



# Heart rate variability as an indicator of pig welfare

by

Mandy Bowling

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## **Declaration**

I declare that this thesis is a record of original work and contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text.

Mandy Bowling

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# Literature Review

## **Introduction**

Sow housing has recently undergone major changes across Australia with the phase out of sow stalls and a move towards group housing (Australian Pork Limited, 2013). These changes have been driven largely by consumer demand for ‘welfare-friendly products’ (Croney *et al.*, 2012). The move from confining sows in stalls during their entire pregnancy to confining sows for only 5 days around mating and one week pre-farrowing (Australian Pork Limited, 2013), has meant that sows will now be housed together. However, there is limited information on how stressful these changes are to sows.

“Stress” has been defined by neuroscientists as “... the condition where the environmental demand exceeds the natural regulatory capacity of the organism” (Koolhaas, 2011). In other words, the normal homeostatic mechanisms operating to return physiological set points to normal limits are overwhelmed by the environmental scenario. Quantifying stress therefore requires the measure of relevant physiological parameters to determine if they are outside of ‘normal’ set point values.

Identifying which parameters are relevant then becomes the issue. Clearly hormonal components of the hypothalamus-pituitary adrenal axis (HPA) such as corticotrophin-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH) and cortisol are prime candidates, as they are central to stress responses (Koolhaas, 2011). Short-term acute stressors may also be expected to influence the catecholamine (adrenaline and noradrenaline) axis (Koolhaas, 2011). These changes in cortisol and adrenaline levels would then be reflected in changes in metabolism (e.g. carbohydrate and lipid metabolism) and physiology (e.g. heart rate, blood pressure, respiration rate). Therefore these are prime candidates for determining the level of stress being experienced by an animal in a given situation. However, they ignore the neural arm of the homeostatic response mechanisms.

Homeostasis is achieved through a combination of both hormonal and neural responses, but to date most studies of stress have concentrated largely on the hormonal component (e.g. Bergamasco *et al.*, 2010). Attempts to overcome this deficiency have concentrated on assessment of behaviour which should reflect the summation of both neural and hormonal status.

While both hormonal status and behaviour are widely used to quantify stress, they have significant limitations as stress indicators. Levels of cortisol in body fluids, such as saliva or blood, are significantly impacted by non-stress related factors such as time-of-day, feeding patterns, and other environmental components (Bergamasco *et al.*, 2010). Perhaps more importantly it is not clear that high cortisol *per se* indicates that an animal is 'stressed'; it may merely reflect that the animal is responding appropriately to the stressor by elevating cortisol which allows it to metabolically 'cope' with the situation.

Similarly, behavioural assessments suffer limitations as indicators of stress. Firstly, they may be subjective and dependent on the observer. Whilst training can remove some of this subjectivity it remains problematic. Perhaps more importantly, and similar to the cortisol problem, how do we know what behaviours are indicative of stress? Many behaviours such as aggression and submission may merely indicate that the animal is 'coping'. There is an urgent need to identify a quantitative measure of stress which truly reflects when animals are not coping.

The best way to identify such a measure is to validate it in humans where we can get a correlation between the measure and the human's perception of their psychobiological state (i.e. their mental and physical feelings). Heart rate variation (HRV), which reflects the neural networking operating in an individual, may provide the solution to this problem.

HRV analysis provides data on the relative influence of the two arms of the autonomic nervous system, the sympathetic and parasympathetic arms (von Borell *et al.*, 2007). It is

important to understand that HRV analysis is not heart rate analysis per se. HRV represents longer-term neural control of body systems and has been used in human medicine to study disease risks and health (von Borell *et al.*, 2007).

HRV analysis has also been studied in many different animal species, such as horses, chickens and cows as potential indicators of stress and animal welfare (von Borell and Veissier, 2007). Most HRV studies in pigs have been as models in biomedical research for human diseases, but some studies have considered its potential role in stress and welfare (von Borell and Veissier, 2007). There are no known current studies on the effects of housing space and mixing stress in sows using HRV, and this review examines the current literature on pig welfare and the use of HRV as an indicator of welfare in pigs.

### *Current Sow Housing*

Impacts of various housing conditions on the health and welfare of sows is a contentious, well discussed issue. The use of sow stalls during gestation has become a major focus with changes being undertaken to replace sow stalls with alternatives, such as group housing. Many countries outside of Australia have already undertaken these changes, with Switzerland banning sow stall use in 2007 and the European Union banning their use in 2012 (Weaver and Morris, 2004). Australia has recently begun to follow this example, with Australian Pork Limited making the decision in 2013 to voluntarily phase out the use of sow stalls across Australia by 2017 (Australian Pork Limited, 2013). This change has been driven largely by consumers increasingly demanding ‘ethical’ production of meat (Croney *et al.*, 2012).

Sow stalls were used in the pig industry because they were found to decrease aggressive interactions between sows during gestation (Salak-Johnson *et al.*, 2012) which can lead to reduced reproduction and productive output. Stalls also allow for easier management of sows because they allow individual feeding of each sow (Salak-Johnson *et al.*, 2012). However, stalls are believed to have several detrimental effects on sows, such as the severe restriction of movement and the inability for sows to express natural behaviours. Lameness, stereotypes and

chronic stress are all attributed to the use of sow stalls (Chapinal *et al.*, 2010). These, and other welfare issues, have led to retailers and consumers driving the change from sow stalls to group housing.

### *Group Housing and Sow Stalls*

In Australia, changes to the use of sow stalls mean that sows can only be confined to a sow stall for the first five days after being mated, and for the last week before farrowing (Australian Pork Limited, 2013). For the remaining time, sows now need to be housed in different housing conditions, such as groups. However, there have been conflicting studies on the benefits of housing sows in groups, compared to housing them in sow stalls. Chapinal *et al.* (2010) found that sows housed together in groups demonstrated fewer stereotypes and were “better rested”, compared to sows housed in sow stalls. From this study, they concluded that sows housed in groups had better welfare without a decrease in production, providing they had good management. However, there have been conflicting studies on sow stalls compared to group housing that have not had the same findings. A study by Salak-Johnson *et al.* (2012) measured the different responses of sows in sow stalls and group housing, and found no significant difference in welfare and production between the groups. They also noted that most scientific reviews have indicated that there is no difference in welfare between sows in sow stalls and those in group housing.

Another study by Li *et al.* (2012) found that aggression can become severe towards first parity sows in group housing systems. This study found that due to the establishment of a hierarchy in group housing, younger sows can have increased injuries and cortisol levels, suggesting reduced welfare. Feed intake was also decreased, and reproductive failure occurred in group housed sows (Li *et al.*, 2012).

These discrepancies between studies demonstrate that determining the most welfare friendly housing system is difficult. The rapid changes of sow housing systems currently observed in the Australian industry means that there is a pressing need to understand the effect these

changes are having on the welfare of sows. Determining the welfare of the sow means determining their response to the stress of group housing which can be difficult, as shown by these conflicting studies.

### *Stress*

Stress has been difficult to define in the past and was first defined by Hans Selye in 1936 as, “the non-specific response of the body to any demand for change” (stress, 2013). This definition was created by Selye after he observed illnesses such as heart attack, stroke, rheumatoid arthritis and kidney disease may be caused by stress, not just pathogens as previously thought (stress, 2013). However, this definition of stress is subjective (American Institute of Stress, 2013) and to usefully quantify stress requires a more objective, reliable and repeatable measure.

### *Stress Perception*

Stress is not simply defined because it is perceived differently by individuals, with varying responses to the same environment (von Borell and Veissier, 2007). Although stress is perceived as negative, it can have positive effects, such as increased production, before it reaches a threshold where it begins to have negative effects on health, immunity, reproduction and production (stress, 2013). There are varying thresholds for different individuals, which will affect how they will respond to stress and when stress will begin to have negative impacts on them.

During a stress response, homeostatic balance is altered (Jaskulke and Manteuffel, 2011) with increased heart rate, respiration rate and adrenal gland activity (Kim *et al.*, 2008b). Individuals with a high threshold for stress are able to return to a homeostatic balance with few ramifications. Those individuals that have a low threshold for stress are not able to respond to the stress as easily, with homeostasis remaining unbalanced (Jaskulke and Manteuffel, 2011). Individuals with an altered homeostatic balance experience negative effects such as decreased growth rate, increased disease, decreased feeding, decreased social

and exploratory behaviours and depression (Rutherford *et al.*, 2006). These effects can negatively affect animal welfare (von Borell and Veissier, 2007).

### *Acute and Chronic Stress*

There are two forms of stress; acute which involves a short-term event and chronic which occurs after exposure to stress over an extended period (Kim *et al.*, 2008a). In humans, chronic stress, can lead to many health problems such as; obesity, hypertension, type II diabetes, coronary heart disease and a suppressed immune system (Kim *et al.*, 2008a). It can also affect the behaviour over time and lead to changes in hormones such as cortisol, and altered balance of the autonomic nervous system (Kim *et al.*, 2008b).

Chronic stress is believed to be a problem in intensively-farmed animals (Dantzer and Mormede, 1983) which may be due to their genetics and the intensive housing conditions they are kept in (Dantzer and Mormede, 1983). In the case of sows, a number of events can lead to chronic stress, including the conditions experienced during confinement and social stress that can occur during group housing (de Jong *et al.*, 2000). This has been indicated by the observation of increased health problems and reduced growth rates in sows mixed into groups (de Jong *et al.*, 2000).

### *The Autonomic Nervous System*

The autonomic nervous system (ANS) is the part of the nervous system that is not under conscious control (Cunningham, 2002). This area of the nervous system controls functions such as heart rate, smooth muscle and glands (Cunningham, 2002). It is divided into two arms; the parasympathetic and sympathetic (Cunningham, 2002). These two arms have opposite functions, with the sympathetic resulting in a rapid response to increase body functions, and the parasympathetic decreasing and slowing down body functions. (Cunningham, 2002). Together, these two branches of the autonomic nervous system maintain homeostasis within the body.

