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The effect of rubber slat mats on cortisol concentrations in stall-housed gilts

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ABSTRACT

It has been established that rubber mats improve lying comfort in stall-housed gilts at low ambient temperatures. The present study investigated whether rubber mats influence stress levels in gilts by improved lying comfort. The study was performed in a commercial pig farm service unit, during the 28-day breeding cycles in autumn and winter. During both cycles, the control and experimental groups of gilts (9 gilts each) were housed in gestation stalls with a concrete slatted floor; in the experimental groups, the floor was additionally coated with adjusted 2-cm thick rubber mat. Stress level was assessed by determination of cortisol concentrations in gilt serum on days 1, 8, 15 and 28 of each cycle, when the postural behaviour of gilts was observed. In both groups of gilts, serum cortisol was significantly lower ($P<0.05$) on day 28 as compared with day 1 in both breeding cycles. However, on day 28, significantly lower ($P<0.05$) serum cortisol was measured in the experimental group as compared with the control group. There was a negative correlation between cortisol concentrations and the time that experimental gilts spent lying in total, and lying laterally ($r = -0.46$ and $r = -0.52$, respectively, $P<0.05$) and a positive correlation between cortisol concentrations and time spent standing (r

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= 0.43, P<0.05). In the control group, there was no significant correlation between serum cortisol concentrations and the postures observed, except for sitting (r = 0.55, P<0.05), but a correlation was recorded between cortisol concentrations and posture changing (r = 0.33, P<0.05). In conclusion, the use of rubber mats proved to be an efficient management tool to reduce stress levels in gilts by improving their lying comfort.

Key words: pig, flooring, rubber mat, stress, cortisol

Introduction

Stress is a biological response elicited when an individual perceives a threat to its homeostasis (MOBERG, 2000). If the intensity and duration of the stressor action exceed the body's adaptive potential, a phase of exhaustion occurs. In addition to susceptibility to infections, this phase is frequently associated with the development of metabolic diseases and behavioural impairments, and even the death of the animal (VUČINIĆ, 2006). The response to stress depends on the type, intensity and duration of stressor action, as well as on the metabolic and health status, age and sexual maturity of the animal (EINARSSON et al., 2008). It is also influenced by individual differences (KEELING and JENSEN, 2014) and previous experience (VON BORELL, 2001). In addition, pigs can be genetically predisposed to stress susceptibility. According to MARPLE and CASSENS (1973), the cortisol turnover rate in the plasma of stress-susceptible pigs is threefold that recorded in normal pigs.

In pigs, stress is often assessed by cortisol concentration, the blood level of which shows circadian variations, being highest in the morning and lowest at night. Circadian cortisol variations become perceivable during the period of the pig's sexual maturation, at the age of 15 weeks, and become persistent after 20 weeks of age (SKARLANDTOVÁ et al., 2011).

Stress *per se* need not have adverse effects on animal welfare, which can be disturbed even when the signs of stress are not perceivable (VON BORELL, 2001). Generally, many difficulties are encountered in the assessment and comparison of various types of stressors and their influence on animal welfare, in particular regarding their prolonged actions (EINARSSON et al., 2008).

Modern artificial conditions in intensive pig production may act on animals as stressors, especially in the case of tight space restriction, as in breeding females housed in gestation stalls (BARNETT et al., 1989; PAVIČIĆ, 1997; ESTIENNE et al., 2006; VUČEMILO et al., 2011), with special emphasis on gilts because this is their first experience of individual housing. The very construction of the stall may contribute to stress levels in breeding females (BARNETT et al., 1991; BERGERON et al., 1996), as well as the type of stall flooring and/or bedding. In commercial pig production, breeding females are mostly kept on a slatted or semi-slatted concrete floor. These floor types are advantageous in terms of durability, hygiene and human resources, but offer poor comfort to the animals (TUYTTENS, 2005; TUYTTENS et al., 2008).

