The effect of parity on salivary cortisol in sows in their transition from group housing to farrowing crates

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Preface

In front of you lies the bachelor thesis "The effect of parity on salivary cortisol in sows in their transition from group housing to farrowing crates" of Marije de Haan. This bachelor thesis is written to complete the study Biology, Nutrition and Health at Aeres University of Applied Sciences in Almere.

The research was conducted at the Department of Farm Animal Health of the Faculty of Veterinary Medicine at Utrecht University. The research question was formulated together with Sanne Roelofs.

Several people contributed to the completion of this bachelor thesis. First, I would like to thank Rebecca Nordquist for giving me the opportunity to do research at the Behaviour and Welfare Research Group at Utrecht University. Second, I would like to thank Sanne Roelofs for her time, input and support during this study. In addition, I would like to thank Maaike Cox from Aeres University of Applied Sciences for her help and feedback and my fellow students for their peer reviews. Finally, I would like to thank the animal caretakers Jan-Adriaan den Hertog, Jan van Mourik and Dirk van der Heide at the Tolakker for helping me with the collection of the data for this study and Christine Oei for performing the lab work.

I hope you enjoy reading this thesis.

Marije de Haan

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Summary

In the Netherlands, about 1.2 million breeding sows are kept for about 3.5 years (eight to nine pregnancy cycles) on average in farming practice. These sows may be exposed to several stressors. With the increasing interest in pig welfare, it is important to investigate these stressors so adjustments in pig farming can be made and pig welfare can be improved.

In this study, the effect of the transition from group housing to the farrowing crates on salivary cortisol is studied and the hypothesis that younger sows with a lower parity would experience this event as more stressful is tested. The transition is divided into two parts. First, selected sows were separated from the main group and housed in a temporary separation pen for a maximum of 17 hours. Second, the sows were showered, weighed and moved to the farrowing crates. For this study, a total of 52 sows were tested. Every week, a group of six to nine sows were sampled over a period of eight weeks. Each sow was sampled once in the group housing two weeks before expected parturition (serving as the baseline cortisol level) and twice on the day of relocating, one week before expected parturition: in the separation pen and in the farrowing crates, after showering and weighing.

The results did not fully confirm the hypothesis that parity affects the acute stress response in the transition from group housing to the farrowing crates. In the transition from group housing to the separation pen, the cortisol levels increased with both a significant effect of parity ($P=0.0426$) and the sampling moment ($P<0.0001$). Unexpectedly, the cortisol levels in the transition from the separation pen to the farrowing crates for both low parity and high parity sows dropped, almost back to baseline level with a significant effect of sampling moment ($P<0.0001$). Parity did not affect these cortisol levels. This outcome suggests that the transition from group housing to the separation pen resulted in an acute stress response. Further research has to be done to investigate what specifically caused this stress response (e.g. social stress caused by changes in group composition or stress caused by changes in the environment), so practical adjustments such as hiding areas and straw-bedding in the temporary pen can be made to reduce stress and improve pig welfare.

Samenvatting

In Nederland worden ongeveer 1.2 miljoen fokzeugen gemiddeld 3.5 jaar (acht tot negen zwangerschapscycli) in fokzeugbedrijven gehouden. Deze zeugen worden blootgesteld aan meerdere stressoren. Vanwege de toenemende interesse in het welzijn van varkens is het van belang om deze stressoren te onderzoeken, zodat er aanpassingen gemaakt kunnen worden in varkenshouderijen om daarmee het welzijn van de varkens te bevorderen.

In dit onderzoek is het effect van de transitie van de groepshuisvesting naar de kraamstallen op cortisol in het speeksel onderzocht en de hypothese dat zeugen met een lagere pariteit deze overgang als stressvoller ervaren getest. De transitie vanuit de groepshuisvesting naar de kraamstallen bestaat uit twee onderdelen. Eerst werden de zeugen gescheiden vanuit de groepshuisvesting, waarna ze tijdelijk in een separatiehok verbleven (maximaal 17 uur). Vanuit dit hok werden de zeugen naar een kleine ruimte gebracht waar de zeugen werden schoongemaakt. Na het schoonmaken werden de zeugen gewogen en verplaatst naar de kraamstallen. In totaal zijn er 52 zeugen getest. Wekelijks werd een groep van zes tot negen zeugen gesampled, in een periode van acht weken. Elke zeug is één keer gesampled in de groepshuisvesting (dienend als baseline) twee weken voor de verwachte partus en twee keer op de dag van de transitie, één week voor de verwachte partus: in het separatiehok en na het schoonmaken en douchen in de kraamstallen.