Under stress, the sympathetic nervous system becomes dominant, preparing the body for a rapid response to the stressor by increasing heart rate, respiration rate and adrenal activity (Kim *et al.*, 2008b). Once the stressor has been removed, the parasympathetic nervous system becomes dominant and respiration rate and heart rate decrease, and the body is able to return to normal homeostasis (Kim *et al.*, 2008b). However, if the stress is maintained over a long period of time, such as in chronic stress, the sympathetic nervous system remains dominant. This results in the body remaining in a state of rapid response and begins to have negative effects, such as illness and behavioural problems (Kim *et al.*, 2008a).

## **Stress Measurements**

Currently, there are several methods to measure stress in pigs, although the measurement of chronic stress is difficult (Chapinal *et al.*, 2010). Some of the current methods used in pigs are cortisol levels in blood and saliva (Bergamasco *et al.*, 2010), the 'backtest' in piglets (Geverink *et al.*, 2002), behavioural observations and injury scoring (Stukenborg *et al.*, 2011).

### *Plasma and Salivary Cortisol*

Cortisol in plasma or saliva is used across species to indicate stress, with changing cortisol levels reflecting physiological stress (Bergamasco *et al.*, 2010), physical stress and the response to a particular environment (Jaskulke and Manteuffel, 2011). However, plasma cortisol is not always considered a reliable indicator of long term stress in animals (Jaskulke and Manteuffel, 2011). This is partly due to difficulty fully understanding the relationship between the hypothalamus-pituitary adrenal axis (HPA) and exposure to a long term stress (Rutherford *et al.*, 2006). It is thought that the HPA axis may become accustomed to stress, affecting cortisol levels and its reliability as a measurement for chronic stress (Rutherford *et al.*, 2006).

Measurements of plasma cortisol are also influenced by a diurnal rhythm, with the time of day affecting cortisol levels (Geverink *et al.*, 2002). Cortisol levels are higher during light hours

and lower during dark hours (Geverink *et al.*, 2002), which can then give a false indication of stress. Cortisol is also a difficult measurement to rely on because it is produced in increased levels during an exciting event, such as feeding (Weaver and Morris, 2004). This means that an increased cortisol response may be due to excitement, rather than stress, and therefore is not always a reliable indicator of stress per se.

### *Backtest*

Another method used in the pig industry to identify pigs prone to stress is the 'backtest' in piglets (Geverink *et al.*, 2002). This test involves a piglet being placed onto its back for one minute and the number of escape attempts scored (Geverink *et al.*, 2002). Piglets can then be assigned with an 'active' or 'passive' coping style, with piglets with an 'active' coping style more influenced by the sympathetic system, and those with a 'passive' coping style influenced by the parasympathetic (Geverink *et al.*, 2002).

This information can then be used to understand how easily the piglet will cope with stress, with those under parasympathetic control believed to cope better (Geverink *et al.*, 2002). This information is useful in piglets, but the 'backtest' technique cannot be applied in older pigs, such as sows, due to their size.

### *Behaviour*

Observation of behavioural responses in animals can be a useful indicator of stress. These observations measure the interactions between pigs, such as fighting, and the number of times they occur (Stukenborg *et al.*, 2011). From these measurements, a dominance index can be created showing more dominant sows and the sows that are less dominant and more stressed (Stukenborg *et al.*, 2011). Although an effective measurement of stress, behavioural observations are not always relied upon as they can be subjective due to individual scorer perceptions. However, behaviour scores do enable the observations of which sows are more likely to be stressed by recording agonistic interactions between sows. This can demonstrate

which sows are dominant, submissive or in between and which are more likely to be stressed (Stukenborg *et al.*, 2011)

### *Lesion Scoring*

Another measurement of sows housed together in groups is lesion scoring (Karlen *et al.*, 2007). Lesion scoring is a measurement of the aggression and fighting that occurs between sows and involves the counting of injuries present over the whole body of the animal (Karlen *et al.*, 2007). The location of the lesion is noted, with lesions occurring on the front third of the body indicating a sow in more aggressive interactions and possibly under a higher level of stress (Stukenborg *et al.*, 2011). While lesion scoring is an objective measurement, it is an indicator of aggression between sows and can be difficult to correlate directly to stress, with sows scoring high for lesions, not necessarily highly stressed and vice versa.

## **Heart Rate Variation**

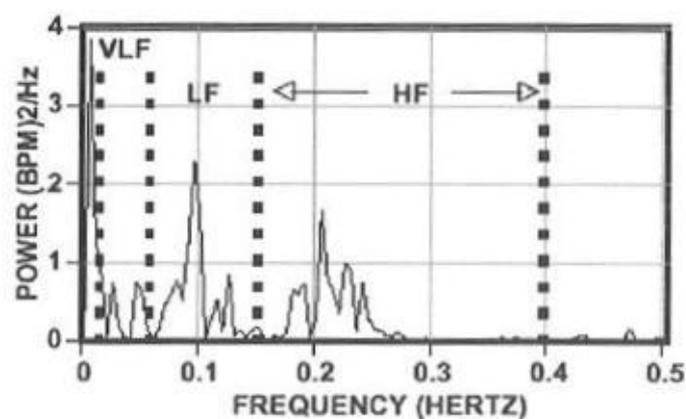
Heart rate variation is a relatively new method to assess stress in animals (Valerie *et al.*, 2012). Variation in heart rate was first noticed in the 18<sup>th</sup> century and has since been used in human physiological, pathological and emotional studies (von Borell *et al.*, 2007). Patterns of HRV have been studied for decades (Francis *et al.*, 2002) with research into HRV as a measurement of stress and psychological state increasing (von Borell *et al.*, 2007). An increase in the use of HRV in production animals, has led to the establishment of the 'Heart Rate and Heart Rate Variability Task Force' by the European Union, to provide guidelines for HRV measurements (von Borell *et al.*, 2007).

### *Heart Rate Variation and the Autonomic Nervous System*

Measuring HRV is effective because it is an instant, quantitative and non-invasive (Kim *et al.*, 2008b) measure of the control of the ANS on cardiac function (Gehrke *et al.*, 2011). HRV is measured by detecting the interval between R peaks in the QRS complex in a cardiac cycle (Nagel *et al.*, 2011a). The influence of the ANS on the heart is caused by the sympathetic and

parasympathetic branches acting on the Sinoatrial node (SA node) (Schmidt *et al.*, 2010b) located in the left atrium of the heart (Cunningham, 2002). The SA node of the heart contains ‘pacemaker’ cells which spontaneously depolarise together to create the initiation of a heart beat (von Borell *et al.*, 2007). The SA node is under the influence of the ANS and therefore, the time each beat and R wave occurs, is controlled by the ANS acting on the SA node (von Borell *et al.*, 2007). The influence of either parasympathetic or sympathetic neurons acting on the SA node of the heart, create the differing inter-beat intervals observed in R-R waves, creating measurable HRV (von Borell *et al.*, 2007).

Parasympathetic influences acting on the heart create a faster response of less than five seconds and sympathetic responses are slightly slower, occurring in up to five seconds and the full effect occurring 20 to 30 seconds later (von Borell *et al.*, 2007). This creates fluctuations in R-R intervals and is measurable by the frequency these fluctuations occur, with higher frequencies due to the parasympathetic system, and lower frequencies due to the sympathetic system (von Borell *et al.*, 2007). These frequencies differ for each species, with specific frequency calculations needed for individual animal species (Poletto *et al.*, 2011).



**Figure 1: Power and frequency created after analysis of heart rate variation. The power detected in each frequency represents either the parasympathetic at higher frequencies or sympathetic at lower frequencies (Medicore, 2013).**

### *Heart Rate Variation and Stress*

HRV has been shown to more effectively measure stress than heart rate alone, and has been used to assess individual psychological, emotional and pathological processes (Poletto *et al.*, 2011). It has also been used to assess stress, behaviour, temperament, emotional state, psychophysiological stress (Bergamasco *et al.*, 2010) and mental stress (Kim *et al.*, 2008b). As well as being able to interpret the overall function of the ANS (Stewart *et al.*, 2008), HRV can be used to assess both acute and chronic stress in animals (Schmidt *et al.*, 2010b).

### *Animal Studies*

In animals, HRV analysis has been used to understand animal behaviours and emotions by quantifying stress, pathology changes, emotional changes, behavioural changes, management practices and different animal temperaments (von Borell *et al.*, 2007). Although much of the HRV research has been in humans, it is believed the same principles can be transferred to many animal species due to similar limbic systems, or emotional centres, in the brain (von Borell *et al.*, 2007).

Many different animal species have been involved in HRV research. HRV has been studied in horses to study the effects of genotype, nutrition, behaviour and environment on stress (Gehrke *et al.*, 2011). Horses have also been used in HRV studies on equipment validation (Valerie *et al.*, 2012), the effects of air transport on stress (Munsters *et al.*, 2013), the effects of different horse riders on stress (Munsters *et al.*, 2012), foetal studies to show the maturation of the ANS (Nagel *et al.*, 2010, Nagel *et al.*, 2011b, Nagel *et al.*, 2011a), training stress in horses (Schmidt *et al.*, 2010a) and long-term road transport stress (Schmidt *et al.*, 2010b, Schmidt *et al.*, 2010c).

Dogs have been used in HRV studies of (von Borell *et al.*, 2007) welfare and equipment validation (Valerie *et al.*, 2012). Other species studied include: calves in pain and stress studies (Stewart *et al.*, 2008), dairy cows during milking changes (Stewart *et al.*, 2008),

chickens to study pecking behaviour (Kjaer and Jorgensen, 2011) and the study of ANS activity in great cormorants (Yamamoto *et al.*, 2009).

#### *Pigs and Heart Rate Variation*

Pigs have been used in a variety of HRV studies in areas of pig welfare and medical research as well as validation studies. Studies have also been conducted on pigs to identify different areas of ANS activity, and when they occur (Poletto *et al.*, 2011). Poletto *et al.* (2011) used blockers of the sympathetic and parasympathetic branches of the ANS, atropine and propranolol, to determine at which frequency activity of the ANS could be identified. This study was able to identify low frequency sympathetic and parasympathetic activity occurs at 0.0 - 0.09Hz and high frequency parasympathetic activity occurs at 0.09-2.0Hz, However, this conflicts with previous studies where the low frequency activity was identified to occur at 0.01 – 0.13Hz and high frequency at 0.13 – 0.41Hz (von Borell *et al.*, 2007), which has generally been accepted in most studies.

