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Microbiological air quality in free-farrowing housing systems for sows

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ABSTRACT

This study investigates air hygienic characteristics of housing systems without fixation for lactating sows, with a focus on microbiological air quality. For animal welfare considerations, free-farrowing systems have been developed to replace those with farrowing crates (FC) and are currently available on the market. A group housing system (GH) for six lactating sows, a single loose-housing system (LH), and a conventional system with FC were tested. By maintaining similar management conditions, microbiological air quality was examined in eight batches with 148 farrowings in total with samplings at the beginning, in the middle, and at the end of the occupancy period of 33 days. No significant differences between the systems were observed in the concentration of total airborne bacteria (TAB), haemolytic streptococci (HS), molds, or yeasts. However, the concentrations of TAB and HS increased significantly over time. Additionally, significant correlations were detected for LH and FC between TAB and HS, and between TAB and carbon dioxide; significant correlations between TAB and inside temperature and total animal weight were revealed only in GH. Significant correlations between TAB and both the dust and inside humidity parameters were found only in FC. Given these results, it was shown that the free-farrowing systems used here do not necessarily lead to poorer microbiological air quality.

1. Introduction

Due to animal welfare concerns and growing public interest, the restriction of sows in farrowing crates (FC) has become a central topic in the debate on animal husbandry in many countries as FC were found to exert a deleterious effect on both the behavior and physiology of these animals (Jarvis, D'Eath, Robson & Lawrence, 2006). Loose-housed farrowing systems (LH) were consequently developed, providing a healthier alternative to traditional FC. They offer increased freedom of movement and improved opportunities for social contact and nest-building behavior, which result in lower stress levels (Jarvis et al., 2006) and fewer stereotypies (Arellano, Pijoan, Jacobson & Algers, 1992). Despite the advantages for sow welfare, however, many farmers remain skeptical as to the feasibility of such systems, mainly due to a higher piglet mortality (Wechsler & Weber, 2007).

One important factor indirectly affecting piglet mortality is the state of hygiene within these respective housing systems (Baxter, Lawrence & Edwards, 2011; Rantzer & Svendsen, 2001; Weber, Keil, Fehr & Horat, 2009). Hygiene management is critical for maintaining the animals' health and for preventing infections, with cleanliness of the animals'

environment and good air quality being important determinants of welfare (EFSA, 2007). In most free-farrowing systems no clear separation between the excreting and feeding area (defined functional areas) is given, which may lead to impaired hygiene (Baxter, Lawrence & Edwards, 2011). Furthermore, husbandry systems for respective production units (i.e., farrowing vs. finishing units) or bedding type (i.e., slatted floors vs. litter) are known to influence the concentrations of bacteria in the air (Banhazi, Seedorf, Rutley & Pitchford, 2008b; Cormier, Tremblay, Meriaux, Brochu & Lavoie, 1990; Sowiak et al., 2012). Additionally, higher animal activity could result in poorer air quality due to fluctuations in the airborne bacteria concentration during the day (Curtis et al., 1975; Kim, Ko, Lee, Park & Kim, 2005).

Air quality can be assessed based on the following parameters: concentrations of carbon dioxide, ammonia, endotoxins, dust, total airborne bacteria (TAB), and fungi (i.e., molds and yeasts). The first four listed parameters were the focus of a recently published study (Lühken et al., 2019), which showed that only in one of three comparisons the dust and ammonia concentrations in GH and the endotoxin concentrations in FC were significantly higher than in the other systems. The current study focuses, therefore, on the latter parameters

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(i.e., TAB, molds, and yeasts). These germs-together with dust particles-are known to form an active bioaerosol (Seedorf et al., 1998) which, in turn, can cause infectious and allergic diseases in animals and humans (Donham, 1991; Kim, Ko, Kim, Kim & Kim, 2008; Robertson, Wilson & Smith, 1990). Additionally, this study evaluates the concentration of haemolytic streptococci (HS). HS is an animal-associated parameter and, as such, remains unaffected by an external entry. αhaemolytic streptococci in particular are known as commensals (Done et al., 2005) but they have recently been ascribed a certain pathogenicity that is reflected in subclinical expressions (Murphy, Cargill, Rutley & Stott, 2012). These streptococci are known to be associated with pneumonia (Palzer, Ritzmann, Wolf & Heinritzi, 2008) and are suggested to exert a negative effect on feed intake (Murphy et al., 2012). Murphy et al. (2012) considered the concentration of HS to be the principal source of poor hygiene, affecting both production parameters and economic performance.

