pigs of different breeds and sexes, with pigs showing generally more fear in a NAT than in a NOT. This is based on a literature survey showing that social isolation in a novel environment is one of the most stressful situations for all social species including pigs, leading animals to suffer from separation anxiety (Forkman et al., 2007).

Material and Methods

Data were collected from animals that were previously analysed with respect to tail biting behaviour and measures of HR and HRV (Zupan et al., 2012). Briefly, 30 pigs (above 110 days old) with intact tails were selected based on the frequency of biting during a 60-min observation period on a farm. The pens were of mixed sex. Pigs were housed at 12 pigs per pen. Pigs were of two crossbreeds: Landrace × Yorkshire (LY) and Landrace/Yorkshire × Landrace/Duroc (LYLD). A particular phenotype consisted of three barrows (LY: $n = 2$; LYLD: $n = 1$) and seven gilts (LY: $n = 3$; LYLD: $n = 4$). After selection on a farm, the pigs were transported to the research facilities where they were weighed and housed individually in pens in the same room. Pelleted concentrate and water were available *ad libitum* via a trough and water nipple, respectively. The body weight of the tested pigs ranged from 28 kg to 70 kg.

The experimental setting was as described in Zupan et al. (2012). Pigs were tested in five blocks of six pigs per block, with two trios per block. Briefly, behavioural and physiological responses by the measures of HR and HRV were recorded during the NOT and the NAT. Animals were

exposed to the tests one time each. After the habituation period, the NOT was carried out in the home pen of a pig (1.25 × 1.79 m). The Polar device, which consisted of a wristwatch computer and a HR belt, to measure HRV, was fitted on the pigs and basal recording followed for duration of 10 min. Afterwards, the test person entered the pen and switched off the wristwatch computer and tied a string with a novel object, i.e., the white container with black painted stripes of a bucket size, to the middle of the pen. The person turned on the Polar device and exited the pen. The continuous 10 min of test period followed.

The NAT was conducted on the following day. Basal HR data were collected as described for the NOT. The pig was then gently herded by the test person from the pen down a short corridor to the test room (1.95 m × 2.80 m) where it was exposed to social isolation. There, the behavioural and cardiac responses of an individual pig were recorded continuously for 10 min. After each test session in a novel room, the pig was returned to its pen and the room was thoroughly cleaned with water and a brush.

Behaviours scored (Table 1) are similar to the definitions of Zupan et al. (2012). The behaviour of the pigs was recorded using the MSH digital video system program (MSH-video, Riga, Latvia, <http://www.guard.lv/eng/index.php>) and continuously scored using a PC and the Observer software package XT 7.0 (Noldus Information Technology 1997). The behavioural scoring of an individual pig started after the test person exited the pen or novel room and lasted for the following 8 min.

The HR monitor system (RS800TM155, Polar Electro Oy, Helsinki, Finland) set up at 1000 Hz sampling rate consisted of a flexible belt with two integrated electrodes, a transmitter, a separate storage device, and corresponding software (Polar Precision Performance Software, version 5). The HR monitor system was designed to store time series of interbeat intervals (IBI) of consecutive heartbeats and validated for pigs (Marchant-Forde et al., 2004). Spurious measures in the IBI were corrected using a correction function within the Polar software with the standard set-up. In the software, pre-defined standard settings in the scissor check box were: filter power = moderate, protection zone = 6, and activation of the check box for elimination

Table 1 - Definitions of behaviours observed in the novel object test (NOT) and novel arena test (NAT)

Behaviour	Definition
Lying (duration)	Lying or sitting without investigation of novel object
Standing (duration)	Standing without investigation of novel object
Walking (duration)	Locomotion of more than one step without investigation of novel object
Object investigation (duration, latency) ¹	Nosing, biting, licking, or manipulating novel object

¹ NOT only.

of supraventricular beats. Data with a corrected error rate above 5% were excluded from the analysis. Recordings with longer sequences of equal values in the tachogram were discarded as well. Then, we selected the first continuous 5-min time window of acceptable quality. A time segment of 10 min (5 min before test period; i.e., basal and 5 min during test period) was selected for analysis.

