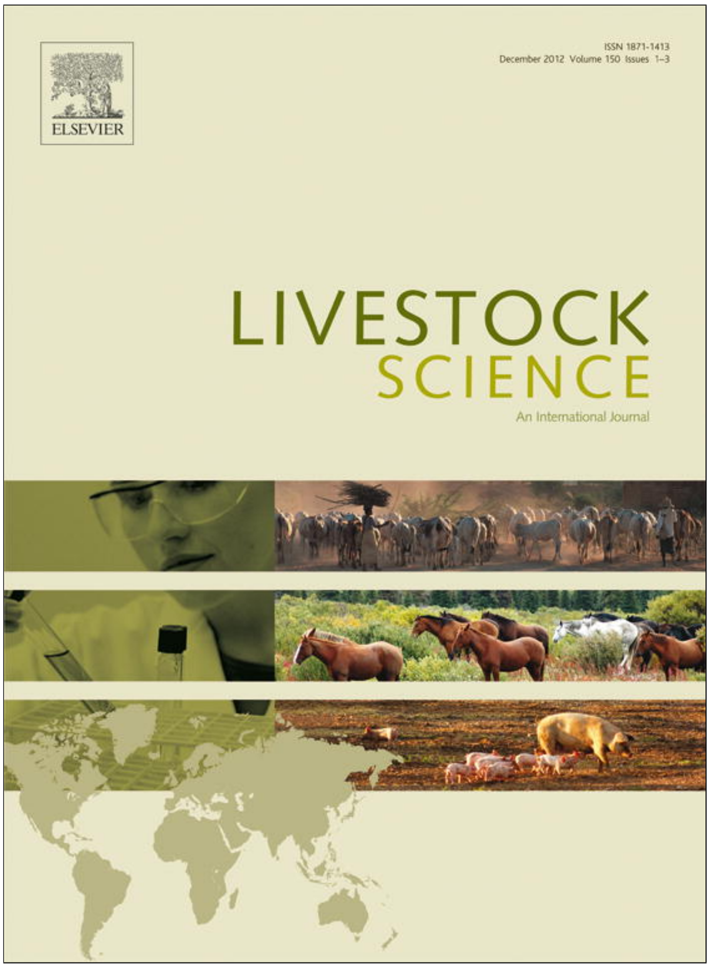
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Meta-analysis on the relationship among feeding characteristics, salivary and plasmatic cortisol levels, and performance of pregnant sows housed in different systems

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ABSTRACT

This study was carried out to evaluate the relationship among the effects of housing systems, the nutritional composition of diets, and feeding program on performance and concentrations of salivary, and plasmatic cortisol in pregnant sows. The database was composed by 22 articles published from 1996 to 2010, totaling 170 treatments, and 1941 animals. Of the 22 studied articles 12 articles evaluated salivary cortisol, 9 evaluated plasmatic cortisol, and 1 article evaluated both salivary and plasmatic cortisol. The meta-analysis was performed by sequential analysis: graphical, correlation, and variance–covariance. Salivary cortisol was positively correlated ($P<0.05$) with feed composition, crude protein, total lysine intakes, and lysine level. Plasmatic cortisol was negatively correlated ($P<0.01$) with metabolizable energy level and positively correlated ($P<0.01$) with level and intake of crude protein. In groups housing on deep bedding, the salivary cortisol level was 10% higher ($P<0.01$) in relation to groups housing on concrete floor. The piglet's weight at birth decreased by 0.002 kg for each increase of a unit nMol L^{-1} in plasmatic cortisol ($Y=1.6032-0.0002X$) or salivary cortisol ($Y=1.6026-0.0002X$), measured in pregnant sows. The feeding (amount of feed per day) and the housing system (individual or groups) influence the levels of cortisol. Cortisol at high levels reduces the reproductive performance.

