Notas Científicas

Physiological indicators of stress in gestating sows under different cooling systems

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Abstract – The objective of this work was to determine physiological stress markers, neutrophil:lymphocyte ratio (N/L) and corticoid concentrations, in gestating sows under different cooling systems. A sprinkling cooling system (SS) and a system based on fan-assisted evaporative cellulose pad (PS) were used. SS showed higher N/L ratio (1.095) than PS (0.850). Corticoid concentrations showed high variability. Corticosteroids are more efficient short-term stress indicators while N/L ratio is a good medium and long-term stress indicator. According to N/L ratio, gestating sows under PS benefit from a higher level of welfare.

Index terms: cooling system, cortisol, neutrophil:lymphocyte ratio, swine, welfare.

Indicadores fisiológicos de estresse em porcas gestantes sob diferentes sistemas de refrigeração

Resumo – O objetivo deste trabalho foi determinar os marcadores fisiológicos de estresse, a razão entre neutrófilos e linfócitos (N/L) e as concentrações de corticoides em porcas gestantes submetidas a diferentes sistemas de refrigeração. Foram utilizados um sistema de refrigeração com aspersores (SS) e um sistema baseado em painel evaporativo de celulose com ventilador (PS). O SS apresentou maior proporção N/L (1,095) que o PS (0,850). As concentrações de corticoides tiveram alta variabilidade. Os corticoesteroides são indicadores de estresse mais eficientes em curto prazo, enquanto a proporção N/L é um bom indicador de estresse em médio e longo prazo. De acordo com a proporção N/L, as porcas gestantes submetidas ao PS se beneficiaram de maior bem-estar.

Termos para indexação: sistema de refrigeração, cortisol, proporção neutrófilos:linfócitos, suínos, bem-estar.

Animal welfare is one of the main worries in swine husbandry under intensive systems. At present, research is being done to study the factors that can affect animal welfare, among them housing conditions, including temperature (Lewis & Berry, 2006), floor type, slat versus straw bedding (Scott et al., 2007), floor space per animal (Morrison et al., 2007), and confinement of pregnant sows (Karlen et al., 2007). Other factors include the duration and conditions of transportation (Gosalvez et al., 2006; Lewis & Berry 2006), social interaction, enrichment of the surroundings (Bracke et al., 2007; Coutellier et al., 2007; Puppe et al., 2007; Scott et al., 2007), and health status of the herd (Lipperheide et al., 1998; Segalés et al., 2004).

In this respect, European Union Directives 2001/88/EC and 2001/93/EC lay down minimum standards for the protection of pigs. EC Directive 2001/93/EC particularly states that "the accommodation for pigs must be constructed in such a way that allows the animals to have access to a lying area physically and thermally comfortable…".

The aim of this work was to determine the effects of the cooling system on sow welfare during gestation in summer.

Several stress markers were measured in pregnant sows accommodated in thermoregulated housing systems using two usual cooling systems: sprinkling and fan-assisted evaporative pads.

This experiment was carried out at a large commercial pigsty located in Los Cánovas, Murcia, Spain, from June to September 2005. The climate of the region is typically semiarid. Air temperature and relative humidity during the study period ranged from a minimum of 10.4ºC and 85% in September to a maximum of 43.6ºC and 60% in August. Annual average rainfall is 270 mm, being almost zero during July and August.

Forty-seven crossbred pregnant sows (Landrace x Large White) were selected according to their farrowing number (n = 5, 6, 6, 6 and 5) in farrowing order from 1 to 5, respectively, and distributed in two adjoining sheds with different cooling systems. Nineteen sows were kept in sheds thermoregulated by a pad system (PS), which consisted of evaporative cellulose cooling pads (one 2x1 m^2 pad per shed) with fan-assisted ventilation (a 4.000 m^3 h^{-1} fan at the opposite sidewall of the pad). The cooling system is switched on automatically when the temperature exceeds 26ºC, and the maximum temperature inside the shed was 33ºC; with maximum relative humidity of 80%. Twenty-eight sows were kept under a sprinkling system (SS), cooled by water sprayers, for thermoregulation on days with temperatures over 26°C. A PVC tube was placed 1.5 m above the heads of the sows, with a nozzle every 2.5 m. Nozzle pressure was 10 atm and water temperature was 26ºC. However, the temperature in the shed reached 37ºC with relative humidity of 70%.