We analysed HR (beats per min, bpm) and HRV in the time domain (the root mean square of successive differences in IBI, RMSSD, ms) and the frequency domain (power of the low-frequency band, LF; power of the high frequency band, HF; and LF/HF ratio; see von Borell et al., 2007). The frequency limits that correspond to von Borell et al. (2007) were set at 0.03 to 0.10 Hz for the LF band and 0.13 to 0.41 Hz for the HF band.

The statistical analysis was performed on SAS (Statistical Analysis System, version 9.1) using total number of recordings (Table 2). Results are shown as means \pm standard error (SE), and all reported P-values are 2-tailed. The data residuals were tested for normality (UNIVARIATE procedure) and when a normal distribution of the residuals could not be assumed, data were either log transformed (Tables 5, 6, and 7) or an analysis was performed using non parametric statistics (behavioural data). Significance was indicated by $P < 0.05$ and a

tendency by $P < 0.10$. The fixed effects investigated were phenotype, breed, and sex. The present study focuses on breed and sex effects, and effects of phenotype are reported in a previous paper (Zupan et al., 2012).

The GENMOD procedure that fits generalized linear models was utilized for records of behaviour in the tests. The dependent variables were count values per animal (excretion frequency) or the duration of time in seconds (other recorded variables).

The GLM procedure was applied for each of the cardiac parameters separately. Pair-wise differences were tested by a multiple post hoc Tukey-Kramer test. Individuals were used as the subject for the repeated statement. The effect of body weight was tested in each model as a covariate but no significant effect was found.

Baseline data were used as covariate in the models. While in the NAT a significant influence of breed on HR was found, walking duration data were added as a covariate in the model to assess the effect of physical activity on HR. Activity could not explain variance in the data.

To investigate Spearman correlation coefficients, procedure CORR was used to assess the relationship between the HRV variables in all tested conditions. In the text, only Bonferroni-corrected statistical significant values are reported ($P < 0.05$, B: $P < 0.004$).

Table 2 - Total number of pigs used for particular statistical analysis

	Test	Fixed effect	N	Level	n
Behaviour	Novel object test	Sex	24	Gilt	15
				Barrow	9
		Breed		LY	14
				LYLD	10
	Novel arena test	Sex	27	Gilt	17
				Barrow	10
Breed			LY	14	
			LYLD	13	
Heart rate variability (baseline)		Sex	13	Gilt	9
				Barrow	4
		Breed		LY	9
				LYLD	4
Heart rate variability (baseline/testing)	Novel object test	Sex	14	Gilt	8
				Barrow	6
		Breed		LY	9
				LYLD	5
	Novel arena test	Sex	11	Gilt	8
				Barrow	3
		Breed		LY	7
				LYLD	4

N - total number of pigs used; n - total number of pigs used for a particular level; LY - Landrace \times Yorkshire; LYLD - Landrace/Yorkshire \times Landrace/Duroc.

Results

In the NOT, it was observed that three animals did not make contact with the novel object. Gilts demonstrated longer duration of lying and standing plus longer duration of contact with a novel object but shorter duration of walking compared with barrows ($P < 0.05$). The behavioural responses in the NAT test did not differ between sexes. Analyzing the breed differences during the exposure to the NOT, the duration of lying, standing, and walking was longer in LY pigs, while the duration of contact with a

novel object was longer in LYLD pigs ($P < 0.05$) (Table 3). A different picture emerged in the NAT, in which a longer duration of standing but shorter duration of walking was shown in LY pigs compared with LYLD pigs ($P < 0.05$) (Table 4).

The combined baseline values of the cardiac activity parameters differed between breeds but not between gilts and barrows (Table 5). In detail, LY pigs had a significantly lower baseline HR and LF with a higher RMSSD and HF than LYLD pigs ($P < 0.05$). When looking at the correlations between parameters of HRV, patterns