Rubber mats have not yet been widely used in pig production. However, studies of their effect on breeding female welfare show favourable results, including improved lying comfort, and sows and gilts find them preferable at low environmental temperatures (TUYTTENS et al., 2008; ELMORE et al., 2010; PAVIČIĆ et al., 2014; OSTOVIĆ et al., 2015). Previous studies have mostly focused on lactating (GRAVÅS, 1979; BOYLE et al., 2000; FARMER et al., 2006; ZURBRIGG, 2006; DEVILLERS and FARMER, 2008) and group-housed pregnant sows (TUYTTENS et al., 2008; ELMORE et al., 2010; CALDERÓN DÍAZ et al., 2013; CALDERÓN DÍAZ and BOYLE, 2014; BOS et al., 2016).

The aim of the present study was to assess whether rubber mats influence cortisol concentrations, as a stress indicator, in stall-housed gilts by offering improved lying comfort.

Materials and methods

The study was performed in commercial production conditions, at a pig farm service unit during the breeding cycles in the autumn (November) and winter (December-January). In each season, the breeding cycle lasted 28 days, which is the period for which breeding females in pig production may be kept in individual stalls after service, according to the COUNCIL DIRECTIVE 2008/120/EC (2008).

The study included 36 gilts of the Large White breed. Of these, 18 gilts were randomly divided into control and experimental groups (n = 9 gilts each) in the autumn breeding cycle. The same procedure was repeated with the remaining 18 gilts in the winter breeding cycle. The control groups of gilts were housed in 1.80×0.60 m gestation stalls with a concrete slatted floor, with 80-mm wide slats and 20-mm gaps. In the experimental groups of gilts, the stall floor was additionally covered with a 2-cm thick textured rubber mat (Gumiimpex-GRP Inc., Croatia), adjusted to the stall size and floor gaps. To ensure that all study gilts entered the service unit at the same time, oestrus synchronization and insemination were previously performed.

In the service unit, the gilts are fed with concentrated feed twice a day from the same troughs used for water supply *ad libitum*. The housing has natural and at least 8-hour artificial illumination and forced ventilation. During cooler months, there is no heating, but appropriate microclimate conditions are ensured by reduced ventilation.

The level of stress was assessed by determination of serum cortisol concentration (μg/dL) in the control and experimental groups of gilts on days 1, 8, 15 and 28 of each cycle. Blood was drawn by the same veterinarian, assisted by the same technicians between 12.30 and 13.00 PM. The gilts were restrained using nose snares, and 5 mL of blood was taken from the *cranial vena cava* into tubes without additives and gel for separation of corpuscular blood elements, in order to avoid their possible interference. Blood sampling was performed according to the principles of aseptic procedure. Upon completion of clotting, blood samples were centrifuged at 1200 g for 10 minutes, and the serum was aliquoted and stored at -20 ºC until analysis. A 100-μL serum aliquot was used to determine cortisol concentration. Measurements were performed on an Immulite 100 autoanalyser (Siemens, Germany) using a competitive chemiluminescent immunoenzymatic assay. Prior to measurements, the manufacturer's calibration curve was adjusted to the reagent used for the measurement, and measurement quality was tested using the standard control sera in low, medium and high concentrations.

Prior to blood sampling, the air temperature in the service unit was measured by a portable digital instrument (Testo Inc., Germany), and the postural behaviour of the gilts was monitored over a 4-hour period using video cameras (Toshiba Camileo P30, China), as previously described (PAVIČIĆ et al., 2014).

All experimental procedures were approved by the Ethics Committee of the Faculty of Veterinary Medicine in Zagreb and the Veterinary Directorate of the Ministry of Agriculture, Republic of Croatia.

Data were statistically processed and analysed by the Statistica v.12 software (StatSoft Inc., 2014). Distribution of the cortisol concentration indicator was tested by the distribution fitting test, which showed that it followed a normal distribution. The effect of factors (group, cycle and day) and their interactions on cortisol concentration (dependent variable) were tested by the Generalized Linear Model - Factorial ANOVA. Factorial analysis of variance was employed to test differences in cortisol concentrations between the control and experimental groups during the days of observation within a particular cycle. The same test was also used to determine differences within a group between different days of the same cycle. Linear correlation was used to test the association of cortisol concentration with air temperature in the service unit, length of holding particular postures, and the number of posture changes. The level of significance was set at $P<0.05$.