The sows were housed in individual galvanized crates with a width of 0.65 m and a length of 2.1 m including the trough, and with a partially slatted concrete floor (30%) at the back. All the females were homozygotes for the dominant halothane allele. For identification purposes, all animals were tattooed and tagged. The sows were fed a standard barley, corn and soybean meal gestation diet. They received one daily meal with 7,000 kcal day^{-1} of digestible energy (ED) up to the 100^{th} day of gestation and with 7,500 kcal day^{-1} ED thereafter, and were automatically fed in individual feeding troughs. Fresh water was always available from the nipple drinker.

Urine and blood samples were taken from sows in the sheds with the different cooling systems from the 14^{th} to the 22^{nd} day of gestation. All samples were taken between 7:30 and 9:30 h, when sows urinate spontaneously. Corticoid concentrations and neutrophil:lymphocyte (N/L) ratio were determined from the urine and blood samples respectively. Urine was sampled before the blood to avoid the stress caused by blood sampling, because acute stress responses modifies the corticoid concentration quickly (Mormède et al., 2007), but not the neutrophil:lymphocyte ratio.

Blood samples were taken from each sow by anterior *vena cava* puncture. The whole procedure took no longer than 30 s, including the 20 s needed for taking the blood sample. The samples were stored at 4ºC for further smears the next day. Relative leukocyte counts (lymphocytes and neutrophils) were made by microscopic examination of blood smears. Smears were stained using the May-Grünwald-Giemsa method. About 100 cells were counted and the neutrophil:lymphocyte (N/L) ratios were determined.

To analyze cortisol and cortisone concentrations, spontaneously voided urine was collected, acidified using 6 mol L^{-1} HCl (1% of urine volume) and frozen at -80ºC. Before analyzing corticoid concentrations, creatinine concentration was determined to adjust the volume of urine used in the corticoid extraction procedure. Creatinine levels in swine urine were determined using a colorometric quantitative reaction (Jaffe's reaction, Kinetic Creatinine Procedure, bioMérieux SA). Extraction and analysis of cortisol and cortisone were done according to Hay and Mormède (2007). The results of these corticoid concentrations were expressed in relation to creatinine concentration (nanograms of corticoid per miligrams of creatinine), in order to account for differences in urine production, as creatinine is excreted at a relatively steady rate.

Data was analyzed using the SPSS 15.0 statistical package (SPSS version 15, 2006). Cortisol and cortisone concentrations were analyzed after log transformation to fit the Shapiro-Wilk test of normality. The effect of the cooling systems on stress markers at the beginning of the gestation period (PS versus SS) was estimated using analysis of variance (proc GLM, SPSS, 2006), in which the farrowing order was included as a fixed effect with two levels, primiparous and multiparous sows.

Physiological welfare marker measurements are shown in Table 1. Significant differences ($p<0.05$) between the two cooling systems were only detected for N/L. The difference for this marker was of 0.245 (12.5% of the average for both systems), unfavorable to SS, as a result of the higher percentage of neutrophils plus the lower percentage of lymphocytes in the SS group. Although a small amount of data was collected for N/L ratio (18–19 data per treatment), it was enough to detect differences between treatments, as this variable showed low variability in comparison to the others (coefficients of variation were of 13.9, 99.45 and 120.23% for N/L, cortisol, and cortisone concentrations respectively).

Several studies with different species have shown that stress conditions (for example, increasing levels of corticosterone in the chicken feed, or weaning in piglets) result in a redistribution of white blood cells involved in the defense and immunological response against antigens, such as an increase of neutrophils (heterophils in poultry), a decrease of lymphocytes and thus, a higher N/L (Gross & Siegel, 1983; Puppe et al., 2007). Heterophil:lymphocyte ratio appeared to be a more reliable indicator of levels of social stress than the corticosteroid levels were (Gross & Siegel, 1983). Thus, these works highlight N/L as a good medium to long-term indicator of exposure to stress, while corticoids are better indicators of immediate stress situations. In this respect, no significant correlations were found between these parameters. However, Puppe et al. (2007), studying the effect of housing treatments after weaning in piglets, observed that the N/L ratio increased at day one after weaning, but declined by the fourth day, being very transient. There are no reports on the effect of heat stress on N/L ratio. In this work, it was observed that the N/L ratio was lower in the PS than in the SS group. Thus, using N/L ratio as a stress indicator, PS sows would exhibit a higher level of welfare, probably because the temperature of the PS sheds did not increase as much as in the SS sheds. This is especially important in the region where this study was undertaken (southeast), which is one of the hottest in Spain, and also because the experiment was performed in the summer. In order to reduce the risk of miscarriage, it is recommended that the sows be kept in individual stalls at the beginning of gestation, and the EC Directive 2001/88/EC allows it for up to four weeks after the service to one week before the expected time of farrowing. Consequently, when the sows must be kept in individual stalls, a well-controlled environment is recommended.