1. Introduction

The pig world production increased based on intensive and confined systems. The discussion of appropriate housing systems for pregnant sows is a current issue related to animal welfare (Remience et al., 2008). The European Union determined by directive 2001/88/CE that from 2013 the sows must remain housed in groups from 28 days of gestation until the transfer to farrowing room. Thus, it became necessary to study production systems that meet the physiological needs of animals by providing the maximum welfare.

Group housing is the most recommended for the expression of natural and social behaviors by the sows (McGlone, 1991). On the other hand, this system can bring productive problems in the beginning of pregnancy, such as differences in body condition among animals of the group and bodily injury (Lovedahl et al., 2005). Differences in behavioral, immunological, physiological, and productive measures may be used as welfare indicators, with the levels of salivary and plasmatic cortisol among them (Anil et al., 2006a).

To determine the relationship of the elevated levels of cortisol and the housing conditions, it is necessary to isolate the effect of time, performing sample collection

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daily with the animal calm. In species with diurnal habits, such as pig, plasmatic levels of glucocorticoids (including cortisol) are naturally higher in the early morning, and decrease throughout the day (Janssens et al., 1995). This change in cortisol concentrations in pigs during the day is known as circadian rhythm (Griffith and Minton, 1991). The sampling for cortisol determination should be performed in the same period of the day, and this effect should be considered in the global analyses.

The relationship between the environment, and the stress levels in sows during pregnancy and lactation can be observed through the levels of cortisol and behavior. Some of these variables may also be influenced by the diet composition and feeding management of sows (Holt et al., 2006). This analysis can be supplemented with productive data, allowing the evaluation of the effect of high levels of circulating cortisol on the productivity of sows. Due of the variability in experimental results, the use of meta-analysis is interesting, because this technique allows integrating different variables and establishing responses adjusted to experimental diversity (Sauvant et al., 2008).

In this context, the objective of this study was to evaluate, by meta-analysis, the relationship between the effects of housing systems, nutritional composition of diets, and feeding management on performance, and concentrations of salivary and plasmatic cortisol in pregnant sows.

2. Material and methods

The articles that comprised the database were selected by the following criteria: presence of cortisol levels (salivary or plasmatic), and use of pregnant sows housed in different systems (individual or collective, and also concrete floor or deep bedding). Considering these criteria only articles published in the last 14 years were used in the database (1996–2010). The data were selected from sections of material and methods and results of each selected paper, and tabulated in a database compiled in electronic spreadsheet. The variables analyzed were experimental features, nutritional composition of diets, feed program, salivary and plasmatic levels of cortisol, and litter performance. The methodology for the definition of dependent and independent variables, and the coding of the data followed the proposals described in the literature (Lovatto et al., 2007; Sauvant et al., 2008).

The analyzed characteristics were related to experimental design, treatments, number of animals in each study, genetic, and available animal/area ratio. The data concerning diet composition were converted to the same unit before analysis. Descriptive statistics are presented in Table 1 (minimum, maximum, and average values found in the studies). The studies also differ in terms of diet composition and feed amount offered to the animals.

The housing systems (individual or groups and concrete floor or deep bedding) were used as grouping qualitative criteria, as a resource to associate homogeneous groups on certain characteristics and to include them in the analytical models as a source of variation. The dependent variables were the levels of salivary and plasmatic cortisol and productive data of sows. The independent variables were the type of housing, type of pen floor, diet, and feeding program. Other encodings were used as moderating variables in the analysis, in order to consider the variability of the compiled studies (*general*, *inter*-, and *intra*-effects). For the *general* codification (article effect), a sequential specific number was assigned for each paper inserted into the database. The *inter*-codification was formed by the general coding plus sequential numbers, in order to assign a specific code for each treatment of the base. The *intra*-codification, similar to that used for the previous effect, was attributed to the groups with repeated measures.