Knowledge of air quality is not only necessary for assessing the health risk posed to animals and workers (Banhazi, Seedorf, Rutley & Pitchford, 2008a; Donham, Haglind, Peterson, Rylander & Belin, 1989; Kim et al., 2008; Murphy et al., 2012; Radon et al., 2002; Roque et al., 2016; Seedorf et al., 1998; Sowiak et al., 2012), but is also of the highest relevance to the increasing resistance of germs to disinfectants (Wong, Zhang, O'Donoghue & Boost, 2013) and antibiotics (Friese et al., 2012). Consequently, housing systems should conform to a high standard of hygiene which, besides other implements, can be achieved through their careful design and construction (Baxter, Lawrence & Edwards, 2011; Rantzer & Svendsen, 2001). With this study, preliminary insights are gained as to microbiological air quality in two commercially available free-housing systems for lactating sows.

2. Materials and methods

2.1. Farm, animals, and experimental conditions

The study was conducted on the premises of the Lower Saxony Chamber of Agriculture in Oldenburg/Wehnen from August 2016 to August 2017. On the farm, 80 reproductive sows of a common breed (db.Victoria, BHZP GmbH, Dahlenburg-Ellringen, Germany) were managed in five-week intervals. Two rooms in the barn were each equipped with one of three housing systems, namely a group housing system (GH) with a common area, a single loose-housing system (LH), or single pens with farrowing crates (FC; all: Big Dutchman International GmbH, Vechta, Germany). The systems provided room for six sows in GH (pen size: 5.0 m²; common area: 14 m²) and LH (pen size: 7.0 - 7.3 m²) and for eight sows in FC (pen size: 5.2 m²). Air conditioning was provided by an automatically adjusting air ventilation system with side vents (Big Dutchman, Germany) and a hot-water based heating. Fully slatted floors were installed in each room with deep slurry pits underneath. Cleaning and disinfection were performed by the standard farm routines following each batch. Due to practical difficulties, a microbiological examination of the cleaning and disinfection success could not be performed in this study. The screening of the surfaces by means of agar contact plates and sock swabs is very susceptible to faults and, in some cases, not suitable, as there are high levels of residual contamination and focal hotspots. The sows were introduced from the gestation house to the farrowing systems one week prior to farrowing (day 1). Sows in GH were separated for the first 24 h followed by a 48-h integration. Afterwards, sows were separated again until five days after birth. Sows in LH were never confined whereas sows in FC were continuously confined. The weaning of the piglets took place on their 26th day of life, representing day 33 after sows were introduced to the systems. The sows left the systems on the same day (day 33). This study was based on the data of eight batches with 148 farrowings in total. Despite efforts to achieve a number of six sows in each housing systems per batch, the number of sows varied in some cases. More detailed information of all relevant test conditions can be found in Lühken et al. (2019).

2.2. Measurements

Sampling took place at day 5, 19, and 33 following introductions of sows to the systems. All air samples for microbiological examination were collected in the mornings with a Coriolis Micro-Air Sampler (Bertin Technologies SAS, Montigny-le-Bretonneux, France). For each sample, the corresponding sterile cone was filled with 15 mL sterile phosphate buffered saline (PBS) and connected to the device. While sampling, the Coriolis Micro-Air Sampler was positioned in around 1.2 m height, adjusted in the direction of the animals, and at least 2.5 m from the air exhaust fan. The Coriolis sampler ran for 60 s with a suction volume of 150 L/min. Samples were cooled during transport and storage and prepared in laboratory the same day. Before further processing, the cone was stirred up and a dilution line (by using 9 mL PBS and 1 mL original dispersion) was applied. The dispersions were streaked out on three agar plates each. For identification of TAB, tryptone soya agar (TSA) of HS sheep blood azide agar and of fungi dichloran glycerol agar 18 (DG18) (all: Thermo Fisher Scientific, Waltham, USA) were used. TSA plates were incubated for 24 h at 37 °C, azide plates for 24-48 h at 37 °C and 5% CO2, and DG18 plates for seven days at 25 $^{\circ}\text{C}.$ Colonies were counted and given as colony-forming units per cubic metre (cfu/m³). To differentiate the streptococci, individual colonies were mixed with a 3% hydrogen peroxide solution and tested for the presence of the catalase enzyme.