De resultaten kwamen niet volledig overeen met de hypothese dat pariteit effect heeft op de acute stressreactie in de transitie vanuit de groepshuisvesting naar de kraamstallen. De cortisollevels namen toe in de transitie van de groepshuisvesting naar het separatiehok. Zowel pariteit ($P=0.0426$) als het moment waarop de sample is genomen ($P<0.0001$) gaven een significant effect op het speekselcortisol. Onverwacht daalden de cortisolniveaus bijna terug naar baseline niveau in de transitie van het separatiehok naar de kraamstallen voor zowel zeugen met een lage als hoge pariteit met een significant effect van het moment van samplen ($P<0,0001$). Pariteit had geen effect op de cortisolniveaus. Deze uitkomst suggereert dat de transitie van groepshuisvesting naar het separatiehok heeft geresulteerd in een acute stressreactie. Verder onderzoek zal uitgevoerd moeten worden om uit te zoeken wat specifiek heeft geleid tot deze acute stressreactie (bijvoorbeeld sociale stress veroorzaakt door veranderingen in de groepssamenstelling of stress veroorzaakt door veranderingen in de omgeving), zodat praktische aanpassingen zoals schuilplekken en stro in het separatiehok gemaakt kunnen worden om stress te verminderen en het welzijn van de varkens te verbeteren.

1. Introduction

Approximately 12.4 million pigs are kept in commercial farming practice in the Netherlands (CBS, 2017). This number is fairly stable every year due to production rights. The population of pigs consists of breeding pigs, piglets and fattening pigs. A total of approximately 1.2 million of these 12.4 million pigs are breeding pigs. Breeding sows are staying in farming practice for approximately 3,5 years (eight to nine cycles) on average. With that, compared to the other sorts of domestic pigs, breeding sows spend the most time in farming practice. The Netherlands produced about 3.8 billion kilograms of meat in the year 2016. About 40 percent from this total amount was pork (CBS, 2017). Pig farming is thus an important sector in the Dutch economy. At the same time, the interest in welfare and living conditions among consumers and in politics is increasing. For example, since the forming of the new cabinet in the Netherlands in 2017, the inspection by welfare assessors in the pig industry has been tightened due to filmed abuse scandals in slaughterhouses and large barn fires. The aim of this tightened inspection is to improve animal welfare and to limit health and environmental risks (Rutte, 2017). Because of the increase in interest in pig welfare and the fact that breeding sows have a relatively long life in the breeding companies, the welfare of these sows is an important issue.

Although there is an increasing interest in animal welfare, the definition of it is rather complex and thus often debated. In addition, animal welfare is perceived differently in different societies. The definition of animal welfare in society is determined by the moral and ethical standards in society (Ohl & van der Staay, 2012). As a result, different assessment methods are being used to monitor animal welfare (Botreau, Veissier, Butterworth, Bracke & Keeling, 2007). In an attempt to scientifically define animal welfare, the Brambell Committee defined animal welfare with the five freedoms, i.e. 1) freedom from hunger and thirst, 2) freedom from discomfort, 3) freedom from pain, injuries and diseases, 4) freedom to express normal behaviour and 5) freedom from fear and distress (Brambell, 1965). However, this definition has some limitations and since the definition of the Brambell Committee, various definitions and assessment methods have been developed (Fraser, 1995; Blokhuis, Jones, Geers, Miele & Veissier, 2003; Botreau, Veissier, Butterworth, Bracke & Keeling, 2007; Welfare Quality, 2009). Although there are a lot of definitions and assessment methods still, the (absence of) stress has always been a part of the definition. Currently, stress is directly measured in animals with physiological measurements serving as biomarkers to assess welfare (Gutiérrez, Escribano, Fuentes & Cerón, 2013; Hemsworth, Mellor, Cronin & Tillbrook, 2014).

The concept stress was first introduced and described by Hans Selye (Selye, 1950). He defined stress as a non-specific response of the body to external challenges. Stress reactions occur when the homeostasis of an animals is at risk (Veissier & Boissy, 2007; Einarsson, Brandt, Lundeheim, & Madej, 2008). These stress reactions are a repertoire of physiological and behavioural adaptive responses. Oxygen and nutrients are directed to the central nervous system and possible stressed body sites, heart rate and blood pressure are increased (physical adaptation) and alertness, cognition and attention are increased, while appetite and feeding behaviour and reproductive behaviour are suppressed (behavioural adaptation) (Chrousos & Gold, 1992; Habib, Gold & Chrousos, 2001; Chrousos, 2002; Charmandari, Tsigos & Chrousos, 2005). The response to stress is influenced by several factors. The response to stress depends on the nature of the stressor (for example, the duration and the intensity of the

stressful event), but individual differences can also trigger different stress reactions. For example, in the case of sows, personality, age and health can give a difference in stress response (Koolhaas et al, 1999).