A study by Kuwahara *et al.* (2004) identified possible issues with HRV recordings while studying miniature pigs in individual and paired housing. They found that there is a diurnal rhythm associated with HRV testing, with a higher HRV in the dark phase and a lower HRV in the light phase (Kuwahara *et al.*, 2004). However, they only found this effect occurred when pigs were housed individually (Kuwahara *et al.*, 2004), which means that HRV measurements need to be recorded at the same time to remove this effect. They also found that the mixing of pigs does affect HRV and that it could be used as an effective measurement of welfare in pigs (Kuwahara *et al.*, 2004).

#### *Heart Rate Variation and Pig Welfare*

HRV as an indicator of pig welfare has only occurred recently, with few studies using commercial pigs (von Borell *et al.*, 2007). Pig welfare has been studied using HRV to look at the effects of tail biting in pigs (Zupan *et al.*, 2012). This study was able to identify different levels of stress and fear in pigs experiencing tail biting compared to those doing the actual

biting. Pigs have also been used in equipment validation studies (Valerie *et al.*, 2012) and have been used extensively in medical studies (von Borell *et al.*, 2007).

de Jong *et al.* (2000) used HRV to study the effects of social hierarchy stress in grower pigs and found pigs lower in the hierarchy were more “stressed” when HRV was used. Another study by Jaskulke and Manteuffel (2011) looked at the difference in HRV in confined and non-confined pigs and found that there was no significant difference between the two groups.

These types of studies into pig welfare need to be continued to understand the stress response of pigs in different housing conditions. HRV has shown to be an effective method of stress measurement in previous studies but needs to be validated further. Currently, there are no studies that have used HRV as an indicator of sow welfare in different housing conditions.

Therefore, the hypothesis for the study is:

- If stress is increased in the sow, then heart rate variation frequency will decrease

The aims for this study are:

- To validate the heart rate variation technique in sows
- To determine if previously identified stressors in sows influence heart rate variation
- To determine how heart rate variation outputs correlate with other accepted measures of stress in animals (cortisol levels and behavioural scores)
- To determine if heart rate variation is altered during acute or chronic stress caused by housing conditions in sows

## **Conclusion**

This review has analysed the current literature to better understand the use of HRV as an indicator of pig welfare in intensively-farmed sows. While HRV has been used in pigs previously, most studies have been in biomedical research. However, there have been some studies that have been successful in using HRV to measure pig welfare in different housing conditions and stressors.

There have been discrepancies in some tests of whether pigs are more stressed in sow stalls or when mixed together in larger social groups, when other measurements of stress were used. This identifies a need to further study how stressed sows are in different housing conditions such as sow stalls, mixing pens and ecoshelters. HRV has not been used in this area and could prove an effective measurement of stress in sows. The relationship between HRV analysis and other measurements of stress would contribute to our knowledge of stress physiology and its quantification in animals.

The use of HRV in pigs is a relatively new measurement and needs more research into its use. Using HRV to indicate stress and welfare in sows in different housing conditions will further add to the body of knowledge on the use of HRV in pigs and the understanding of stress in sows to further improve sow welfare.

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# Thesis

## **Abstract**

The housing of gestating sows has undergone major changes in Australia, with conversions from individual stalls to group housing. This is largely driven by community perceptions of improved animal welfare. Heart rate variation (HRV) was employed to indicate stress in sows associated with changes in the autonomic nervous system. Sows were tested for HRV in stalls, group pens (2m<sup>2</sup>, 4m<sup>2</sup> and 6m<sup>2</sup>) and ecoshelters. Three blocks of 18, multiparous Large White x Landrace sows were sampled on day 5 in stalls, 7 in pens and 70 in ecoshelters, relative to mating (day 0). External heart rate monitors were placed on sows for 30 minutes to obtain an electrocardiogram (ECG) trace of 5-10 minutes. Saliva cortisol samples and injury scores were collected from sows on the same day. Results were analysed using a general linear model with fixed effects of day, block and parity. A significant effect of housing ( $P < 0.05$ ) on HRV (LF ms<sup>2</sup>), saliva cortisol concentration (nmol/L) and injury score was identified. These measures indicated sows experienced heightened stress in group pens when compared with stalls and ecoshelters. A significant positive correlation ( $r = 0.51$ ;  $P < 0.01$ ) was found between LF and saliva cortisol. This suggests that HRV may be used as an indicator of stress in sows. Variation in HRV measures was large and most likely explained by sow interference, thus future studies should use a non-invasive method of heart rate monitoring.

## **Introduction**

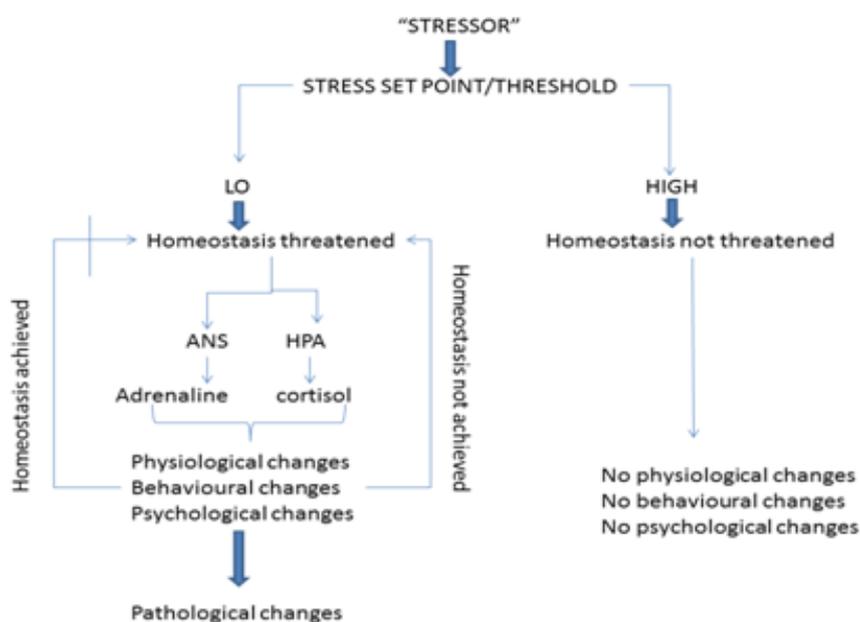
The housing of gestating sows across Australia has recently changed dramatically due to increased consumer concern for animal welfare. This has resulted in an industry led phase out of sow stalls throughout Australia by 2017 (Australian Pork Limited, 2013). However, this change in husbandry has occurred much sooner for some, with major supermarkets already selling ‘sow stall free’ meat which has meant that pork producers have rapidly converted traditional sow stall housing to group housing systems. This follows a similar trend in other countries, such as Switzerland and the European Union which phased out sow stalls in 2007 and 2013 respectively (Weaver and Morris, 2004).

This rapid conversion of gestation housing has meant that there is still research that needs to be conducted in order to better understand sow welfare. Stalls have been an effective method of sow housing in the past as sows are unable to fight, benefiting welfare and reproductive output (Salak-Johnson *et al.*, 2012). They also allow for individual feeding and medical treatment. However, stalls have also been shown to compromise welfare due to restriction of movement leading to lameness, stereotypic behaviour which is indicative of stress, and the inability of sows to express ‘natural’ behaviours (Chapinal *et al.*, 2010).

Group housing of sows is seen as a welfare improvement over individual stall options as confinement is reduced and social interactions are increased (Chapinal *et al.* 2010). Sows in groups show fewer stereotypes (Chapinal *et al.*, 2010), which would suggest that welfare may be improved. However, there have been studies that have shown there is no difference in sow health or welfare in group housed sows compared to when they are in stalls (Salak-Johnson *et al.*, 2012). Furthermore, the aggression directed towards lower parity sows whilst in group housing may lead to reduced welfare in younger, smaller animals (Li *et al.*, 2012). There is also no conclusive research into the most appropriate size pen and design to promote sow welfare (Salak-Johnson *et al.*, 2012). At present, we have a limited understanding of sow

welfare and optimal housing conditions. With rapid changes occurring to sow housing systems, this needs to be addressed to ensure that changes in sow housing are indeed accompanied by improved animal welfare outcomes.

Stress in animals, including pigs, has been defined as, ‘a disturbance in homeostasis’ (Jaskulke and Manteuffel, 2011). During a short term or acute stress response, homeostasis is quickly restored to normal and there are no long-term effects on the individual (Jaskulke and Manteuffel, 2011). However, if the stress remains over a longer period of time such as with a chronic stress (Kim *et al.*, 2008a), homeostasis set points are altered resulting in decreased growth, increased disease and behavioural changes (Rutherford *et al.*, 2006) (Figure 1).



**Figure 1: Physiological changes caused by stress and the effect on different individual homeostasis thresholds**

Each individual has a unique ‘stress threshold’ which determines their response to a stressor. (von Borell and Veissier, 2007). Under the same stressor, individuals will respond differently (Jaskulke and Manteuffel, 2011), with homeostasis of lower stress threshold individuals becoming threatened. This leads to physiological, behavioural, psychological and pathological changes.

Measuring stress in animals can be difficult, and most methods are directed at simple metrics, such as plasma cortisol levels, reflecting the endocrine response to a stressor. During a stress response, hormonal control involves activation of the hypothalamus-pituitary adrenal axis (HPA axis) and release of cortisol (Koolhaas, 2011). The effects of cortisol are to enable animals to respond rapidly to a stressful situation through changes in metabolism, including the transportation of glucose for energy to respond to a perceived threat (Cunningham and Bradley, 2007). Cortisol levels can be measured in blood, saliva, urine and faeces to indicate an acute stress response, but are not always a reliable indicator of a chronic stress response (Rutherford *et al.*, 2006). Additionally, other factors such as sample collection itself, and diurnal variation in the cortisol concentration may interfere with the interpretation of results. Other measures of stress include shifts in immunological parameters and behavioural responses.

Behaviour can be used to determine if an animal is experiencing stress, as changes in behavioural patterns are often reported when adverse conditions are experienced, such as mixing of pigs or changes to stocking density (Stukenborg *et al.*, 2011). An issue arises however as behavioural responses can be influenced by a range of factors outside of stress and are therefore often difficult to interpret (Stukenborg *et al.*, 2011). Examples of external influences on behaviour include genetics, growth stage (Stukenborg *et al.* 2011) and pregnancy (Marchant-Forde and Marchant-Forde, 2004), as well as experience and the environment in which the animal is housed. This variety of influences on behaviour can make it a complex method of understanding stress in animals.