Table 3 - Effect of sex and genotype on behaviour during the novel object test

Behaviour	Fixed effect	Level	Mean \pm SE	χ^2 test	P-value
Lying (duration)	Sex	Gilt	164.5 \pm 46.2	40.59 ^{df=1,21}	<0.0001
		Barrow	149.1 \pm 60.9		
	Genotype	LY	216.4 \pm 47.8	803.19 ^{df=1,21}	<0.0001
		LYLD	77.9 \pm 58.0		
Standing (duration)	Sex	Gilt	45.0 \pm 15.3	6.48 ^{df=1,21}	<0.05
		Barrow	42.6 \pm 20.2		
	Genotype	LY	58.7 \pm 15.8	181.96 ^{df=1,21}	<0.0001
		LYLD	23.6 \pm 19.3		
Walking (duration)	Sex	Gilt	13.8 \pm 6.8	18.61 ^{df=1,21}	<0.0001
		Barrow	22.8 \pm 9.0		
	Genotype	LY	21.4 \pm 7.1	29.14 ^{df=1,21}	<0.0001
		LYLD	11.3 \pm 8.6		
Latency to contact a novel object	Sex	Gilt	4.2 \pm 1.9	0.21 ^{df=1,17}	>0.10
		Barrow	3.6 \pm 2.7		
	Genotype	LY	3.6 \pm 2.1	0.42 ^{df=1,17}	>0.10
		LYLD	4.3 \pm 2.5		
Contact (duration)	Sex	Gilt	183.3 \pm 37.4	14.53 ^{df=1,21}	<0.001
		Barrow	172.4 \pm 49.3		
	Genotype	LY	79.8 \pm 38.7	1864.25 ^{df=1,21}	<0.0001
		LYLD	318.5 \pm 46.9		

SE - standard error.

LY - Landrace \times Yorkshire; LYLD - Landrace/Yorkshire \times Landrace/Duroc.

The data were modeled for a particular behavioural variable using phenotype, breed, and sex as tested effects. Results are presented in seconds and standard errors.

Table 4 - Effect of sex and genotype on behaviour during the novel arena test

Behaviour	Fixed effect	Level	Mean \pm SE	χ^2 test	P-value
Lying (duration)	Sex	Gilt	56.1 \pm 22.2	0.12 ^{df=1,24}	>0.10
		Barrow	54.4 \pm 29.6		
	Genotype	LY	55.9 \pm 24.4	0.38 ^{df=1,24}	>0.10
		LYLD	55.1 \pm 26.1		
Standing (duration)	Sex	Gilt	254.8 \pm 18.3	0.16 ^{df=1,24}	>0.10
		Barrow	255.3 \pm 24.3		
	Genotype	LY	266.2 \pm 20.1	14.52 ^{df=1,24}	<0.0001
		LYLD	242.9 \pm 21.4		
Walking (duration)	Sex	Gilt	169.1 \pm 9.9	0.72 ^{df=1,24}	>0.10
		Barrow	170.3 \pm 13.3		
	Genotype	LY	157.9 \pm 10.9	23.68 ^{df=1,24}	<0.0001
		LYLD	182.0 \pm 11.7		

SE - standard error.

LY - Landrace \times Yorkshire; LYLD - Landrace/Yorkshire \times Landrace/Duroc.

The data were modeled for particular behavioural variable using phenotype, breed, and sex as tested effects. Results are presented in seconds and standard errors.

Table 5 - Effect of sex and genotype on mean heart rate (HR) and heart rate variability parameters during baseline measurements

Parameter	Fixed effect	Level	Mean ± SE	F-value	P-value
HR (bpm)	Sex	Gilt	153.6±5.3	0.15 ^{df=1,11}	>0.10
		Barrow	156.6±5.9		
	Genotype	LY	149.5±3.8	5.50 ^{df=1,11}	<0.05
		LYLD	165.6±5.7		
RMSSD (ms)	Sex	Gilt	22.7±4.5	0.40 ^{df=1,11}	>0.10
		Barrow	18.6±5.1		
	Genotype	LY	24.1±3.8	5.05 ^{df=1,11}	<0.05
		LYLD	15.5±5.7		
log10LF	Sex	Gilt	1181.2±214.8	0.04 ^{df=1,11}	>0.10
		Barrow	630.2±322.2		
	Genotype	LY	1292.6±198.4	6.84 ^{df=1,11}	<0.05
		LYLD	379.5±297.5		
log10HF	Sex	Gilt	136.7±39.0	0.45 ^{df=1,11}	>0.10
		Barrow	73.5±43.9		
	Genotype	LY	149.9±33.0	6.84 ^{df=1,11}	<0.05
		LYLD	43.8±49.3		
LF/HF ¹	Sex	Gilt	1070.5±140.9	0.01 ^{df=2,10}	>0.10
		Barrow	1146.4±158.4		
	Genotype	LY	1150.9±119.3	0.12 ^{df=2,10}	>0.10
		LYLD	965.4±177.9		

SE - standard error.