Results

The results of serum cortisol concentrations in the control and experimental groups of gilts during the breeding cycles under study are shown in Fig. 1, and the correlations of cortisol concentrations with service unit air temperature, duration of posture and number of posture changes in gilts, in Table 1. The air temperature in the service unit decreased continuously over the study days of both breeding cycles, ranging from 17.5 to 15.5 °C in the autumn cycle, and from 17.4 to 15 °C in the winter cycle.

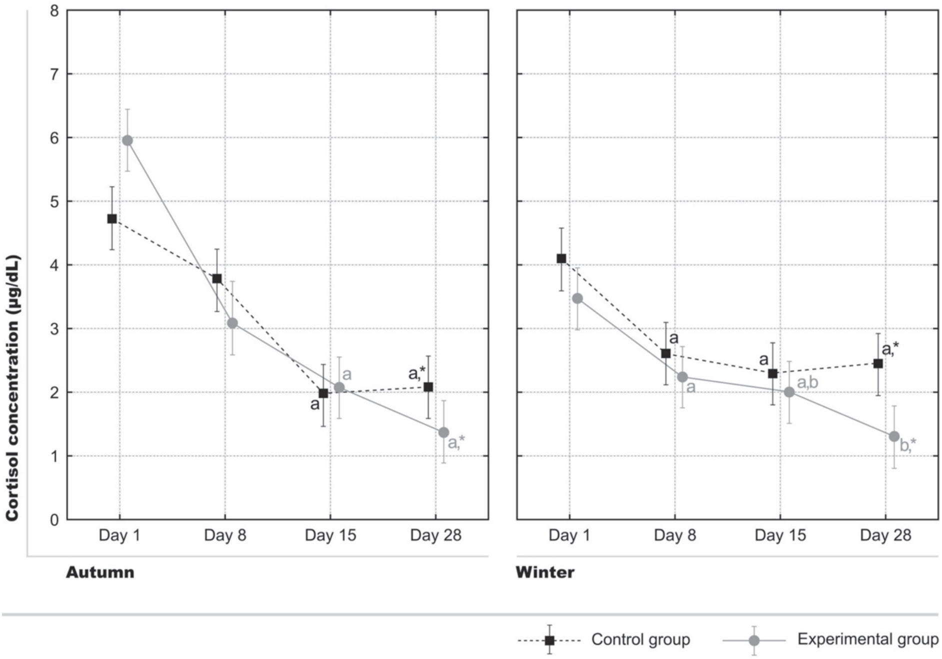

Fig. 1. Mean serum cortisol concentration in the control (concrete stalls) and experimental (rubber stalls) groups of gilts, during different 28-day production cycles in autumn and winter (9 gilts *per* day and group). [a,b] All values are significantly different (P<0.05) between the different days of the same cycle within a particular group, except for those marked with the same letter; [*]Significantly different values (P<0.05) between the control and experimental groups on the same days of a particular cycle.

As illustrated in Fig. 1, in both the control and experimental groups of gilts, lower serum cortisol concentrations were recorded at the end of cycle as compared with the beginning of cycle, in both seasons observed. During the autumn cycle, the serum cortisol concentrations were significantly lower (P<0.05) on each consecutive day of measurement in both groups, with the exception of day 28 *versus* day 15. Apart from the significant decrease (P<0.05) at week 1, serum cortisol concentrations in the winter cycle did not differ significantly between the subsequent days of measurement, in the control group. A similar pattern was also observed in the experimental group in the winter season, with a significant decrease (P<0.05) in cortisol concentrations at the end of the cycle. Significant between-group differences (P<0.05) in cortisol concentrations, with lower values found in the experimental gilts, were recorded on day 28 of both cycles.