Whilst endocrine and behavioural parameters are used to quantify stress, homeostasis is also achieved by the involvement of the nervous system. To date, attempts to quantify neurological control of a stress response have been scant. The advent of a relatively new method of assessing neurological status has paved the way for this deficiency to be overcome.

The method, known as heart rate variation (HRV) analysis, has been used in human and animal ethological research (Valerie *et al.*, 2012). HRV measures neurological control over the heart and is calculated by assessing the difference between consecutive R peaks, or R-R intervals (von Borell *et al.*, 2007). Over time the difference, or variation, between R-R intervals is calculated to give an overall representation of the variation in heart rate and can be used to indicate both acute and chronic stress (Schmidt *et al.*, 2010c).

HRV is influenced by both the sympathetic and parasympathetic branches of the autonomic nervous system (Cunningham, 2002). During a stress response, the sympathetic nervous system dominates heart rate, while the parasympathetic is in control during times of rest and acts to decrease heart rate (von Borell *et al.*, 2007). These two branches are constantly working against each other to maintain homeostasis (Kim *et al.*, 2008b). However, during a stressful event the sympathetic nervous system becomes dominant over the parasympathetic nervous system (Kim *et al.*, 2008a). This dominance in sympathetic control allows rapid responses to danger, but if maintained over a longer period of time, can cause reduced growth, reduced reproductive output and increased disease susceptibility (Rutherford *et al.*, 2006).

Interpretations of HRV analyses are difficult and still the subject of debate in the literature, with acute and chronic stress having different effects on HRV parameters. It is generally accepted that during an acute stress response such as tail biting, sympathetic control increases (Zupan *et al.*, 2012) due to activation of the 'flight or fight response'. This results in an elevation in heart rate, a decrease in parasympathetic indicators RMSSD and SDNN (Zupan *et al.*, 2012), and an increase in the low frequency variation spectrum. During a chronic stress response, there is thought to be suppression in HRV (Medicore, 2013) (Kim *et al.*, 2008b) due to a change in responsiveness of the autonomic nervous system to stress. This has been found to occur in human athletes and people with depression and anxiety (Kim *et al.*, 2008a) who become less able to respond to stressors effectively. These differences in acute and chronic

stress responses become important when understanding the neurological control during an acute stress response and when managing a chronic stress.

Heart rate variation has been shown to be a useful measure of stress in both humans and animals. It has been utilised in investigations of pig welfare (von Borell *et al.*, 2007). Heart rate variation has been used to study stress involving tail biting (Zupan *et al.*, 2012), social hierarchy (de Jong *et al.*, 2000), confinement (Jaskulke and Manteuffel, 2011), cognitive enrichment (Zebunke *et al.*, 2013) and transport (Peeters *et al.*, 2008) in pigs. However, to date there has not been a study into the use of HRV to detect stress in sows under different housing conditions.

This study was conducted to validate the HRV technique in sows using external heart rate monitors, to determine if HRV is altered during acute or chronic stress caused by and to determine if HRV correlates with other measures of stress in sows. It was hypothesised that under certain housing conditions, some sows will be more stressed than others due to an increased sympathetic influence and lower stress threshold, and that overall sows will be more stressed in group mixed environments compared to in individual stalls.

## **Materials & Methods**

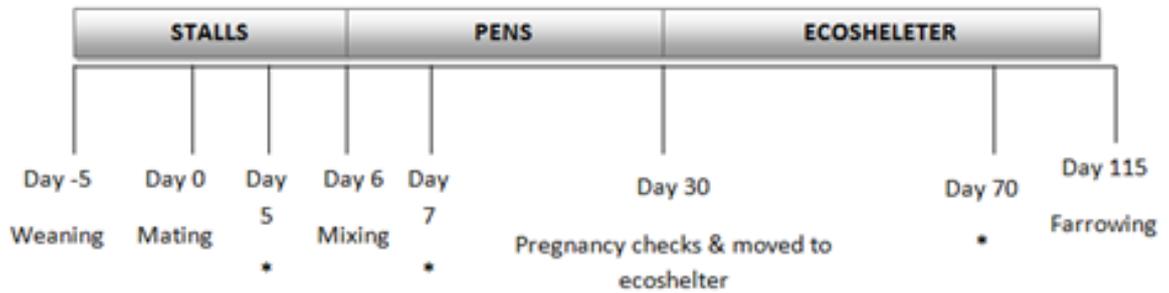
### *Sow Management and Housing*

For this study the guidelines of the 'Code of Practice for the Care and Use of Animals for Scientific Purposes (Canberra 2004) was followed. The study was also approved by the University of Adelaide Animal Ethics Committee (Animal Ethics Number: S-2013-076) and was conducted at the University of Adelaide's Piggery, Roseworthy, South Australia.

Fifty four Large White x Landrace mixed parity sows were utilised in three replicate blocks each containing 18 sows from May until October, 2013. At weaning, sows were housed in individual stalls and underwent daily heat detection until a standing heat reflex was observed, at which point they were artificially inseminated. Between three and five days post-insemination, sows were mixed into partially-slatted concrete group pens that each housed six animals. Group pens differed in size and thus sows were either allowed a space allowance of 2m<sup>2</sup>, 4m<sup>2</sup> or 6m<sup>2</sup> per sow. Sows remained under these conditions for five days after grouping, at which time pen size was standardised to 2m<sup>2</sup> per sow. Sows were housed in group pens until day 30 which marked pregnancy scanning. Sows deemed pregnant were then re-located to a straw based eco-shelter (336m<sup>2</sup>) with shoulder stall feeding stations that housed 40 sows for the remainder of gestation (a further 80 days).

### *Sow Measurements*

The following measures were collected from sows under each of the three housing conditions described above. Measurements were collected on days five (stall housing), seven (group housing) and 70 (eco-shelter housing) of gestation between the hours of 13:00 and 16:00 (Figure 2).



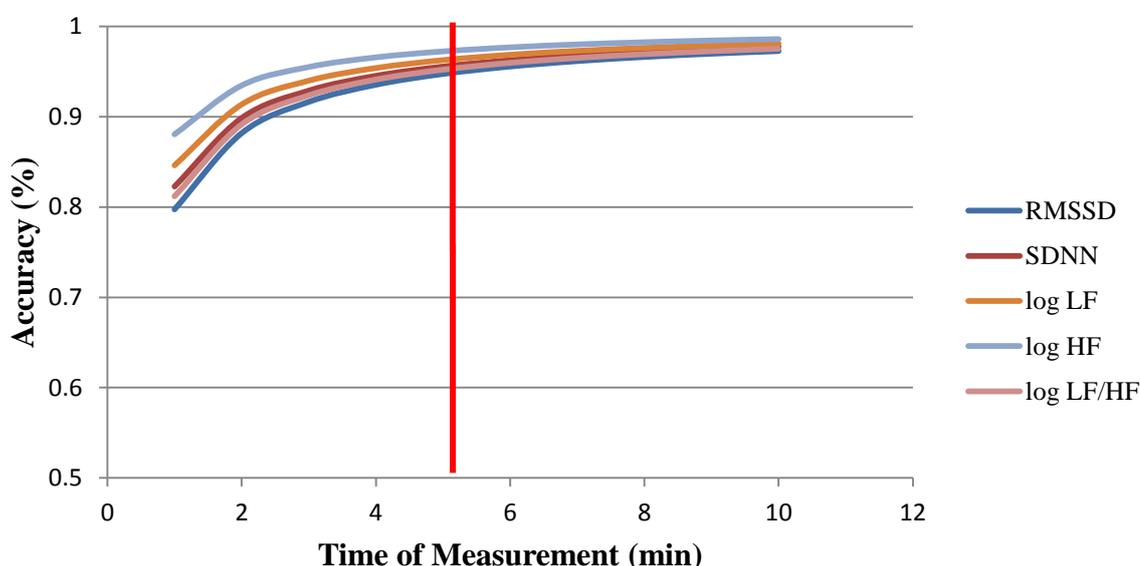
\* = HRV, saliva cortisol and injury scores collected

**Figure 2: Timing of measurements taken from sows after artificial insemination (mating) on day zero. The first measure was taken on day five in sow stalls, the second on day seven in group mixing pens and the final measure on day 70 in an ecoshelter.**

### *Electrocardiograms*

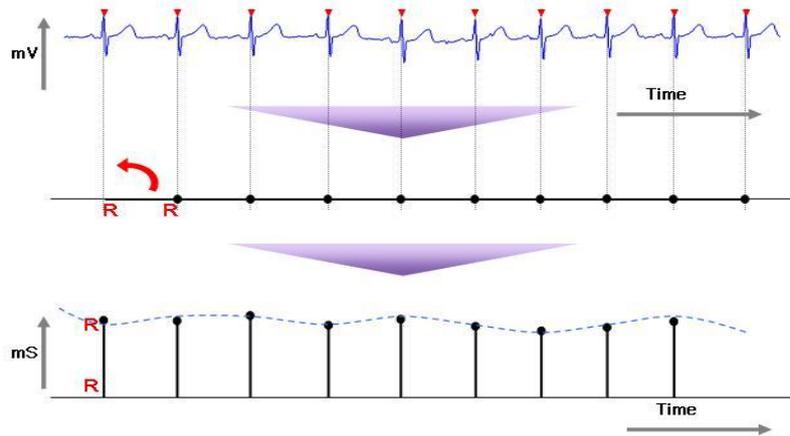
Electrocardiograms (ECG's) were taken non-invasively from each sow on the above mentioned pre-defined days. Sows were prepared for recordings by shaving an area of approximately 6 cm by 6 cm behind the foreleg, on the chest, using disposable razors and Sorbolene cream. The shaved area was then cleaned with Cholorhex C 0.1% and 70% ethanol to remove dirt, hair and dried skin that might have interfered with the electrode and skin contact. Once the area was cleaned and dried, an Ambu blue sensor-L electrode (Surgical and Medical Supplies Australia Pty. Ltd) was placed on each side of the sow and stuck as firmly as possible. Once the electrodes were in place, the Bluetooth ECG and Activity Monitor (Alive Technologies Australia Pty. Ltd) was attached to the electrodes using 1.8m MLA0310 Lead Wires (ADInstruments PowerLab Systems, Australia) . Once the wires were attached to the electrodes, the monitor was turned on and recording began. The monitor was placed inside a case and the wires and monitor strapped to the sow using 5 cm x 2.5 cm Tensoplast elastic adhesive tape (Lyppard Australia Pty. Ltd.) with all wires covered to prevent wires being chewed by other sows. Once the monitor was securely in place, the sow was left for a minimum of 30 minutes before the monitor was removed.