The methods of measurements of the following parameters are described in more detail by Lühken et al. (2019) and are only briefly mentioned here. Temperature and humidity were measured by a high-precision PCE-THB 40 device (PCE Deutschland GmbH, Meschede, Germany). The dust samples were collected by SKC Universal PCXR8 pumps (SKC Inc., Eighty Four, PA, USA), which ran for 24 h at a flow rate of 2.5 L/h (3.6 m³ in total), and endotoxin concentrations were subsequently analyzed with the Kinetic-QCLTM Kinetic Chromogenic LAL Assay (Lonza Group Ltd., Basel, Switzerland). Colorimetric tubes (Dräger GmbH, Lübeck, Germany) were used to determine concentrations of ammonia and carbon dioxide.

2.3. Statistical analysis

All results were statistically analyzed with SAS software for Windows 9.4 (SAS Institute Inc., Cary, USA). Data were logarithmically transformed for subsequent analysis. Data analysis for the parameters of concentration of TAB per $\rm m^3$ of air, concentration of HS, concentration of molds, and concentration of yeasts was conducted separately using a mixed linear model (MIXED procedure). System, batch, day, and the interaction between system and day, as well as between system and batch, were included as fixed factors. Multiple pairwise comparisons were calculated using Tukey – Kramer tests. Spearman's correlations were used for TAB concentration with the following variables: concentration of HS, molds, yeasts, dust, endotoxins, carbon dioxide, ammonia, outside temperature, inside temperature and humidity, and total animal weight. Statistical significance was considered at p < 0.05.

3. Results

3.1. Total airborne bacteria

TAB ranged from 4.22-5.67 log cfu/m³ (GH), 4.03-5.37 log cfu/m³ (LH), and 3.43-5.58 log cfu/m³ (FC). On day 5 and 19 the mean

TAB concentration was highest in LH and on day 33 in GH and FC, but housing system type revealed no significant influence (p>0.5). A significant influence of the day of occupancy (p<0.001) and batch (p=0.05) was observed. Pairwise comparisons revealed that concentrations of TAB were significantly lower on day 5 than on day 19 (p<0.001) and 33 (p<0.001), but there was no detectable interaction between system and day.

3.2. Haemolytic streptococci

The occurrence of HS was excluded in 4 of 72 samples and maximum single values were 4.78 log cfu/m³ (GH), 4.64 log cfu/m³ (LH), and 4.92 log cfu/m³ (FC). Highest mean values were found on day 5 in LH, on day 19 in GH, and on day 33 in FC. As with TAB concentrations, the housing system showed no significant influence (p > 0.05), but day of occupancy (p < 0.01) and batch (p < 0.01) were found to be significant. Pairwise comparison revealed that concentrations of HS were lower on day 5 than on day 33 (p < 0.01), but there was no interaction detectable between system and day.

3.3. Fungi (molds and yeasts)

Molds were not detectable in 7 of 72 samples and maximum single values were 3.43 log cfu/m³ (GH), 3.50 log cfu/m³ (LH), and 3.64 log cfu/m³ (FC). Highest mean concentrations were found on day 5 and 19 in FC, and on day 33 in LH. The housing system type had no significant effect (p>0.05). Concentrations of fungi differed significantly by day of occupancy (p<0.05). Pairwise comparison revealed that concentrations of molds were significantly higher on day 5 than on day 19 (p<0.05), but there was no interaction detectable between system and day.

No yeasts were detected in the air in 11 of 72 samples of GH and LH, and in 13 samples of FC. Maximum single values were 3.54 log cfu/m³ (GH), 3.78 log cfu/m³ (LH), and 3.52 log cfu/m³ (FC). Mean concentrations were highest in LH on day 5 and 19, and in GH on day 33. The housing system type showed no significant effect (p>0.05) whereas the batch did (p<0.001). The concentration of yeasts did not vary significantly over time.

3.4. Correlations

Significant correlations (Table 2) were detected between TAB and HS (LH: r=0.68; FC: r=0.63) and between TAB and carbon dioxide (LH: r=0.52; FC: r=0.47). Only in FC, correlations between TAB with the parameters dust (FC: r=0.68) and inside humidity (FC: r=0.53) were significant. Only in GH, significant correlations were found

Table 2Spearman's correlation coefficients for TAB concentration with different variables according to system.

Correlation variable System	TAB (cfu/m³ GH	LH	FC	
HS	0.37	0.68**	0.63**	
Molds	-0.17	-0.09	0.06	
Yeasts	0.02	-0.14	-0.05	
Dust	0.18	0.35	0.68**	
Endotoxins	0.13	0.32	0.20	
Carbon dioxide	0.05	0.52**	0.47*	
Ammonia	0.01	0.36	0.12	
Outside temperature	-0.02	-0.18	-0.13	
Inside temperature	0.42*	-0.20	0.14	
Inside temperature, 120 h	0.18	-0.06	0.07	
Inside humidity	-0.03	0.13	0.00	
Inside humidity, 120 h	-0.22	-0.09	-0.53**	
Total animal weight	0.58**	0.31	0.27	

^{*} p < 0.05.

between TAB with the parameters inside temperature (GH: r = 0.42) and total animal weight (GH: r = 0.58).