The Hypothalamic-Pituitary-Adrenal (HPA) axis plays a key role in the stress response (Moberg & Mench, 2000; Smith & Vale, 2006; Koolhaas et al, 2011). Corticotropin-releasing factor (CRF) regulates the HPA axis by initiating a process, which results in the release of glucocorticoids from the adrenal cortex (Smith & Vale, 2006), resulting in secretion of steroid hormones from the adrenal gland (Moberg & Mench, 2000). Stress responses can therefore be assessed by determining the activation of the HPA axis, by measuring the secretion of glucocorticoids like cortisol in blood plasma, saliva, hair, milk, urine and feces (Cook, 2012; Casal, Manteca, Pena, Bassols & Fàbrega, 2017). The detection of corticosteroid hormones, especially cortisol, is most widely and frequently used as a biomarker of an animal's stress response (Hellhammer, Wüst, & Kudielka, 2009; Neethirajan, Tuteja, Huan & Kelton, 2017). While cortisol in blood, saliva and milk samples provide information about a short-term stress response, cortisol in urine and feces is accumulated in hours or days. Cortisol in hairs are accumulated over a period of weeks, and can therefore be used for measuring long-term stress. Measuring acute stress responses gives an indication of which specific parts of an animal's environment are a source of stress. That is why for instance salivary cortisol has an advantage over hair cortisol when assessing animal welfare, because long-term stress measurements do not provide information about which specific stressors have been responsible for an increased stress response. Measuring acute stress can therefore provide information with which adjustments can be made in the animal's environment to reduce stress and improve animal welfare.

Sows on breeding companies have to deal with multiple sources of stress. In the Netherlands, it is mandatory to group house sows instead of keeping sows in individual gestation crates. This has derived from welfare interests: sows are able to perform and express normal behaviour in the group housing, improving their welfare. The group housing also causes problems: the number of sows in the groups in which the sows are kept is much higher than the groups that are formed in the wild (Einarsson et al, 2008). As a result, sows form their own groups within the large group with a smaller number of sows, as they would form their groups with several sows and their offspring in the wild. Furthermore, in the wild, groups try to avoid other groups. This is nearly impossible for the sows in commercial group housing, resulting in aggressive behaviour (Spoolder, Geudeke, van der Peet-Schwering & Soede, 2009).

On average, a sow is inseminated 2,5 times per year. For the protection of the piglets and the animal caretakers, the sows are brought from the group housing to stalls where they are housed individually in farrowing crates, one week before the expected parturition. In most commercial group housing systems, on the day of relocation to the farrowing crates, the sows are cleaned first. After cleaning they are weighed, and from there they walk to the farrowing crates. This is where the sows stay during the lactation period, i.e. up to four weeks after the parturition. This transition can create a socially unstable environment, which could result in aggressive behaviour. When the sows are removed from the group and placed in a new group before moving to the farrowing crates, they are separated from their familiar group they formed within the large group, and placed with relatively unfamiliar sows. This new social

grouping in a limited space can result in aggressive behaviour (Soede et al, 2006). The transition to the farrowing crates can therefore be a stressful event for the sows.

Different studies focussing on (repeated) regrouping of pregnant sows show an increase in stress, aggressive behaviour and an impaired reproductive performance, early in the pregnancy (Turner, Hemsworth, & Tilbrook, 2005; Soede et al, 2006; Spoolder et al, 2009; Greenwood, Plush, van Wettere & Hughes 2014). The fighting and other aggressive behaviour which occurs due to regrouping results in a physiological stress response (Arey & Edwards, 1998; Soede et al, 2006; Coutellier et al, 2007). Soede et al (2006) describes an increase in cortisol levels just after the regrouping of primiparous (first-parity) sows. A day after regrouping the cortisol levels had returned to the levels before regrouping, indicating that the sows had an acute stress response, but did not develop any chronic stress response.

In the aforementioned study, the cortisol level in primiparous sows was measured right after mixing the groups. However, a review by Verdon et al (2015) suggested that the way in which the group is divided, in terms of the distribution of different parities, can have an effect on aggression and stress in the group (Verdon et al, 2015). Younger sows are more subordinate and are more likely to get injured due to aggression than older sows when placed in a new group (Li, Wang & Johnston, 2012). Older sows fight each other over dominance, but also express aggression towards the younger subordinate sows (Verdon et al, 2015). In a study by Li et al (2012) it was investigated whether aggression decreased when the groups are sorted based on parity: the results of the study indicated that primiparous sows had fewer injuries when they were put together instead of in a group with multiparous sows. Ison et al (2014) studied the physiological stress response in salivary cortisol of primiparous sows after mixing with multiparous sows. The primiparous sows in the mixed group with multiparous sows showed a much higher cortisol level than the primiparous sows from the control group, suggesting that the distribution of low parity and higher parity over groups affects the stress response in primiparous sows. Hoy et al (2009) substantiates that a higher parity is accompanied by a higher social rank in the hierarchy, but adds that this only applies till parity four: after this the weight of the sow plays the determining factor for the social rank, i.e.: the heavier the sow, the higher the social ranking (Hoy, Bauer, Borberg, Chonsch & Weirich, 2009). Furthermore, younger sows, partly due to their lower rank, often have a higher cortisol level because they sustain more injuries due to aggression of the dominant sows. In addition, the older dominant sows are able to choose the more preferable areas to lay down and have easier access to different recourses (Strawford, Li & Gonyou, 2008; Ison et al, 2014).