Recordings were taken for a minimum of 30 minutes to help ensure an uninterrupted reading of 5-10 minutes was recorded and sows were relaxed and undisturbed. This required twenty-one sows having HRV parameters measured five times for one minute. The variance for the total 105 measures was then divided between pigs and within individual pigs. The repeatability was calculated as between pig variance as a proportion of the total variance and the accuracy was calculated as the correlation between the estimated value for a pig and the true value. For all of the HRV measures, there was over 90% accuracy at 5 minutes of the ECG measurement (Figure 2). From this we determined that a 5-10 minute section was to be used for HRV analysis.



**Figure 3: Relationship between time of measurement and accuracy as calculated as the correlation between the estimated value for a pig and the true value for HRV measures RMSSD, SDNN, log LF, log HF, and log LF/HF. The red line indicates that a 5 minute trace achieves 90% accuracy.**

Recordings were then analysed for variation in the R to R interval in each QRS complex in the ECG reading (Figure 4). This variation was calculated using the LabChart 7 program (ADInstruments PowerLab Systems Australia) to give an overall representation of the R-R interval variation and indicate the level of heart rate variation in each individual sow.



**Figure 4: Identification of R peaks and the variation in R-R interval over time where the time between consecutive R peaks is measured to give the variation in R-R intervals over time (Medicore, 2013).**

Recordings used were completely uninterrupted with minimal other electrical noise interference and clearly visible R peaks. The selected section was analysed using the HRV function of Labchart, where R peaks were automatically detected and the variation analysed. The sensitivity of the program was adjusted so all R peaks were included and T peaks were not picked up for analysis. The measurements recorded from this analysis are indicated in Table 1.

**Table 1: Heart rate variation measurements calculated using LabChart software and used as an indicator of heart rate variation in individual sows (Medicore, 2013)**

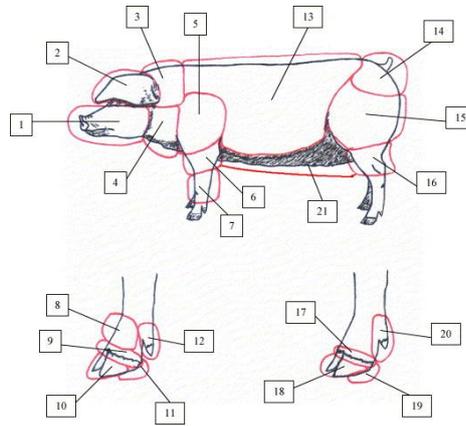
Measurement	Indication of Heart Rate Variation
<b>RMSSD</b>	an estimate of parasympathetic regulation of the heart
<b>SDNN</b>	measure of beat to beat intervals
<b>Heart Rate</b>	number of beats/per minute
<b>Number of heart beats</b>	total number of heart beats
<b>LF</b>	strong indicator of sympathetic activity
<b>HF</b>	indicator of parasympathetic activity
<b>LF/HF</b>	overall balance between the parasympathetic and sympathetic
<b>HF (nu)</b>	emphasises parasympathetic regulation
<b>LF (nu)</b>	emphasises sympathetic regulation

### *Saliva Sampling*

Saliva samples were collected from sows on the pre-determined days in the afternoon between 12:30 and 13:00 to account for the cortisol diurnal rhythm (Geverink *et al.*, 2002). Sows were sampled using a saliva salivette (Salivettes, Sarstedt Australia, South Australia, Australia), where the swab was removed from the tube and a cable tie tied around it. The swab was placed into the corner of the mouth of the sow with as little disruption as possible, and moved around to stimulate saliva production. The time the sample collection began was recorded with each sample taking no longer than two minutes to collect in order to ensure that the cortisol in the sample was not elevated due to handling. After collection, used swabs were placed back into Salivette tubes and centrifuged for 20 minutes at 5000rpm. After centrifuging, saliva collected at the bottom of the Salivette was pipetted into ependorf tubes and stored at -10°C until analysis. Saliva samples were sent for cortisol analysis which was conducted using a radioimmunoassay for cortisol by the Animal Biology Department at the University of Western Australia. Porcine saliva was modified for use in the radioimmunoassay by adding 75µL of buffer to 100µL of saliva. Limit of detection was 0.9 nmol/L and the mean intra and inter-assay coefficients of variation were 2.5% and 4.8% respectively.

### *Injury Scoring*

Injury scores were collected as a possible subjective indicator of the number of aggressive interactions experienced. The scoring system used throughout the trial was adapted from (Karlen *et al.*, 2007). Specific areas on the sow were scored for injuries such as scratches, fresh wounds, abscesses or old scars. The areas counted for injuries included head, ear, upper and lower neck, shoulder, side, legs and rump and both sides collated to give a total injury score for that sow on that day. The diagram used for recording injuries to sows in each housing condition is shown in Figure 5.



**Figure 5: Injury score template used to count and score injuries on individual sows where the total number of scratches, cuts and abscesses on each area of the sows body is counted (Karlen *et al.* 2007).**

*Behavioural Observations*

Behaviour of sows was observed for sows in the group pens and the ecoshelters. Each sow was marked with a unique symbol so individual identification during an aggressive interaction could occur. Video cameras were used to record sows in each group pen for six hours in the morning from feeding at 07:00 until 13:00. The videos were then analysed for aggressive interactions between sows and incidents where sows were displaced by other sows. In the eco-shelters sows were observed for the hour around feeding which occurred at 07:00.

The interactions observed via video recordings in the group pens or direct observations in the eco-shelters were then scored to provide an indication of social dominance hierarchy (Table 2).

**Table 2: Classification of sow dominance hierarchy based on the amount of aggression each sow delivered to other sows, or received from other sows.**

Hierarchy	Classification
<b>Dominant (D)</b>	Delivered more aggression than they received
<b>Sub-dominant (SD)</b>	Received more aggression than they delivered
<b>Submissive (SM)</b>	Delivered no aggression

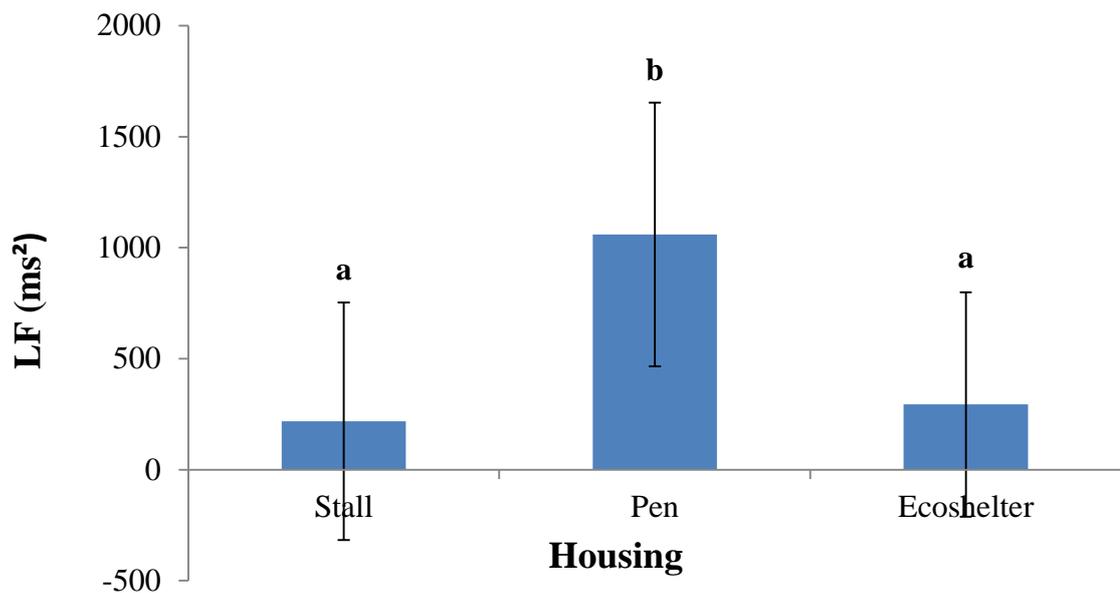
### *Statistical Analysis*

Statistical analysis was performed using the SPSS 2.0 statistics program (IBM, Chicago USA). For the analysis  $P < 0.05$  was deemed significant. The effect of housing design, space and social hierarchy on HRV measures was analysed using a general linear model with the fixed effects included in the model of replicate, sow parity, housing, space (nested within pen housing), and social hierarchy. Any significant two-way interactions were also included. Pearson's correlation analysis was conducted to examine relationships between HRV measures and other indicators of stress. The HRV parameters SDNN, RMSSD, LF, HF, LF/HF, LF (nu) and HF (nu) were log transformed before analysis to normalise distribution. For saliva cortisol values, the results were transformed using log, and injury score values were square-root transformed. Back-transformation of all data occurred for the presentation of results.

## Results

### *Effects of Housing on HRV*

There was a significant effect of housing treatment on HRV as measured by LF ( $\text{ms}^2$ ) ( $P < 0.05$ ). Sows housed in pens had an increased LF ( $\text{ms}^2$ ) ( $1059 \pm 593.8$ ) indicating a higher sympathetic influence, than those housed in either individual stalls ( $218 \pm 535.2$ ) or an ecoshelter ( $294 \pm 505$ ) (Figure 6).