The database was composed by papers developed at different institutions (Table 2). The database had 170 treatments and 1941 gestation sows, with an average of 34 sows per treatment (ranging from 5 to 320 females per treatment) and 88 sows for study (from 6 to 640 sows per study). The study included sows of different genetic types, which are as follows: Landrace (9.1%), Yorkshire × Landrace (36.4%), Landrace × Large White (18.2%), Yorkshire (4.1%), and commercial breeds (31.8%). In 59% of the treatments, sows were fed only once a day and in 41% of cases the sows received 2 meals daily, with an average intake of 2.25 kg of feed per day (ranging from 1 to 3 kg/d). The average calculated intakes were 453 g/d of crude protein and 21 g/d of total lysine.

From the total, 75% of the treatments used pregnant sows housed in individual stalls and 25% housed in collective pens. The floors of the pens were grouped in

Table 1
Number of sows, cortisol levels (salivary and plasmatic), and calculated composition of gestation diets in the database.

Variables	Total	Number of diets[a]	Minimum	Maximum	Average	Standard-deviation
Animals	1941	–	5	320	34	49.42
Salivary cortisol (nMol L^{-1})	–	–	0.00	60.00	8.36	17.24
Plasmatic cortisol (nMol L^{-1})	–	–	5.66	200.00	64.90	55.76
Metabolizable energy (kcal kg^{-1})	–	80	2073	3300	2687	327.75
Net energy (kcal kg^{-1})	–	22	2006	2293	2150	136.49
Crude protein (%)	–	96	12.40	18.40	15.40	1.77
Crude fiber (%)	–	36	2.70	14.80	8.75	3.78
Lysine (%)	–	30	0.47	0.97	0.72	0.16
Calcium (%)	–	14	0.80	0.98	0.89	0.06
Total phosphorus (%)	–	14	0.46	0.77	0.62	0.09

[a] Number of diets in the database that showed the nutrient composition.

Table 2
Description of the papers used in the database.

Author	Country	Animals (*No.*)	Genetics
Spoolder et al. (1996)	United Kingdom	96	Commercial genetic
Zanella et al. (1998)	Germany	24	Landrace × Large White
Mcglone and Fullwood (2001)	United States	42	Commercial genetic
De Leeuw et al. (2003)	Netherlands	48	Commercial genetic
De Leeuw and Ekkel (2004)	Netherlands	48	Commercial genetic
Otten et al. (2004)	Germany	6	Landrace
Razdan et al. (2004)	Sweden	11	Yorkshire × Landrace
Anil et al. (2006a, 2006b)	United States	25	Yorkshire × Landrace
Anil et al. (2006a, 2006b)	United States	310	Yorkshire × Landrace
Holt et al. (2006)	United States	68	Commercial genetic
Hulbert and Mcglone (2006)	United States	160	Commercial genetic
Niekamp et al. (2006)	United States	24	Commercial genetic
Séguin et al. (2006)	United States	74	Yorkshire
Jansen et al. (2007)	United States	96	Yorkshire × Landrace
Karlen et al. (2007)	Australia	640	Landrace × Large White
Sorrells et al. (2007)	United States	48	Yorkshire × Landrace
Lay et al. (2008)	United States	43	Yorkshire × Landrace
Olivieiro et al. (2008)	Finland	38	Yorkshire × Landrace
Remience et al. (2008)	Belgium	68	Landrace
Damgaard et al. (2009)	Denmark	28	Yorkshire × Landrace
Mosnier et al. (2009)	France	20	Landrace × Large White
Ison et al. (2010)	United Kingdom	24	Landrace × Large White

Table 3
Correlations between the levels of salivary and plasmatic cortisol, variables of nutrition and feeding program variables of gestating sows.