As shown in previous paragraphs, a lot of research has been done into the effects of both chronic and acute stress due to regrouping on reproduction performance of sows (Turner et al, 2005; Soede et al, 2006; Spoolder et al, 2009; Greenwood et al, 2014;). However, little is known about the acute stress in sows during transition from the group housing to the farrowing crates, a week before the expected parturition. This study focuses on the stress physiology in sows in their transition from group housing to the farrowing crates, taking into account the difference in parity. The research question that follows from this is:

What is the effect of parity on salivary cortisol in sows [Yorkshire x Dutch Landrace] in their transition from group housing to the farrowing crates?

It is expected that primiparous and second-parity sows will have a higher increase in cortisol because of their lower rank. In addition, primiparous sows may experience the transition as

more stressful because they have not experienced it before (complete novelty). This can also result in not fully cooperating with the animal caretakers, which may result in exerting extra pressure on the sows by the animal caretaker.

The goal of this study is to gain insight in the stress physiology of sows in their way to the farrowing crates, taking in consideration the difference in parity. With the outcomes, this study aims to establish a bridge between science and professional practice, so management can possibly be adjusted. For example, the planning of insemination could be adjusted to minimize the mixing of different parities in a group (i.e. primiparous and second-parity relocated together) or adjustments in the sow's environment could be made. Finally, this study aims to contribute to further research into stress in sows in their transition to the farrowing crates.

2. Materials and Methods

2.1 Animals and housing

The research was conducted at the breeding company "De Tolakker" at the Faculty of Veterinary Medicine. The company is also used for research purposes and education. For this research, a total of 52 sows [Yorkshire x Dutch Landrace] was tested. A group of six to nine sows was relocated to the farrowing crates every week over a period of eight weeks. The selection of sows was based on the program of the company, meaning that the sows that were available were tested.

About 230 sows are kept on De Tolakker. An average of 160 carrying sows are kept in loose housed groups, with straw bedded parts and electronic feed stations (Intellitek ESF, Fancom bv Panningen, the Netherlands). Also, the sows are able to go outside. The other sows are located in the farrowing crates or in the insemination stall. Water is available ad libitum; all sows were fed once daily.

The farrowing pens are partly slatted, 181 x 238 centimetres in total size and the crates within the pens are about 190 x 84 centimetres at its widest part (figure 1).

Figure 1 Sow in farrowing pen

2.2 Transition to farrowing crates

First, the sows were separated from the group a day before relocating to the farrowing crates. This did not happen at a fixed time, because it was determined by the electronic feeding system, which automatically separated the sows from the group. The time of separation varied per sow, depending on the time they entered the feeding station the day before moving. The sows stayed in this temporary separation pen for a maximum of 17 hours. On the day of relocation to the farrowing crates, the sows were divided into groups of three to seven, depending on the total group size, and put in the shower room (185 x 395 centimetres) by an animal caretaker and in some cases veterinary medicine students, to be cleaned. After that, the sows were weighed, and from there they walked to the farrowing crate.

2.3 Saliva collection

Per sow, a total of three saliva samples were taken: the first sample served as baseline and was therefore measured in the group housing, two weeks before the expected parturition (S1). The second and third sample were taken on the same day a week later, one week before expected parturition. The second sample was carried out just before showering, when the sows were separated from the group, but before relocating to the individual pens (S2). And finally, the third sample was performed just after cleaning, weighing and moving to the farrowing crates (S3). The transition from group housing to the separation pen was already in process at the start of data collection. To optimize the data collection, S2 and S3 were taken from the first group. Therefore, the baseline cortisol level of the first group is missing.

The goal was to take samples at fixed times, due to the circadian rhythm demonstrated in cortisol in pigs (Ekkel et al, 1996, Ruis et al, 1997). However, the times at which the pigs were showered and relocated also depended on the animal caretaker and veterinary students present, resulting in a small difference in the times at which the samples were taken. The total amount of time it took to take the samples also differed, depending on the group size. Saliva collection was carried out over eight weeks (week 42 to week 49) to come to a suitable sample size. Therefore, the test did take longer than the time that an individual sow was followed.

Saliva collection was conducted as described by van der Staay, Schoonderwoerd, Stadhouders & Nordquist (2015). The sows had to chew on cotton buds (Cotton Swabs 150 mm x 4mm WA 2PL; Heinz Herenz, Hamburg, Germany) until they were thoroughly moistened. After saliva was collected, the swabs were placed in special centrifuge tubes with inner cases (Salivette, Sarstedt, Germany) and were rapidly centrifuged (Sigma 4K10, supplier: Salm en Kipp bv, Breukelen, the Netherlands) at 3524 g for 10 minutes at 10 °C to obtain the saliva. The collected saliva was stored in tubes at -20 °C until cortisol concentration was measured by a Coat-a-Count radioimmunoassay, according to manufacturer's procedure (Coat-a-Count cortisol TKCO, Siemens Healthcare Diagnostics BV, The Hague, The Netherlands). All the collected samples were assayed on the same day.