LY - Landrace × Yorkshire; LYLD - Landrace/Yorkshire × Landrace/Duroc.

RMSSD - root mean square of successive R-R intervals; log10LF - log transformed values tested for power of the low-frequency band; log10HF - log transformed values tested for power of the high-frequency band; LF - power of the low-frequency band; HF - power of the high-frequency band.

¹ Calculated on the original values of LF and HF.

Table 6 - Mean heart rate (HR) and heart rate variability parameters of pigs of different sexes and genotypes while pre-tested (baseline) and tested in the novel object test

Parameter	Fixed effect	Level	Mean ± SE		F-test	P-value
			Baseline	Testing		
log10HR (bpm)	Sex	Gilt	152.6±7.9	142.5±7.7	0.03 ^{df=5,8}	>0.10
		Barrow	152.7±8.9	160.5±8.6		
	Genotype	LY	146.8±6.9	143.6±6.8	1.03 ^{df=5,8}	>0.10
		LYLD	163.2±9.6	162.2±9.3		
RMSSD (ms)	Sex	Gilt	13.6±2.7	13.3±2.4	0.07 ^{df=5,8}	>0.10
		Barrow	9.3±3.0	8.6±2.7		
	Genotype	LY	13.7±2.4	12.2±2.1	0.78 ^{df=5,8}	>0.10
		LYLD	8.2±3.3	9.6±2.9		
LF	Sex	Gilt	860.9±249.3	1238.1±444.6	0.01 ^{df=5,8}	>0.10
		Barrow	620.7±287.9	585.5±516.6		
	Genotype	LY	160.7±56.1	1331.1±412.9	0.02 ^{df=5,8}	>0.10
		LYLD	31.1±75.3	217.7±555.7		
HF	Sex	Gilt	146.4±67.4	137.5±54.9	0.00 ^{df=5,8}	>0.10
		Barrow	71.7±75.8	57.7±61.8		
	Genotype	LY	160.7±59.5	137.8±48.5	0.25 ^{df=5,8}	>0.10
		LYLD	13.1±82.2	41.1±66.9		
LF/HF ¹	Sex	Gilt	1078.8±181.8	625.8±109.2	0.12 ^{df=5,8}	>0.10
		Barrow	1143.3±204.4	1022.4±123.3		
	Genotype	LY	1096.7±160.4	750.6±96.8	0.09 ^{df=5,8}	>0.10
		LYLD	1123.9±221.5	877.1±133.6		

SE - standard error.

LY - Landrace × Yorkshire; LYLD - Landrace/Yorkshire × Landrace/Duroc.

log10HR - log transformed values tested for heart rate; RMSSD - root mean square of successive R-R intervals; LF - power of the low-frequency band; HF - power of the high-frequency band.

¹ Calculated on the original values of LF and HF.

Table 7 - Mean heart rate (HR) and heart rate variability parameters of pigs of different sexes and genotypes while pre-tested (baseline) and tested in the novel arena test