As shown in Table 1, a positive correlation was determined between serum cortisol concentrations and service unit air temperature in both the control and experimental groups of gilts (r = 0.65 and r = 0.76, respectively, P<0.05). A positive correlation with cortisol concentrations was also recorded for the length of time gilts spent in a sitting posture and the number of posture changes in the control group (r = 0.55 and r = 0.33, respectively, P<0.05), and with a standing posture in the experimental group (r = 0.43, P<0.05), where a significant negative correlation with cortisol concentrations was found for the time spent lying in total, and lying laterally (r = -0.46 and r = -0.52, respectively, P<0.05).

Table 1. Correlation (r-values) of serum cortisol concentrations with the service unit air temperature and postural behaviour of gilts in the control (concrete stalls) and experimental (rubber stalls) groups

	Cortisol (μg/dL)	
	Control group	Experimental group
Temperature (°C)	0.65*	0.76*
Standing (min)	-0.19	0.43*
Sitting (min)	0.55*	0.12
Total lying (min)	-0.08	-0.46*
Lying laterally (min)	0.06	-0.52*
Lying sternally (min)	-0.14	0.06
Posture change (n)	0.33*	0.01

*P<0.05

Discussion

Animal welfare, among other things, depends on the type of floor in their housing (BERGERON et al., 2008; MILLS et al., 2010). The floor as the main part of the stable, influences animal welfare by its design and material, which is then manifested in species specific behaviour, occurrence of lesions and diseases, and convenience (TUYTTENS, 2005; KYMÄLÄINEN et al., 2009). The type of flooring is of particular importance when animals are kept in a restricted area, such as in the case of individual housing of pregnant females in pig production (GJEIN and LARSSEN, 1995), whereas the lack of bedding in breeding sow and gilt housing has been one of the most controversial welfare issues in commercial livestock production (BARNETT et al., 2001; KARLEN et al., 2007).

Straw as a litter, for instance, has many advantages for pigs, stimulating them to explore the environment by rooting, and improving their physical and thermal comfort, thus reducing stress and the expression of undesirable behaviours, such as frustration, restlessness and stereotypies (BAGARIĆ et al., 2013). However, straw is rarely used in commercial farms because it makes production more expensive, requires more human work, and cannot be used in production systems with slatted floors. An additional disadvantage of straw is that it favours the development of particular pathogens (TUYTTENS, 2005; VAN DE WEERD and DAY, 2009; BOS et al., 2016).

As an alternative to straw, in this study the effect was assessed of rubber flooring on stress levels in stall-housed gilts, following our previous studies on its effect on their postural (PAVIČIĆ et al., 2014) and stereotypic behaviours during different seasons (OSTOVIĆ et al., 2015). The level of stress was assessed by determination of cortisol concentrations in gilt serum.

Cortisol concentration is relatively easy to determine in pigs, but its measurement may suffer from diurnal, seasonal and sampling method variations. Blood sampling *per se* can stimulate cortisol release (POL et al., 2002; SIARD et al., 2003; McGLONE et al., 2004). However, in spite of the method limitations, blood cortisol concentration is used as an indicator of stress in pigs (ESTIENNE et al., 2006; AVEROS et al., 2007; FAGUNDES et al., 2008; BILANDŽIĆ et al., 2010; SUTHERLAND et al., 2010; VUČEMILO et al., 2011; TENBERGEN et al., 2014).

In the present study, serum cortisol concentration was determined in gilts accommodated in a service unit 28 days after insemination, during the cooler autumn and winter seasons. The highest serum cortisol concentration during both seasons was measured on day 1 in the control and experimental groups of gilts, indicating that the initial experience of stall housing, including gilt handling and moving, was stressful for the animals. These results are consistent with the studies by BERGERON et al. (1996) and LYNCH et al. (2000). The latter authors found that, in comparison with the gilts housed in groups, those housed individually displayed a significantly higher rate of trying to turn around and run away, bar biting, slipping, grunting, squealing and defecating during the first hour of the new accommodation.