2.4 Statistical analysis

For the statistical analysis, the difference between S1 and S2 (effect of social grouping = $\Delta 1$) and S2 and S3 (effect of showering and the transition to the farrowing crates = $\Delta 2$) was examined. The group of sows was divided into two groups: the primiparous and second-parity sows formed one group (n=20), and the sows with a higher parity formed the second group (n=32).

The effect of parity on average change in cortisol was analysed with a mixed model analysis of variance (ANOVA), with the fixed effects Parity and Sample Time and the random effect Subgroup, to account for effects of day of sampling and group composition during sampling.

3. Results

The average cortisol levels at the sample moments and changes in cortisol levels over time are shown in table 1. Since the first samples of the first group are missing, the total number of sows is less than the total number of sows used for measuring the average cortisol level at the second and third sample moment (respectively 18 for S1 for primiparous and second-parity sows compared to 20 for S2 and 27 for S1 compared to 32 for sows with a parity >2).

Table 1 Mean cortisol levels in ng/ml and mean cortisol changes in ng/ml over time

	Parity 1 & 2			Parity >2		
	x̄	**Sd**	**SE**	**x̄**	**Sd**	**SE**
Cort.S1 (n=18, n=27)	4.95	5.42	1.28	2.04	0.82	0.16
Cort.S2 (n=20, n=32)	15.84	18.35	4.10	15.43	18.40	3.25
Cort.S3 (n=20, n=32)	3.78	2.66	0.60	2.68	2.00	0.35
Δ cort. 1&2	6.36			10.88		
Δ cort. 2&3	-12.06			-12.74		

3.1 Effect of separation from group

The average cortisol level in the group housing is higher for primiparous and second-parity sows (4.95 compared to 2.04 for sows with a higher parity). However, the average cortisol levels at the second sample moment are relatively close to each other, resulting in a bigger increase in cortisol over time for sows with a parity >2, as shown in figure 2.

Both the parity and the moment of sampling have a significant effect on cortisol ($P < 0.05$). At the second sample moment, saliva cortisol is increased for both groups of sows compared to the first sample moment ($F_{1.88} = 74.35$, $P < 0.001$). The parity of the sows affected the cortisol levels significantly ($F_{1,88} = 4.23$, $P = 0.0426$).

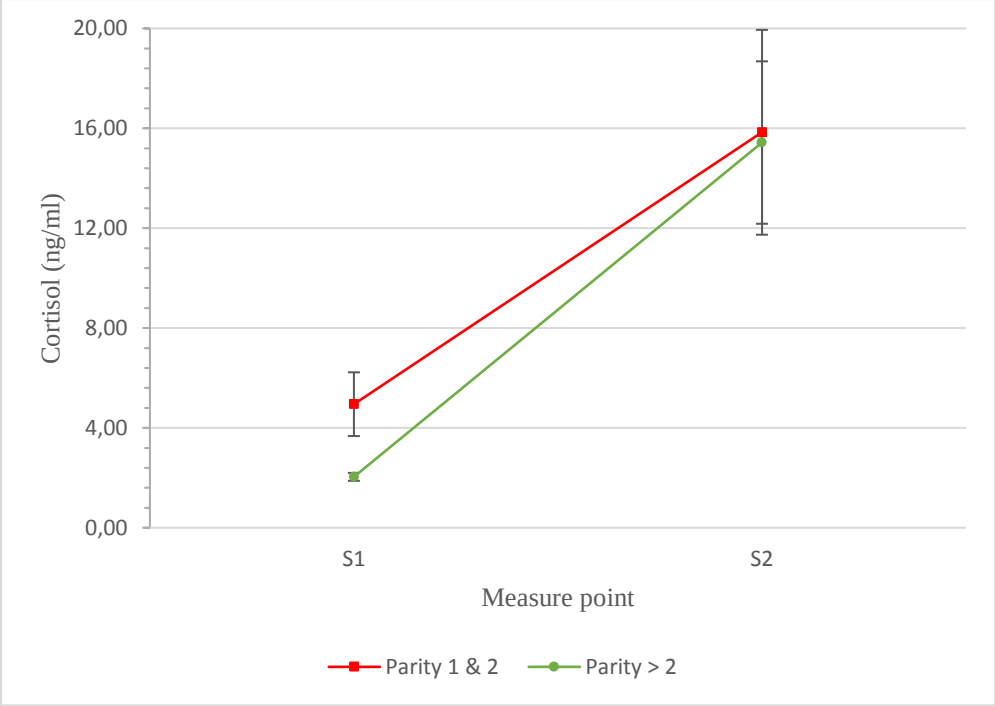

Figure 2 Mean levels of cortisol (± SEM) in the group housing (S1) and after being separated from the group (S2).