Variables	Salivary cortisol levels (nMol L^{-1})	*P*	Plasmatic cortisol levels, (nMol L^{-1})	*P*
Nutritional variables				
Crude protein (%)	0.245	0.118	0.771	0.000
Crude fiber (%)	−0.130	0.511	–	–
Total lysine (%)	0.835	0.000	–	–
Calcium (%)	−0.025	0.938	–	–
Total phosphorus (%)	−0.025	0.938	–	–
Feed intake (kg d^{-1})	0.458	0.001	−0.177	0.263
Metabolizable energy (kcal kg^{-1})	−0.476	0.003	−0.830	0.000
Crude protein intake (g d^{-1})	0.589	0.000	0.509	0.001
Lysine intake (g d^{-1})	0.617	0.011	–	–
Feed management				
Number of feeding[a]	0.008	0.946	0.405	0.003

P, significant at 5% of probability.
[a] Number of meals daily.

deep bedding (30% of the treatments) and concrete floor (70%). The average area available per animal was 2.42 m^2 (ranging from 1.20 to 7.50 m^2 per sow). Of the total number of females studied, it was possible to evaluate the productivity of the piglets of 1454 sows, with an average of 10 piglets born per sow (ranging from 8 to 12 piglets).

The meta-analysis followed 3 sequential analyses. The graphical analysis of dispersion was used for controlling the quality of database and observing biological coherence of data, outlier data and possible trends. The Pearson correlation analysis among several variables was used to identify the related factors in the base, and variance–covariance analysis was used to compare groups and obtain the prediction equations. In analysis of variance–covariance, the models included the codification for general, inter- or intra-effect (Lovatto et al., 2007) and codifications for housing system (individual or collective and concrete floor or deep bedding). The number of daily meals, number of sows per group, area available per animal, and sampling time were tested in the models for the analysis of variance, but they were later excluded once the variables did not present significant effect ($P > 0.05$) in the models. These variables were tested in two ways: (1) considering the isolated effect of each of these characteristics, and (2) considering the effect of the characteristic and moderating codification. The regression equations were obtained by variance–covariance analysis using the General Linear Model procedure. All analyses were performed using Minitab (2007).

3. Results

The correlations between the variables of nutrition, feed program, and levels of salivary and plasmatic cortisol are presented in Table 3. Salivary cortisol showed positive correlations with feed intake (0.458; $P < 0.01$), level of total lysine (0.835; $P<0.01$), crude protein intake (0.589; $P<0.01$), and lysine intake (0.617; $P<0.05$). The metabolizable energy level presented negative correlation (−0.476; $P<0.01$) with the levels of salivary cortisol. Plasmatic cortisol was negatively correlated with the level of metabolizable energy (−0.830; $P<0.01$), and positively correlated with the level (0.771; $P<0.01$) and intake of crude protein (0.509; $P<0.01$), and with the number of daily feedings (0.405; $P<0.01$).

The equations to estimate the levels of salivary and plasmatic cortisol are presented in Table 4. Through the equations, it was possible to observe that plasmatic cortisol levels presented an inverse relationship with feed intake. Thus, on increasing the feed consumption, a decrease in plasmatic cortisol levels is expected. The metabolizable energy showed a different behavior, so as its concentration increased in the diet, the level of salivary and plasmatic cortisol also increased. The increase in the dietary level of crude protein was related with a reduction in salivary and plasmatic cortisol level. In addition, the size of the animals (body weight and metabolic weight) was a good explanatory variable for the estimation of salivary cortisol.

The type of floor (deep bedding or concrete floor) ($P<0.05$) and the type of housing (individual or groups) ($P<0.01$) influenced the levels of salivary and plasmatic cortisol (Table 5). For the salivary cortisol values, the effect ($P<0.05$) of the type of floor, type of housing, and the interaction between these factors were observed. The sows housed individually in deep bedding showed 47% lower ($P<0.01$) salivary cortisol levels than those housed individually in concrete floor. Under the same conditions, the plasmatic cortisol levels were 52% higher ($P<0.01$) for sows housed in deep bedding in relation to those housed in floor concrete. In group housing, the salivary cortisol of animals in deep bedding was 10% higher ($P<0.01$) compared to sows housed in concrete floor pens.