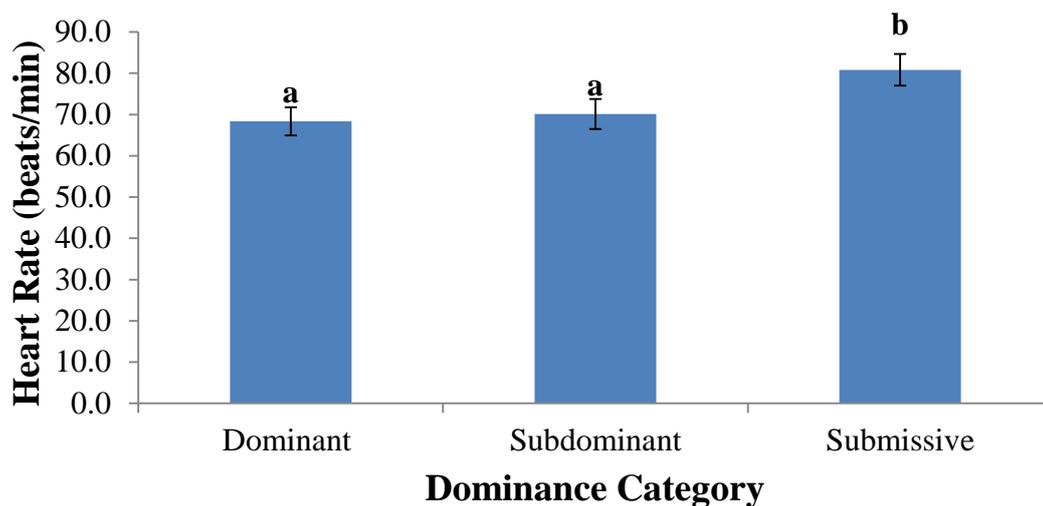


**Figure 6: Low frequency heart rate variation ( $\text{ms}^2$ ) for sows housed in stalls, group pens and ecoshelters. Values are means  $\pm$  SEM. Differing superscripts (a, b) represent significant difference ( $P < 0.05$ ).**

The other HRV parameters analysed, HF ( $\text{ms}^2$ ), LF:HF, RMSSD (ms), SDNN (ms), LF (nu) and HF (nu) were not significantly different between housing treatments ( $P > 0.05$ ).

### *Effects of Social Hierarchy on HRV*

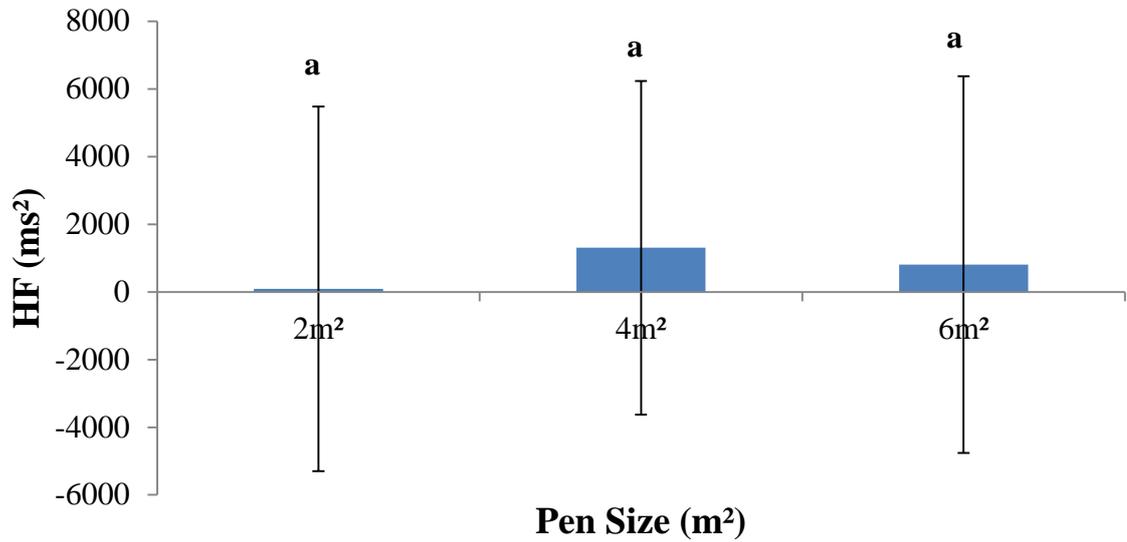
None of the HRV parameters measured were influenced significantly by the hierarchy classifications allocated (dominant, subdominant and submissive). Sows classed as submissive tended ( $P = 0.13$ ) to exhibit a higher heart rate ( $81 \pm 3.8$ ) than subdominant ( $70 \pm 3.7$ ) or dominant ( $68 \pm 3.4$ ) sows, (Figure 6).



**Figure 7: Heart rate (beats/min) for sows classed as dominant, sub-dominant or submissive. Values are mean + SEM. Differing subscripts (a, b) represent difference ( $P < 0.1$ ).**

### *Effect of Space Allowance on HRV*

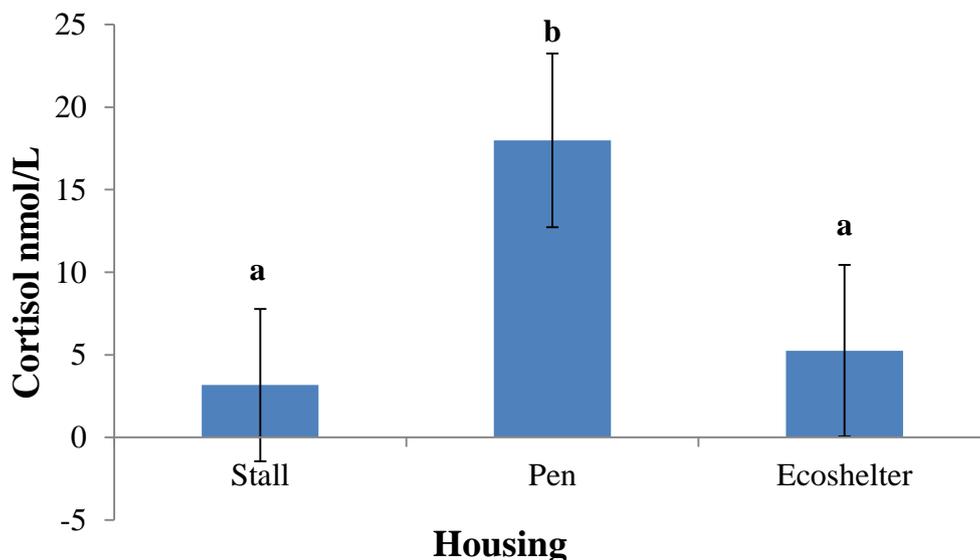
There was no significant difference between the three pen space allowance treatments ( $2\text{m}^2$ ,  $4\text{m}^2$  and  $6\text{m}^2$ ) for any of the HRV parameters measured, although HF ( $\text{ms}^2$ ) approached significance ( $P = 0.061$ ). Sows housed in the  $4\text{m}^2$  group pens tended to have a higher HF ( $1306 \pm 4929.8$ ) than sows in the  $2\text{m}^2$  and  $6\text{m}^2$  ( $91 \pm 5387.6$  and  $807 \pm 5566.5$  respectively), which suggests increased parasympathetic influence in sows with access to  $4\text{m}^2$ .



**Figure 8: High frequency heart rate variation (ms<sup>2</sup>) for sows housed in 2m<sup>2</sup>, 4m<sup>2</sup> 6m<sup>2</sup> pens. Values are mean + SEM.**

*Effect of Housing on Saliva Cortisol Concentration*

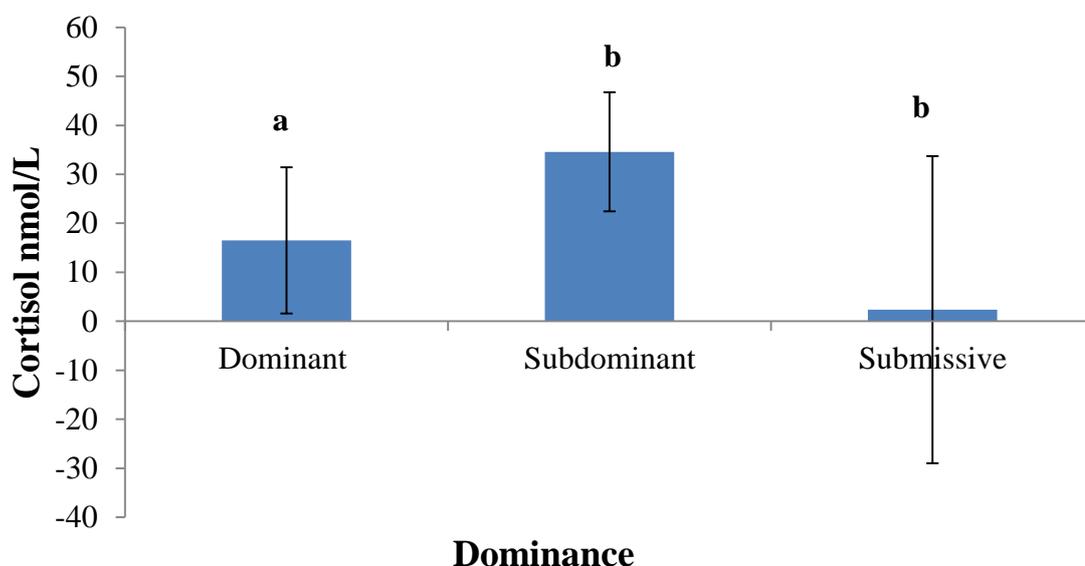
A significant difference ( $P < 0.05$ ) was found in saliva cortisol (nmol/L) between the three experimental housing treatments. When sows were housed in group pens, they had a significantly higher cortisol ( $18 \pm 5.3$  nmol/L) than when they were housed in individual stalls ( $3.2 \pm 4.6$  nmol/L) or the ecoshelter ( $5.2 \pm 5.2$  nmol/L; Figure 9).



**Figure 9: Saliva cortisol concentration (nmol/L) for sows housed in stalls, group pens and ecoshelters. Values are mean + SEM. Differing subscripts (a, b) represent significant difference ( $P < 0.05$ ).**

### *Effect of Social Hierarchy on Saliva Cortisol Concentration*

There was also a significant difference ( $P < 0.05$ ) in salivary cortisol between dominant, subdominant and submissive sows. Sows classed as subdominant had a higher salivary cortisol ( $34 \pm 12.1$  nmol/L) than those classed as dominant ( $16 \pm 14.9$  nmol/L) or submissive ( $2.4 \pm 31.4$  nmol/L; Figure 9).