3.2 Effect of transition to the farrowing crates

There is a significant effect of the moment at which the sample was taken ($F_{1.95} = 77.26$, $P < 0.001$), i.e. the effect of the showering, weighing and transition to the farrowing crates.

The difference in the average cortisol level between the low parity sows and high parity sows before showering is very small, i.e. 15.84 ng/ml for sows with a low parity and 15.43 ng/ml for sows with a higher parity. The same goes for the difference between the low parity and high parity sows in the mean decrease after showering and weighing, i.e. 12.06 ng/ml for the primiparous and second-parity sows and 12.74 ng/ml for the sows with a higher parity (as shown in figure 2). There is no significant effect of the parity.

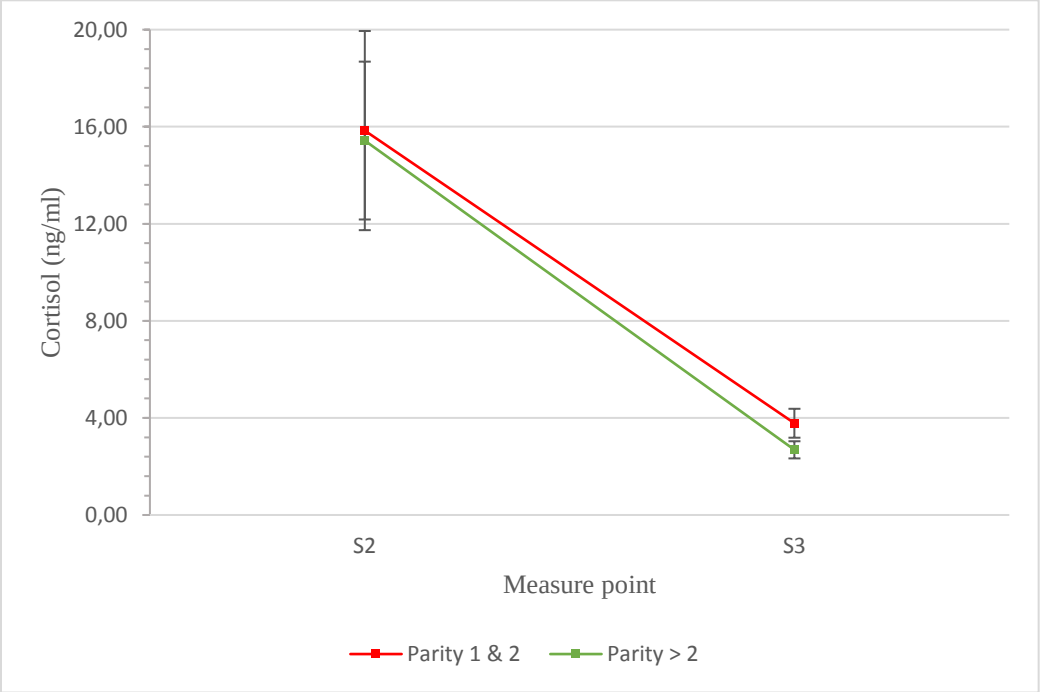

Figure 3 Mean levels of cortisol (± SEM) before the transition to the farrowing crates (S2) and after being showered and weighed (S3).

The standard deviation and the standard error of the mean show that the dispersion of the cortisol level in the separation pen is much greater than the dispersion of the cortisol levels in group housing and after moving to the farrowing crates, as shown in table 1 and figure 2 and 3. The measurements of the samples taken in the separation pen give a standard deviation and standard error of respectively 18.35 and 4,10 for low parity sows and 18,40 and 3,25 for high parity sows compared to the 5.42 (Sd) and 1,28 (SE) for low parity sows and 0,82 (Sd) and 0,16 (SE) for high parity sows in the group housing and 2.66 (Sd) and 0.60 (SE) for low parity sows and 2,00 (Sd) and 0,35 for high parity sows after moving to the farrowing crates.

4. Discussion

The aim of this research is to describe the effect of parity on the stress physiology of sows in the transition from group housing to the farrowing crates. This transition is divided into two parts and stress responses are measured in salivary cortisol: the transition from the group housing to a smaller pen where the sows are separated from the group, and the transition from this pen to the farrowing crates, between which the sows are showered and weighed. When comparing the low parity (1 & 2) and high parity (>2) sows, it was predicted that the sows with a lower parity would experience both transitions as more stressful than the sows with a higher parity. Furthermore, it was predicted that both transitions would result in an increase in cortisol for both sows with a low and a high parity.

The most notable results from this study are an increase in cortisol from the group housing to group separation and a decrease from group separation to the farrowing crates. In the first transition, there is both a significant effect of parity and the sampling moment, at the second transition there is only a significant effect of the sample moment.