With regard to corticoid concentrations, creatinine values higher than 300 ng mg^{-1} were considered outliers. Hay & Mormède (1997) showed lower mean values (28.1 or 6.2 ng mg^{-1} creatinine for Meishan or Large White sows respectively), but higher values (up to 300 ng mg^{-1} creatinine) can be reached when the animals are injected with adrenocorticotropic hormone (Pol et al., 2002). Likewise, these markers tended to an asymmetric distribution (Figure 1), because one group of animals had baseline values, while others showed peaks of these hormones concentrations, probably due to nonspecific response to acute challenges (Mormède et al., 2007). Cortisol and cortisone concentrations showed high variability, which can be due to peaks of these hormones when the animals faced a stressful

Table 1. Effect of the cooling system (SS, sprinkling system; PS, pad system) on stress markers of gestating sows: least square means (LSM) and standard error (SE) of neutrophil-lymphocyte ratio (N/L) and cortisol and cortisone concentrations (ng mg^{-1} creatinine)[(1)].

Cooling	N/L ratio			Cortisol		Cortisone	
system	n	LSM	SE	n	LSM	n	LSM
SS	18	1.095a	0.014	27	22.699	27	4.487
PS	19	0.850b	0.015	13	53.951	13	15.205

[(1)]Means followed by equal letters do not differ by Student t-test, at 5% probability.

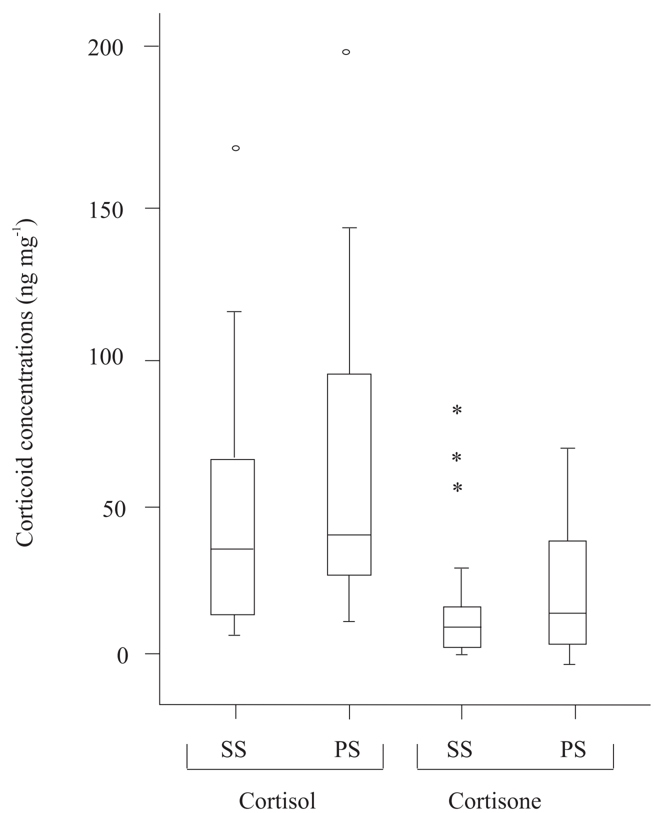

Figure 1. Box and whisker of corticoid concentrations (ng of corticoid per mg of creatinine) on gestating sows under two different cooling systems. SS, sprinkling system and PS, pad system.

situation. Because of this, no significant differences were observed between the types of cooling systems. In Marple et al. (1972), plasma corticoids significantly increased in response to low temperature and humidity with respect to environmental control, and they fell significantly when humidity or temperature increased. Corticosteroid hormone secretions show high variability, which is influenced by diurnal and seasonal rhythms, feed intake and environmental factors, such as temperature and humidity (Mormède et al., 2007).

In conclusion, the neutrophil:lymphocyte ratio was shown to be a good medium to long-term heat stress marker. However, corticoid concentration showed higher variability and is a more efficient stress marker in the short term. According to the N/L ratio, gestating sows cooled by a cellulose pad cooling system benefit from a higher level of welfare than those cooled by a sprinkling cooling system.

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