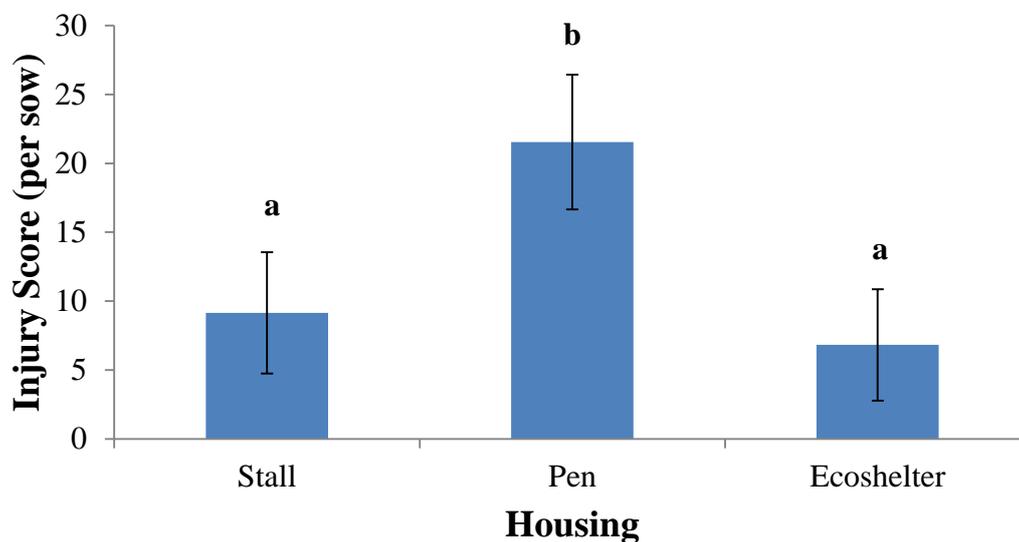
**Figure 10: Saliva cortisol concentration (nmol/L) for sows classed as dominant, subdominant or submissive. Values are mean + SEM. Differing subscripts (a, b) represent significant difference ( $P < 0.05$ ).**

### *Effect of Space Allowance on Plasma Cortisol Level*

There was no significant difference in salivary cortisol concentration between the group pen space allowances ( $2\text{m}^2$ ,  $4\text{m}^2$  and  $6\text{m}^2$ ).

### *Effect of Housing on Injury Score*

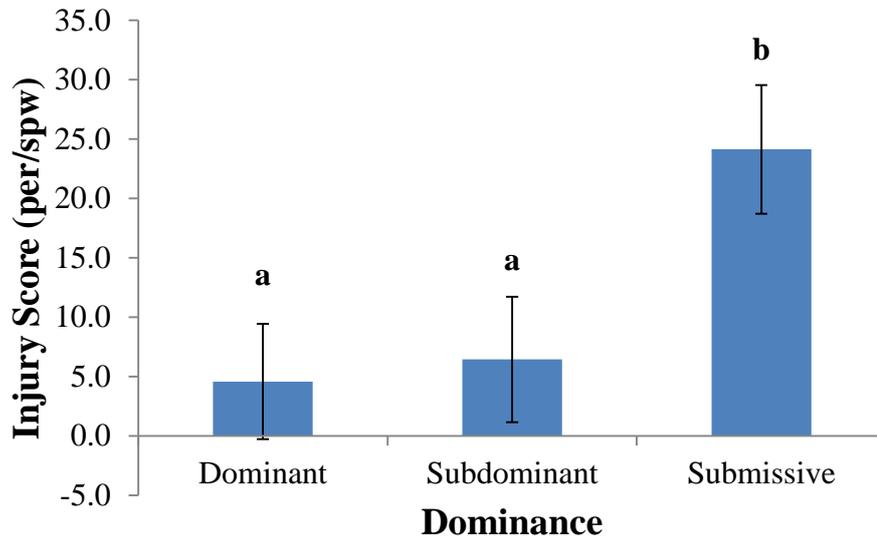
Housing treatment significantly influenced injury scores ( $P < 0.05$ ). When sows were housed in group pens they had a significantly higher injury score ( $21 \pm 4.9$ ) than when they were housed in individual sow stalls ( $9.1 \pm 4.4$ ) or an ecoshelter ( $6.8 \pm 4$ ; Figure 11).



**Figure 11: Injury score (per sow) for sows housed in stalls, group pens and ecoshelters. Values are mean + SEM. Differing subscripts (a, b) represent significant difference ( $P < 0.05$ ).**

### *Effect of Social Hierarchy on Injury Score*

There was a trend towards significance ( $P = 0.12$ ) in injury scores between dominant, subdominant and submissive sows. Sows that were classed as submissive tended to have higher injury scores ( $24 \pm 5.4$ ) than those classed as dominant ( $4.6 \pm 4.9$ ) or subdominant ( $6.5 \pm 5.3$ ; Figure 12).



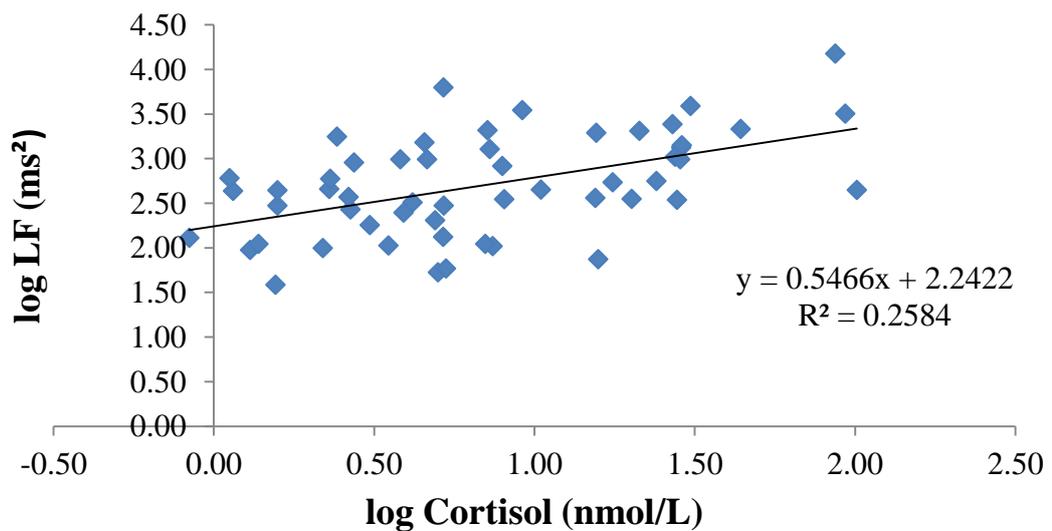
**Figure 12: Injury score (per/sow) for sows classed as dominant, sub-dominant or submissive. Values are Mean + SEM. Differing subscripts (a, b) represent significant difference ( $P < 0.10$ ).**

*Effect of Space Allowance on Injury Score*

There was no significant difference in injury scores between the three group pen sizes.

*Heart Rate Variation (HRV) and Saliva Cortisol Concentration*

Log saliva cortisol concentration (nmol/L) and log LF ( $\text{ms}^2$ ) were positively related to one another ( $r = 0.51$ ,  $P = 0.001$ ; Figure 13).

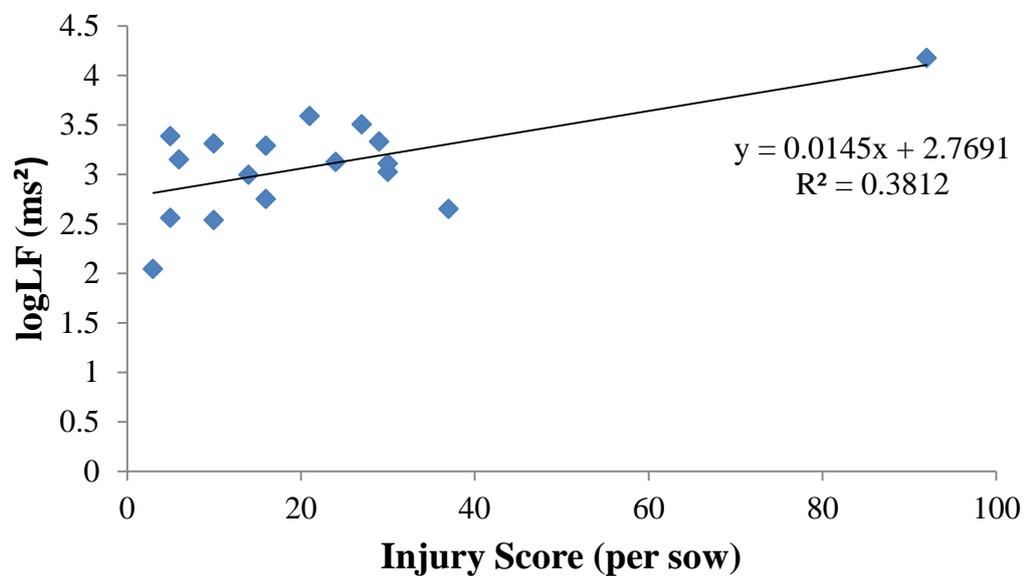


**Figure 13: Relationship between log LF ( $\text{ms}^2$ ) and log salivary cortisol concentration (nmol/L).**

There were no significant correlations between saliva cortisol and the other HRV measures, SDNN (ms), RMSSD (ms), LF (ms<sup>2</sup>), HF (ms<sup>2</sup>), LF (nu) and HF (nu).

#### *Heart Rate Variation and Injury Score*

A significant ( $P = 0.008$ ) positive relationship ( $r = 0.62$ ) was also found between LF (ms<sup>2</sup>) and injury scores under group pen housing conditions only. As LF (ms<sup>2</sup>) increases, there is also an increase in injury score counted on sows (Figure 14). Note the point of high leverage. There was no *a priori* reason to omit this data point.



**Figure 14: Relationship between log LF (ms<sup>2</sup>) and injury score (per sow).**

There were no other significant relationships found between any other HRV measurement parameters and injury score.