The transition from group housing to group separation shows both a significant effect of the parity ($P=0.0426$) and the moment at which the sample was taken ($P<0.0001$). This is in accordance with both the first and second prediction. Although the sows with a lower parity have higher cortisol levels in the group housing, the mean increase in cortisol is bigger for sows with a higher parity. Also, the average cortisol level of the older sows (with a higher parity) never exceeds the average cortisol level of the sows with a lower parity. However, the age of the sows can play a role in this effect (Ruis et al, 1997), i.e.: the baseline cortisol level decreases when pigs get older. This can explain the significant difference. In addition, the cortisol levels in the separation pen are much more dispersed, indicating that the stress response varies a lot per individual. This can be caused by personality (Ruis et al, 2000). The large increase in cortisol suggests that being separated from the group is experienced as a stressful event for pregnant sows. This corresponds with the literature, which demonstrates that the regrouping of sows results in social stress (Turner, Hemsworth, & Tilbrook, 2005; Soede et al, 2006; Spoolder et al, 2009; Greenwood, Plush, van Wettere & Hughes 2014). However, not only a change in group composition can contribute to the stress reaction of the sows, also the effect of a different and novel environment and the lack of control can cause this increase in salivary cortisol (de Jong et al, 1998; Broom, 2008). The separation pen in which the sows have to wait before being showered, weighed and relocated is bare floored, smaller (in absolute measurements) than the group housing and does not have access to straw-bedded areas and the outside area.

Since acute stress was measured the morning after the sows were separated from the group, the effect of surprise can be eliminated as the cause of an increase in cortisol. In addition, the sows are not handled by animal caretakers when they are staying in the separation pen, nor when they are being relocated from the group housing to the separation pen, since the sows are separated by the electronic feed station. Therefore, the effect of human-animal interactions can also be eliminated as a potential stressor. As described above, a change in group composition could lead to stress responses in sows (Turner, Hemsworth, & Tilbrook, 2005; Soede et al, 2006; Spoolder et al, 2009; Greenwood, Plush, van Wettere & Hughes 2014; Ott et al, 2014). Unfortunately, these studies did not describe absolute salivary cortisol data, making it impossible to compare the data and cortisol increases in recent data. Therefore, no

statement can be made about the contribution of the change in group composition to the absolute cortisol increase. However, Coutellier et al (2007) studied the effect of regrouping and relocating on salivary cortisol during the growing-finishing period in fattening pigs. The treatment groups (which were mixed and relocated) showed an average salivary cortisol level of approximately 20 ng/ml after the second regrouping and relocating compared to the average cortisol level of approximately 8 ng/ml in the control group. After the twelfth regrouping and relocation, the cortisol levels of the treatment groups dropped back to 9.04 ± 1.04 ng/ml, compared to the 5.83 ± 0.62 ng/ml of the control group, so the cortisol level and the difference between the treatment group and control group reduced after several regrouping and relocating sessions (Coutellier et al, 2007). When comparing these outcomes to the current study, it can be suggested that the regrouping and relocating of the sows contributed to the acute stress response. However, the average baseline cortisol level measured in the study by Couttelier et al in the habituation period was already higher for the treatment group compared to the control group (respectively approximately 14 and 10 ng/ml). Furthermore, other elements such as housing, provision of straw, difference in routine, breed and animal caretakers can affect differences in cortisol levels in the different studies.

The novel environment and the lack of straw could also have contributed to the acute stress response (de Jong et al, 1998; Tuyttens, 2005; Day, Van de Weerd & Edwards, 2008). Further research has to be done to investigate what elements specifically contribute to the acute stress response measured in salivary cortisol in the separation pen (e.g. social stress caused by being separated from the original group and the change in group composition or stress caused by changes in the environment).

The transition from group separation to the farrowing crates only shows a significant effect of the moment at which the sample was taken (P<0,0001). The average cortisol level drops, almost back to the average cortisol level at the first sample moment. This is not in accordance with the formulated prediction, since an increase in cortisol and a significant effect of parity was expected. In addition, these results are contrary to previous studies, in which was suggested that the way in which groups are divided, in terms of distribution of different parities, can affect aggression and stress in the group (Verdon et al, 2015). Because the sows are put together in a small room when they are cleaned, it was expected that sows with a lower parity would experience this event as more stressful. Younger sows experience more stress because they are lower in rank and thus often the target of aggression (Li, Wang & Johnston, 2012; Verdon et al, 2015). Ison et al (2014) also describes the effect of mixing primiparous sows with multiparous sows on the physiological stress response. The study showed that primiparous sows had a much higher salivary cortisol level when compared to the control group (Ison et al, 2014). It is not clear what caused the contrast in results in current study with the literature. In mentioned studies, hierarchy and aggression are important factors in the stress response. Therefore, the degree of aggression and the hierarchy in the group of sows in the transition to the farrowing crates should be further investigated using behavioural observations.