Parameter	Fixed effect	Level	Mean \pm SE		F-test	P-value
			Baseline	Testing		
HR (bpm)	Sex	Gilt	150.6 \pm 8.8	159.5 \pm 6.5	1.94 ^{df=5,5}	>0.10
		Barrow	151.7 \pm 13.2	155.7 \pm 9.7		
	Genotype	LY	149.9 \pm 9.3	152.4 \pm 6.8a	10.18 ^{df=5,5}	<0.05
		LYLD	152.8 \pm 13.5	169.0 \pm 9.9b		
RMSSD (ms)	Sex	Gilt	13.9 \pm 2.1	13.7 \pm 2.0	0.02 ^{df=5,5}	>0.10
		Barrow	7.8 \pm 3.2	11.4 \pm 2.9		
	Genotype	LY	12.2 \pm 2.2	13.7 \pm 2.1	0.48 ^{df=5,5}	>0.10
		LYLD	12.3 \pm 3.3	11.9 \pm 3.1		
log10LF	Sex	Gilt	1151.2 \pm 346.8	1515.0 \pm 607.7	0.02 ^{df=5,5}	>0.10
		Barrow	450.6 \pm 561.9	1228.4 \pm 984.5		
	Genotype	LY	1176.2 \pm 387.8	1706.2 \pm 679.4	0.02 ^{df=5,5}	>0.10
		LYLD	582.0 \pm 505.6	965.5 \pm 885.8		
log10HF	Sex	Gilt	128.1 \pm 32.3	176.3 \pm 68.5	0.05 ^{df=5,5}	>0.10
		Barrow	52.7 \pm 48.2	139.1 \pm 102.2		
	Genotype	LY	114.9 \pm 33.8	209.6 \pm 71.7	0.04 ^{df=5,5}	>0.10
		LYLD	94.7 \pm 49.5	90.1 \pm 104.9		
LF/HF ¹	Sex	Gilt	890.9 \pm 119.9	960.3 \pm 127.8a	10.75 ^{df=3,7}	<0.05
		Barrow	857.4 \pm 179.0	1013.5 \pm 190b		
	Genotype	LY	995.6 \pm 125.6	885.9 \pm 133.8	2.04 ^{df=3,7}	>0.10
		LYLD	682.4 \pm 183.8	1130.3 \pm 195.7		

SE - standard error.

LY - Landrace \times Yorkshire; LYLD - Landrace/Yorkshire \times Landrace/Duroc.

RMSSD - root mean square of successive R-R intervals; log10LF - log transformed values tested for power of the low-frequency band; log10HF - log transformed values tested for power of the high-frequency band; LF - power of the low-frequency band; HF - power of the high-frequency band.

¹ Calculated on the original values of LF and HF.

of Spearman correlation showed that in LY pigs, LF was positively correlated with HF ($r_s > 0.5$) and negatively with HR ($r_s < -0.7$). Heart rate was also negatively correlated with HF ($r_s < -0.7$), while a positive correlation was found ($r_s > 0.6$) between RMSSD and HF. In LYLD pigs, a strong positive correlation was found between RMSSD and LF/HF ratio ($r_s > 0.8$). Furthermore, in gilts, HR was strongly negatively correlated with LF and HF (both $r_s < -0.8$) and LF was positively correlated with HF ($r_s > 0.6$).

The pigs did not develop significant different autonomic responses under NOT (Table 6). Regarding the correlation analysis, it was revealed that in gilts during the test period, LF was strongly positively correlated with both RMSSD and HF, and HF was positively correlated with RMSSD (overall $r_s > 0.9$). In both breeds, HF was found to be strongly positively correlated with RMSSD ($r_s > 0.9$) and LF ($r_s > 0.8$), while in LYLD pigs, an additional positive correlation was found between RMSSD with LF ($r_s = +1$). In the NAT (Table 7), the LF/HF ratio was affected by sex. Barrows showed a significantly higher increase in LF/HF ratio than gilts did ($P < 0.05$). During the test period, the ratio was found to be negatively correlated with HF ($r_s = -1$) in barrows, while RMSSD was positively correlated with LF ($r_s = +1$). In gilts, RMSSD was strongly

positively correlated with HF ($r > 0.9$) and negatively with HR ($r_s < -0.8$). We found a significant difference between breeds in HR during test period. A higher HR was observed in LYLD pigs than in LY pigs ($P < 0.05$) and the results were not affected by walking activity of the animals (F-value = 0.17; $df = 5, 5$; $P > 0.10$). Pigs did not differ in other HRV parameters. With LYLD pigs, the correlation coefficient revealed that during social isolation RMSSD was strongly positively correlated with both LF and HF ($r_s = +1$).