The equations used to estimate the effect of cortisol on the piglet performance are described in Table 6. The increase

Table 5
Salivary and plasmatic cortisol (nMol L^{-1}) obtained by meta-analysis for sows in different housing systems.

	Salivary		Plasmatic	
	Deep bedding	**Concrete floor**	**Deep bedding**	**Concrete floor**
Individual	1.16	2.19	103.00	49.46
Groups	18.68	16.86	–	–
RSD	0.54		5.20	
Probabilities				
Housing	0.001		–	
Floor	0.030		0.026	
Housing × Floor	0.002		–	

RSD, residual standard deviation.

Table 6
Equations to estimate the influence of cortisol on the productive performance of sows.

Variables	Equation[a]	RSD	R^2
Salivary cortisol (co-variable)			
Mummified	0.1361 + 0.0007X	0.0508	86.26
Dead[b]	1.1762 + 0.0022X	0.3610	61.81
Weaned	9.2217 − 0.0030X	0.1733	91.93
Birth weight (Piglet, kg)	1.6026 − 0.0002X	0.0445	71.18
Birth weaned (Piglet, kg)	6.9478 − 0.0003X	0.0557	97.31
Plasmatic cortisol (co-variable)			
Births	10.5270 − 0.0050X	0.6717	76.79
Stillbirths	0.7759 + 0.0010X	0.2512	75.17
Birth weight (Litter, kg)	1.6032 − 0.0002X	11.060	99.11
Birth weaned (Litter, kg)	6.8189 − 0.0004X	14.329	99.90

RSD, residual standard deviation.

[a] Estimated equations for sows housed in individual stalls or groups pen in concrete floor or deep bedding. The average level of salivary and plasmatic cortisol observed during pregnancy were used to assess its effect on the litter characteristics.

[b] Dead piglets from birth to weaning.

Table 4
Equations to estimate the levels of salivary and plasmatic cortisol (nMol L^{-1}) of sows housed in individual or group pens with concrete floor or deep bedding.

Variables	Equations	RSD	R^2
Salivary Cortisol			
Live weight (kg)	1.6181 − 0.003594X	0.15	96.55
Metabolic weight (kg)	1.8830 − 0.018480X	0.15	96.56
Feed intake (kg d^{-1})	−4.6463 + 3.271800X	0.53	91.46
Metabolizable energy (kcal kg^{-1})	5.1880 + 0.001837X	1.89	99.22
Crude protein (%)	10.8600 − 0.003200X	1.95	99.07
Crude fiber (%)	1.8406 + 0.000320X	1.07	71.06
Crude protein intake (g d^{-1})	−4.7261 + 0.024982X	0.41	95.44
Plasmatic cortisol			
Live weight (kg)	46.0000 + 0.039600X	23.71	91.52
Metabolic weight (kg)	42.7000 + 0.210000X	23.71	91.52
Feed intake (kg d^{-1})	35.5500 − 0.100000X	16.64	94.34
Metabolizable energy (kcal kg^{-1})	43.7000 + 0.000210X	18.31	93.55
Crude protein intake (g d^{-1})	35.5600 − 0.000700X	16.64	94.34

RSD, residual standard deviation.

of 1 nMol L^{-1} in plasmatic cortisol level represented a reduction of 0.005 in the number of piglets born alive; of 0.0002 kg in the birth weight; of 0.0004 kg in weight of piglets at weaning; and an increase of 0.001 in the number of stillbirths. The increase of 1 nMol L^{-1} in salivary cortisol levels represented an increase of 0.0007 and 0.0022 in the number of mummified and dead piglets, respectively. Likewise, every 1 nMol L^{-1} of salivary cortisol caused a reduction of 0.003 in the number of piglets weaned and 0.0002 and 0.0003 kg in the birth and weaning weights, respectively.