Unfortunately, the total number of tested primiparous sows is relatively low (7 of a total of 52 sows), resulting in the combination of primiparous and second-parity sows. The average cortisol levels of the primiparous sows are higher at all measuring points compared to the average of the group (partly caused by age, as cited above). But this can also be caused by personality, since there is a huge outlier. Because of the small group size, no statement can be

made about this. It could therefore be interesting to further investigate effect of parity om the physiological stress in sows in the transition to the farrowing crates, with a larger group of primiparous sows.

To conclude, although there is a significant effect of the parity as well as the moment of sampling on the first transition from group housing to group separation, this is not visible in the average increase in saliva cortisol and can be explained by the difference in age, with younger sows having a higher cortisol in general. In the second transition, the cortisol level is only affected by the sampling time, the parity did not affect salivary cortisol. The cortisol levels in the second transition dropped, almost back to baseline level. The most notable outcome of the study is therefore acute stress response caused by separation of the group housing in a temporary pen, measured in salivary cortisol. Further studies must be conducted to investigate what specific part of the separation caused this acute stress response.

5. Recommendation for future research

Overall, the study showed that stress mainly increased with the separation of the selected group from the group housing. What specifically caused this acute stress response should be investigated. To improve the welfare of the sows, several follow-up studies on adjustments in the separation pen can be performed to find a practical solution to reduce the stress in sows.

5.1 Hiding areas

Separation from the group leads to a stressful situation for the sows. Furthermore, the regrouping of sows can lead to aggressive behaviour (Turner et al, 2005; Soede et al, 2006; Spoolder et al, 2009; Greenwood et al, 2014). The fighting and other aggressive behaviour results in a physiological stress response (Arey & Edwards, 1998; Soede et al, 2006). In order to make it less stressful for sows with a lower rank and to reduce aggressive behaviour, it can be effective to create hiding areas in the pen where the sows temporarily stay before relocating to the farrowing crates.

Bulens et al (2017) studied the effect of hiding walls on the behaviour and performance of fattening pigs (Bulens, Van Beirendonck, Van Thielen, Buys & Driessen, 2017). In this study, T-shaped hiding walls were placed in the pen. Behavioural observations were conducted and skin lesions were examined. The aggressive behaviour did not increase in comparison to the control group, but the T-shaped walls were associated with a reduction of stress levels in general, because less pen manipulation and less belly nosing was observed. To investigate this further, a study on the effect of hiding walls or hiding areas on salivary cortisol in sows could be performed. Also, the results from the observations showed a higher percentage of lying behaviour in the pens without the hiding walls, which suggests that the walls might interfere with the lying behaviour of the sows from the treatment group. Thus, the location of the walls is important (Bulens et al., 2017, Bulens, van Beirendonck, Van Thielen, Buys & Driessen, 2017) and the optimal location could be investigated.

5.2 Straw-bedding

When the selected sows are separated from the group, they leave the group housing with access to large straw-bedded areas and an outside area to move to a smaller, bare floored pen. So, there is not only a major transition in group composition, but also in environment. In addition, several studies have shown the importance of the provision of straw for pig welfare (Ekkel, Spoolder, Hulsegge & Hopster, 2003; Tuyttens, Wouters, Duchateua & Sonck, 2004; Tuyttens, 2005; Day, Van de Weerd & Edwards, 2008).

First of all, the provision of and the access to straw-bedded areas is associated with better comfort (Tuyttens, 2005). Due to the fact that sows spend the majority of the time lying down (Ekkel, Spoolder, Hulsegge & Hopster, 2003), it is important that they can do so comfortably. Because straw is expensive, costs a lot of extra work and the hygiene is worse compared to bare floors, it is important to look for alternatives for smaller pens like the pen where the sows are located before moving to the farrowing crates. One of these studied alternatives is the synthetic mattress. Mattresses like these have already been used for cattle (Tuyttens, Wouters, Duchateau & Sonck, 2004). In this study, the preference of sows between the mattresses and the bare concrete floor is investigated. The results of the study suggest that the mattresses improved the lying comfort of the sows. However, research has to be done into the durability of the mattresses and whether the provision of mattresses have long-term effects on health and the ambient temperature.

In addition to the provision of straw affecting the lying comfort, it has several effects on the behaviour of pigs. For instance: the presence of straw reduces the frequency of manipulative social behaviour such as aggressive behaviour, nosing other pigs and tail biting (Day, Van de Weerd & Edwards, 2008). The presence of straw provides the pigs stimuli for explorative behaviour, leading to a reduction in undesirable behaviour (Tuyttens, 2005). This may cause the sows to perform less aggressive behaviour towards each other.

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When you have checked your report using this checklist, you must add this to your report as an appendix. No form means no mark.
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- ☐ Is a concise version of the entire report
- ☐ Contains conclusions
- ☐ Does not contain personal opinions
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- ☐ Is chapter 1*
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9. The (construction of the) core:
- ☐ Chapters, paragraphs and subparagraphs are numbered and clearly structured (with a maximum of three levels) *
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