## **Discussion**

### *Effects of Housing on HRV*

This study demonstrated HRV can detect an acute stress response in sows under different housing conditions. LF was significantly higher in sows when they were housed in a group-mixing environment, indicating an increased sympathetic influence (von Borell *et al.*, 2007). This shows that when sows are housed together in groups, the stress response is heightened compared to when they are housed in stalls or an ecoshelter.

The increase in stress from stalls to pens is probably due to the mixing of unfamiliar sows in a pen, leading to competition over space and food resources (Tönepöhl *et al.*, 2013). In stalls sows are likely less stressed because they are individually housed and do not have to compete for space and food (Chapinal *et al.*, 2010). The group pen measurements were taken two days after the sow stall measure, and the dramatic change in LF indicates that the sows are experiencing an acute stress response and that LF as a measure of HRV is able to detect this change. The other measures of HRV did not detect this difference between housing types which may be because LF along with HF and LF:HF, are frequency measures. These frequency measures have been shown to reflect sympathetic and parasympathetic influence with the use of agents to stifle either sympathetic or parasympathetic influence (Poletto *et al.*, 2011). The RMSSD and SDNN however, measure the statistical changes in HRV over time (Poletto *et al.* 2011) and do not relate as easily to sympathetic activity (Marchant-Forde and Marchant-Forde, 2004).

A previous study by Marchant-Forde and Marchant-Forde (2004) found an increase in LF and a decrease in HF, SDNN, and RMSSD as pregnancy progressed, indicating an increased sympathetic influence which contrasts the decrease in LF identified presently. This previous study did use gilts, not sows which may affect the HRV outcome.

Large errors in HRV measures between treatments were identified which is likely due to a handling effect, not a limited number of animals as a power calculation was used and other trials have used smaller numbers of animals and not had large variation (Marchant-Forde and Marchant-Forde, 2004). This may be why the other HRV parameters did not detect a difference between housing.

#### *Effects of Social Hierarchy on HRV*

Although no significant effects of social hierarchy were found, there was a tendency for HR to vary depending on social hierarchy. Submissive sows tended to have higher HR than subdominant and dominant sows indicating that submissive sows are experiencing an increased stress response and subsequent increased sympathetically-induced increased HR. This may be because they are being outcompeted by more dominant sows and are less able to remain calm in the presence of more dominant sows. Submissive sows are also reproductively affected, likely due to increased stress, with reductions in farrowing rate, and a lower litter size compared to more dominant sows (Hoy *et al.*, 2009). Further studies on the use of HR in socially-distinct animals are needed to elucidate this trend.

#### *Effects of Space on HRV*

No significant effects of space allowed per sow on HRV were detected. However, HF was close to significance with sows in the 4m<sup>2</sup> pens tending to exhibit increased HF than sows in the 2m<sup>2</sup> and 6m<sup>2</sup> pens. An increased HF indicates an increase in parasympathetic influence (von Borell *et al.*, 2007) suggesting that sows in 4m<sup>2</sup> pens had a trend towards having a higher parasympathetic influence. This means that when sows are housed in a 4m<sup>2</sup> group pen, they may be able to cope better than when housed in a 2m<sup>2</sup> or 6m<sup>2</sup> pens. It is difficult to speculate as to why these sows are experiencing a decreased stress response. Aggression in sows has been shown to increase as floor space decreases (Salak-Johnson *et al.*, 2012) which would indicate the 2m<sup>2</sup> pen to have the highest HF and parasympathetic influence. The result was not significant and the number of animals in pens was lower and so it is difficult to fully

interpret these results but perhaps the 6m<sup>2</sup> pens allowed too much space for sows resulting in resource guarding and more fights between sows. It could also be higher than the 4m<sup>2</sup> pen because the increased space allows for longer fights and increased stress. The large errors associated with treatment effects in the space analysis make interpretation of results difficult. This large error is most likely explained by the sampling methods employed, with sows seeming agitated at times in the pens due to disruption of sows during monitor attachment. Attaching monitors to sows was difficult and often took longer than anticipated. Sows may have been more restless because they were mixed into pens two days before and had been mated the previous week as well as being moved out of stalls. The increased agitation of sows in pens meant more interaction during handling of sows, which may have led to greater variance. The variation may have also been caused by smaller numbers of animals in pen measurements. HF was the only HRV measure to show a trend towards a significant space effect in space which may indicate that the frequency domain parameters (HF and LF) are more sensitive parameters of HRV than the time domain (RMSSD and SDNN) parameters.

#### *Effects of Housing on Saliva Cortisol Concentration*

A difference in saliva cortisol concentration between the three housing treatments was established with sows sampled in group pens having higher salivary cortisol concentrations than when they were sampled in stalls or the ecoshelter. This increase in cortisol concentration occurred two days after the initial stall measure and this increase may be indicative of reduced welfare with an over 40% increase in individual sow cortisol levels (Cronin *et al.*, 1991). In the ecoshelter, sows had a lower cortisol level than when they were housed in pens, indicating decreased stress which may have been because they have increased space to escape more dominant sows, have already established a strong hierarchy or it may be due to a pregnancy effect (Parent *et al.*, 2012).

### *Effects of Space on Saliva Cortisol Concentration*

Saliva cortisol concentrations differed between dominance hierarchies under group housing. Sows that were classed as sub-dominant had an increased saliva cortisol concentrations compared to sows classified as submissive or dominant. An increase in cortisol in the sub-dominant sows demonstrates that these sows were more stressed during their time in mixing pens. Fighting to constantly maintain their position meant that these sows were experiencing an increased HPA axis response to cope with stress, resulting in an increased cortisol level (Koolhaas, 2011).

### *Effects of Housing on Injury Score*

Injury scores were also significantly higher in pens than in stalls or an ecoshelter. This result was expected because when sows were housed in individual stalls they were unable to interact and fight. However, in mixing pens, sows were closely housed together which lead to fighting for the establishment of dominance hierarchies (Seguin *et al.*, 2006). In the ecoshelter, injury scores were also reduced, which may be because sows had been in the shelter for a longer period of time and already had established hierarchies, or because pregnancy lowered stress thresholds reducing aggression between sows (Parent *et al.*, 2012).

### *Effects of Social Hierarchy on Injury Score*

Sows classed as submissive tended to have higher injury scores than sub-dominant or dominant sows. This differs from the salivary cortisol result, which indicated sub-dominant sows were more stressed. The lower ranking sows were likely to have a higher number of injuries because they were being bitten by higher ranking sows during the initial establishment of a hierarchy at mixing (Seguin *et al.*, 2006). Submissive sows were also likely attacked by other sows during fights over space and food access, leading to an increased injury score.

### *HRV and Saliva Cortisol*

One aim of this study was to correlate HRV with other measures of stress, such as saliva cortisol concentration. This was successfully achieved with a significant correlation identified between log LF and log saliva cortisol concentration. This positive relationship indicates that as cortisol increases in response to the influence to the HPA axis, the influence of the sympathetic nervous system also increases, as indicated by LF (Medicore, 2013). This is an important result as it shows that LF is acting as a reliable indicator of stress responses. The other HRV parameters did not show a significant correlation with saliva cortisol which may also indicate that LF is a more sensitive HRV measure and able to detect HRV easier (as discussed earlier).

### *HRV and Injury Score*

The relationship between HRV and injury score was also measured during the study. A significant positive correlation was identified between log LF and injury score and as with the saliva correlation, this correlation indicated an increase in sympathetic dominance as injury score increases. This relationship showed that the more scratches and aggression a sow is involved in, the more stressed they become. Aggression has previously been shown to result in stress as sows are being attacked and unable to escape more dominant sow aggression, resulting in stress in these sows (Karlen *et al.* 2007).

### *HRV, Saliva Cortisol and Injury Score*

The main aim of this study was to determine if a difference in HRV between sows housed in stalls, group pens or an ecoshelter could be established and use HRV as a possible indicator of stress under these housing conditions. The LF as an indicator of HRV measure was able to detect a significant difference in stress level between housing treatments. Saliva cortisol concentration and injury scores also indicated a significant difference between housing treatments. All of these measures were increased when sows were mixed into group housing,

indicating a higher stress response. The significant result for all three stress parameters is important as results are in the same anticipated direction, confirming HRV measures, and more specifically LF variation, can detect an acute stress response in sows.

The results for social hierarchy were conflicting and difficult to interpret. Although not significant, heart rate and injury score showed a trend towards submissive animals experiencing higher stress levels. However, saliva cortisol concentration was higher in subdominant sows, suggesting that these animals were most stressed. The higher injury scores of the submissive sows may have been caused by the aggressive interactions they were involved in during the initial mixing period (the day before sampling), but as a hierarchy was established, become much less involved lowering their salivary cortisol levels. However, the subdominant animals may continue to fight over a longer period, continuing the elevation of cortisol. It may therefore be of interest to examine the relationship between cortisol concentrations, injury score and social hierarchy over a longer time period. These results may mean that the saliva cortisol concentration is a more reliable indicator of stress under short term conditions of group housing.

#### *Future Research*

From all the HRV parameters, only LF and HF differed with any effects examined in the present investigation. This may be due to these measures being a more sensitive measure of HRV and detecting changes in variation at a neurological level. In this study there were large variations between measures which made it more difficult to detect significance. This is probably due to the methodology and sampling technique, with sows being handled and measurements interfered with by handlers and other pigs. It is probably not due to the number of animals because a power calculation was performed prior to the study and even though there were less animal results than planned for, the sample size was larger than in other pig studies which had lower variation (Marchant-Forde and Marchant-Forde, 2004). These errors

in sampling could be improved by using implantable, internal monitoring devices where sow ECG's are recorded without interference, thereby reducing errors and variation.

## **Conclusion**

The aim of the current investigation was to determine if HRV could detect the stress response commonly observed in sows under different housing, social and space allowance conditions. Indeed, some HRV measures (specifically LF and HF) did respond as expected, and relationships between these measures and other stress indicators (cortisol and level of injury) were identified. Variation in the HRV measures was larger than expected. This is thought to be attributed to sampling error rather than insufficient animal numbers, and could be addressed through implantable telemetry. Given the strong agreement with HRV and other stress measures, future investigations should focus on the use of heart telemetry to monitor sow welfare.

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