4. Discussion

The amount of feed offered or the feed restriction does not have clear effects on the plasmatic cortisol level when measured in sows housed under thermal comfort conditions (18–20 °C) (Doberenz et al., 2006). Our study also did not find a significance ($P>0.05$) in the correlation between feed intake and plasmatic cortisol level. However, the restricted diet during pregnancy is pointed as one of those factors responsible for stereotyped behavior and, consequently, for high cortisol levels, because diets do not cause satiation in animals for more than 1 or 2 h (Spoolder et al., 1996). Diets with a high percentage of crude fiber can supply higher volume, while increasing the time of feeding and providing longer satiety time (Meunier-Salaün et al., 2001). The effect of nutrient concentration and feeding frequency on concentrations of salivary cortisol in pregnant sows were not observed in previous studies (Holt et al., 2006; Mcglone and Fullwood, 2001). However, in our study, the correlations observed between nutritional variables and cortisol levels were significant for several features, indicating that the relationship needs to be evaluated in other studies.

The environmental characteristics have an influence on the levels of salivary and plasmatic cortisol (Damgaard et al., 2009). In several species it has been demonstrated that chronic or repeated stress can change the regulation of the hypothalamic-pituitary–adrenal (Janssens et al., 1995). High levels of cortisol were already found in sows housed in individual crates for 28 days, suggesting chronic stress, which does not occur in animals housed in collective pens (Anil et al., 2006a).

The high concentrations of cortisol may be related to the incidence of stereotyped behaviors and also with the reduced immune efficiency, which increases the susceptibility to disease (Jarvis et al., 2006). On the other hand, quiet environments during feeding (provided by individual feeders) can help to maintain low levels of circulating cortisol. Moreover, the highest number of feeding times and the availability of space at feeders can reduce disputes and, consequently, the cortisol levels tend to be normal (Anil et al., 2006b).

Adequate facilities contribute to reducing the stress of pregnant sows. In group pens and with dispute of space in a collective feeder, the sows have greater variation in weight and body condition scores and higher levels of circulating cortisol (Lovedahl et al., 2005). When housed in pens with more space and individual feeders, these levels are lower, especially after the establishment of the hierarchy (Jansen et al., 2007). Aggression during mixing groups is associated with increased levels of cortisol in pigs, but these levels decrease after establishing the hierarchy. Heavier and older sows often dominate the group and the subordinate avoid them, reducing the fighting and stress of the group (Anil et al., 2006b). The increase in size and body weight causes discomfort and difficulty of changing posture, especially for sows housed in pens (Anil et al., 2006a).

Environmental stressors such as temperature, management, facilities, and social interactions increase the secretion of adrenocorticotropic hormone (ACTH), which stimulates the adrenal gland to produce cortisol and sexual steroids (Otten et al., 2004). Their presence in high concentrations inhibits or alters the secretion of gonadotropin hormones, causing infertility or low productivity performance (Spoolder et al., 1996). In general, reproductive performance is impaired by severe stress, reducing the litter size (Hulbert and Mcglone, 2006).

The new regulations imposed by the European Union and the demands of the consumer market in relation to animal welfare influence the facilities and managements in the pig production systems. The type of housing (individual or groups), environment, nutritional management, and social interactions alter the levels of circulating cortisol in pregnant sows. High levels of cortisol alter the secretion of gonadotropin hormones, causing infertility and reducing the productive performance of sows.

5. Conclusions

The feeding (amount of feed per day) and the housing system (individual or groups) influence the levels of cortisol. Sows housed individually in deep bedding present lower levels of circulating cortisol. Sows housed in groups in deep bedding present higher levels of cortisol. Cortisol at high levels reduces the reproductive performance.

Conflict of interest statement

Raquel Melchior declares that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript "Meta-analysis on the relationship among feeding characteristics, salivary and plasmatic cortisol levels, and performance of pregnant sows housed in different systems".

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