Discussion

The most important finding of this study is the difference in basal cardiac activity across breeds but not sexes. Landrace \times Yorkshire (LY) pigs had lower HR with higher RMSSD, LF, and HF when compared with Landrace/Yorkshire \times Landrace/Duroc (LYLD) pigs. In addition, sex and breed affected behavioural responses during exposure to the NOT, whilst during exposure to the NAT only breed had an effect. Considering the cardiac responses in the NAT, HR increased more from baseline to testing in LY pigs than in LYLD pigs. Sympathovagal dominance also increased more from baseline to test period in barrows (increased LF/HF) than in gilts.

We observed no difference between gilts and barrows in time or frequency domain parameters during basal, non-challenging conditions. This could provide evidence of similar allostatic loads (Korte et al., 2007) in the sexes. On the contrary, high baseline levels of vagal tone could be associated with healthy individuals, while low vagal activity may reflect poor allostasis (Korte et al., 2007). By showing higher RMSSD and HF, LY pigs may have had a less suppressed vagal activity than LYLD pigs and may thus be better adapted to different challenges.

During exposure to a novel-challenging situation in the NOT, gilts were witnessed as more exploratory and attentive than barrows, which supports our tested hypothesis. They showed longer duration of time spent actively nosing, biting, licking, or manipulating the novel object and longer duration of standing. It can be argued that showing longer duration of lying, during which animals often spent time manipulating the litter and most likely having their attention directed towards the novel object, is an additional evidence of gilts being more explorative and paying more attention to the novel object that they can manipulate. The behavioural changes observed across sexes together with the lower duration of walking may suggest that gilts may be less fearful than barrows (Forkman et al., 2007). It has been often reported that changes in behaviour follow the changes in physiology, so we would have expected differences in cardiac activity between sexes, but no differences were found. Since a lower motor activity level found in our gilts can be, according to the classical models of motivation (Hughes and Duncan, 1988), seen as a response to a perceived lack of control over the environment, which is stress-induced, we find this less likely when taking into account both behavioural and physiological measurements.

The reverse picture to that seen in the NOT emerged in the NAT, in which gilts showed similar behavioural responses to the social isolation in the novel environment but different cardiac activity compared with barrows. In the NAT, the increased LF/HF ratio values from baseline to testing in both gilts and barrows are neuro-physiological evidence of comparable mechanisms underlying behavioural responses when exposed to a novel situation. However, since gilts had a significantly higher LF/HF ratio than the barrows, it is possible to argue that the mechanism was regulated on a different level. The fact that we found no changes in RMSSD and HF, in parameters that were previously used as indicators of vagal activity in cattle (Després et al., 2002; Kovács et al., 2014) and pigs (Zebunke et al., 2011), leads us to assume that our results point to a general increase in sympathetic activation. The

increased LF/HF ratio by variations in sympathetic input was interpreted earlier in relation to a stressful context in sheep (Coulon et al., 2011). If we consider the changes in the sympathetic system to represent the arousal dimension of effect with negative valence (Yeates and Main, 2008), then the degree of aversiveness of the stimuli in the NAT was greater in barrows than in gilts as indicated by the greater increase in LF/HF. Owing to this and other reports (Porges, 1995; Friedman and Taylor, 1998), it is reasonable to think that barrows showed higher stress susceptibility during exposure to stressors in the NAT for having a higher LF/HF compared to gilts.

Our behavioural results indicate that environmental stimuli in the NOT (but not in the NAT) induced different behavioural responses in gilts when compared with barrows, which goes in line with a review by Forkman et al. (2007), who documented that specific responses of an individual depend on the nature of the novelty-related stimulus due to fluctuations in environmental or motivational variables. Furthermore, our results may also indicate different concepts of optimal exploratory strategy in gilts compared with barrows. As found for the behavioural responses, cardiac responses were not consistent over the two fear tests. While autonomic regulation of the heart varied in pigs of different sexes in the NAT, no such difference was found in the NOT. According to our results, we cannot fully confirm the previous findings showing that stress affects the sexes differently (Kritas and Morrison, 2007), but it is suggested that the response depends on the type of stressor presented.