In both groups of gilts, serum cortisol concentrations were significantly lower at the end of the breeding cycles as compared with day 1. This finding can be ascribed to the gilts' adaptation to the stalls with time (BERGERON et al., 1996; ANIL et al., 2006), but also to the decrease in air temperature in the service unit, i.e. a positive correlation between service unit air temperature and serum cortisol concentrations was recorded in both groups of gilts. However, comparison of serum cortisol concentrations between the groups on day 28 of both breeding cycles yielded a significantly lower level in the experimental group *versus* the control group. These results can be explained by the improved gilt lying comfort on rubber mats as compared with a concrete floor during the cold period, since the rubber mat provides thermal insulation, in particular when the ambient temperature draws closer to the lower limit of the pig thermoneutral zone of 14 °C, as also argued by ELMORE et al. (2010).

As previously demonstrated (PAVIČIĆ et al., 2014), the lower the air temperature in the service unit, the longer the experimental group animals spend lying down. In the present study, a negative correlation was found between the time the experimental gilts spent lying down in total, and lying laterally, as an indicator of lying comfort in the pigs (EKKEL et al., 2003), and serum cortisol concentrations. On the other hand, a positive correlation between serum cortisol concentrations and the time spent standing was recorded in the experimental group. There was no significant correlation between serum cortisol concentrations and duration of postures in the control group of gilts, with the exception of the positive correlation for sitting. However, a positive correlation was found between cortisol concentrations and posture change in the control group of gilts, which may be explained by their higher level of discomfort on the concrete floor (PAVIČIĆ et al., 2006). The positive correlation of serum cortisol concentrations and the time spent sitting in the control gilts may be related to the above finding, since pigs are known to use sitting as an interim posture when changing from lying to a standing posture, and *vice versa* (LI and GONYOU, 2007; PAVIČIĆ et al., 2014).

Conclusion

The use of rubber mats in individual gilt housing in the service unit was shown to be an efficient management tool in reducing the level of stress through improved gilt lying comfort.

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SAŽETAK

Kao što je prethodno utvrđeno, gumene podne obloge tijekom niskih okolišnih temperatura poboljšavaju udobnost pri ležanju pojedinačno držanih nazimica. U ovom radu istraživano je utječu li gumene podne obloge na stupanj stresa nazimica kroz poboljšanu udobnost pri ležanju. Istraživanje je provedeno u pripustilištu komercijalne svinjogojske farme tijekom 28-dnevnog proizvodnog ciklusa u jesen i zimi. Tijekom oba ciklusa kontrolna i pokusna skupina nazimica (9 nazimica svaka) bile su smještene u odjeljcima za pojedinačno držanje tijekom gravidnosti, s betonskim rešetkastim podom. U pokusnim skupinama pod je dodatno bio prekriven prilagođenom 2 cm debelom gumenom podnom oblogom. Stupanj stresa procjenjivao se određivanjem koncentracije kortizola u krvnom serumu nazimica 1., 8., 15. i 28. dana svakog ciklusa, kada je promatrano i posturalno ponašanje nazimica. Koncentracija kortizola u serumu nazimica obiju skupina bila je značajno manja ($P<0,05$) 28. u odnosu na 1. dan obaju proizvodnih ciklusa. Ipak, značajno manja vrijednost ($P<0,05$) utvrđena je 28. dana u pokusnoj skupini nazimica. Između koncentracije kortizola i vremena koje su nazimice pokusne skupine provele ukupno ležeći, odnosno ležeći na boku, ustanovljena je negativna povezanost ($r=-0,46$, odnosno $r = -0,52$; $P<0,05$), a vremena provedenog u stojećem položaju pozitivna povezanost ($r = 0,43$; $P<0,05$). U kontrolnoj skupini između koncentracije kortizola i promatranih položaja, izuzev sjedenja ($r = 0,55$; $P<0,05$), nije bilo značajne povezanosti, no bilo je između koncentracije kortizola i izmjena položaja ($r = 0,33$; $P<0,05$). Zaključno, uporaba gumene podne obloge pokazala se učinkovitim proizvodnim sredstvom za smanjenje stupnja stresa nazimica kroz njihovu poboljšanu udobnost pri ležanju.

Ključne riječi: svinja, pod, gumena obloga, stres, kortizol