Analyzing breed, LYLD pigs in comparison with LY pigs had a longer attention towards the novel object and a lower level of avoidance or fearfulness as indicated by showing longer duration of contact with a novel object in the NOT. Showing more lying, standing, and walking as indicators of less goal attention, a lower excitement for a novel object is furthermore confirmed in LY pigs. In the NAT, the exposure to novel stimuli elicited alterations in behavioural responses in LY pigs showing shorter latency to excrete, more standing, and less walking compared with LYLD pigs. Following the argument that a decreased motor activity level is an indicator of a perception of decreased control over the environment, which is stress-induced, while an increased level reflects an attempt of the animal to extricate itself from its surroundings prior to the onset of the learned helplessness (Hughes and Duncan, 1988), LY pigs seem to be more stressed in the NAT while less in the NOT when compared with LYLD pigs. From the measurements of baseline cardiac activity, we would expect LY pigs to be less stressed or more adaptive in the challenging situations,

but this was evident only during exposure to a novel object that they could manipulate in a familiar environment.

We reported a significant increase in HR from baseline to testing in LYLD pigs, which was greater than what we observed for LY pigs during the exposure to stressors in the NAT. We thus found an impact of the breed on the magnitude of change that may represent the net interaction between vagal and sympathetic regulation (Camm et al., 1996). Increased HR is usually accompanied by vagal withdrawal (Sato et al., 2004) and this was numerically confirmed in our study by the lower RMSSD and HF from baseline to testing. Similar HR dynamics by fear-related response of HR and sympathetic activation has been described before (Stiedl et al., 2009). An alteration in HR but not in LF/HF ratio was reported by Visser et al. (2002) when investigating a reaction to psychological stress in horses. Based on our results and if we consider the changes of the sympathetic system to represent the arousal dimension of effect proposed by Yeates and Main (2008), we propose that LYLD pigs were more aroused by the stimuli in a socially isolated novel environment when compared with LY pigs.

In the NOT, both sex and breed had a significant impact on pig behaviour, with an exception of the latency to approach a novel object, while in the NAT it was only breed. A display of similar latency can be explained by the fact that pigs are in general curious animals that like to approach and explore new objects in their familiar environment. Interestingly, in the NOT, the results reflected neither sex nor breed influence on physiological responses in pigs, but they did in the NAT. It seems that in a novel situation that provokes an attentive response associated with excitement or fear, pigs have a similar attentive state regardless of their genetic background. This leads us to think that the exposure to a novel object may not be stressful for pigs in our study or that stimuli in the NAT were more stressful compared with the stimuli in the NOT, which supports our hypothesis. Another explanation for the obtained results is a small sample size used that may have prevented detection of existing genetic influence on autonomic regulation in the NOT.

A strong positive correlation was found between LF on the one hand and RMSSD and HF on the other hand during baseline conditions in gilts and the two investigated breeds. The same correlations were witnessed during test period in the NOT. In the NAT, the positive correlation was found in both sexes and in LYLD pigs. Finding these correlations emphasizes the strong association of LF with vagal activity in pigs from pens with tail biting outbreaks. The same relationship has been reported in humans with anxiety disorder such as panic (Friedman and Thayer, 1998).

The cardiac activity of different sexes and breeds did not respond to all stress in the same way because the response was dependent on the type of stressors presented. It is justified to assume that responses to stressors in a novel environment found in the current study led to an expression of emotion, possibly, fear, which was accompanied by elevated sympathetic tone. Although previous experience (i.e., phenotypic effects) could be problematic for the validity of baseline and reactive measures and present a confounding influence that cannot be excluded from the considerations here, our results give evidence that the sympathetic tone is more involved as a mediator of stress to the heart than vagal tone.

Conclusions

Pigs display complex behavioural and physiological interactions that characterize responses to social isolation in familiar and novel environments with differences relating to genetic background. Barrows seem to be less explorative than gilts and to have a higher level of fearfulness. When barrows are socially isolated in a novel environment, they have a higher low/high frequency band power ratio. Landrace/Yorkshire \times Landrace/Duroc pigs are animals that perform more manipulative behaviours when occupied with a novel object, which may indicate a lower level of fearfulness than Landrace \times Yorkshire pigs. By showing higher heart rate responses, Landrace/Yorkshire \times Landrace/Duroc pigs also seem to get more aroused by novel stimuli in a socially isolated novel environment. Taken together, heart rate variability data can be an informative physiological measure related to presumed psychological processes in different sexes and breeds of pigs previously housed in pens in which tail biting occurs.

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