4. Discussion

4.1. Total airborne bacteria

The present study is the first of its kind comparing three different housing systems for lactating sows with regard to microbiological air quality. The samplings were carried out over an entire year in eight repetitions (batches). The investigation of each batch at the beginning (day 5), mid-point (day 19), and end of the occupation (day 33) also accounted for variations in the microbiological contamination of the air over time. Nevertheless, no significant influence of the housing system type on the air parameters TAB, HS, fungi, and yeast was found.

Concentrations of TAB were comparable to those reported for farrowing systems in other studies despite the reportedly large fluctuations (Cormier et al., 1990; Curtis et al., 1975; Seedorf et al., 1998). The differences between system types in this study are apparently not so decisive that they would constitute a measurable difference in TAB concentration, as it does for the comparison of farrowing vs. finishing units (Cormier et al., 1990; Curtis et al., 1975). The main cause is likely to be the similarly controlled environment (ventilation, temperature, and humidity) in all three systems (Lühken et al., 2019). This is

Table 1
Means of concentration of TAB, HS, molds, and yeasts according to day and system (MV¹, SD²).

	TAB (log cfu/m ³)		HS (log cfu/m ³)		Molds (log cfu/m ³)		Yeasts (log cfu/m ³)	
	MV	SD	MV	SD	MV	SD	MV	SD
Day 5								
GH	4.52	± 0.39	3.27	± 1.38	2.94	± 0.39	1.14	± 1.58
LH	4.58	± 0.33	3.32	± 1.37	2.67	± 1.15	1.53	± 1.67
FC	4.32	± 0.42	3.24	± 1.36	3.01	± 0.53	1.07	± 1.51
Day 19								
GH	5.02	± 0.35	3.95	± 0.53	1.97	± 1.26	1.04	± 1.48
LH	5.05	± 0.42	3.92	± 0.67	2.22	± 1.38	1.47	± 1.64
FC	5.01	± 0.26	3.81	± 1.59	2.42	± 1.03	1.31	± 1.46
Day 33								
GH	5.18	± 0.26	4.15	± 0.28	2.51	± 1.08	2.25	± 0.96
LH	4.95	± 0.25	4.09	± 0.33	3.00	± 0.36	1.66	± 1.39
FC	5.18	± 0.37	4.43	± 0.41	2.90	± 0.28	1.40	± 1.57

¹ MV: mean value.

^{**} p < 0.01; TAB = total airborne bacteria; HS = haemolytic streptococci; GH = group housing system; LH = single loose-housing system; FC = pens with farrowing crate; cfu = colony forming units.

² SD: standard deviation. TAB = total airborne bacteria; HS = haemolytic streptococci; GH = group housing system; LH = single loose-housing system; FC = pens with farrowing crate; cfu = colony forming units.

corroborated by the finding that there is apparently no major health risk for animals in free-farrowing compared to conventional systems due to airborne diseases, as the mass of viable bacteria in air compromises local defense mechanisms (Robertson et al., 1990).

By investigating the stable air on three dates we confirmed the assumption that TAB concentrations significantly increased over time, which was also reported by Costa, Colosio, Gusmara, Guarino and Sala (2014). A similar increase was determined for dust concentrations, which were explained by the increasing soiling of the systems (Lühken et al., 2019) and which may also explain the increase of TAB. Moreover, bacteria are associated with dust particles, forming a bioaerosol, (Seedorf et al., 1998). Thus, an increase in dust concentration could already result in a parallel increase in TAB concentration. The strong fluctuations of TAB concentrations in the same houses were also found by others (Cormier et al., 1990; Curtis et al., 1975). This finding could be explained by the farmer's activities (e.g., feeding, maintenance), the varying prevalence of diseases, or by excretion of microorganisms, as was suspected by Cormier et al. (1990). A seasonal effect of outside temperature on the adaption of ventilation rates (Curtis, Drummond, Grunloh, Lynch & Jensen, 1975) was refuted by the significant negative correlation between TAB concentration and outside temperature. Cormier et al. (1990) also failed to detect such an effect, which they attributed to the short period of the study (January-April; Cormier et al., 1990). However, this explanation does not suffice for our study, which was conducted throughout the year.

4.2. Haemolytic streptococci

The concentration of HS was found to be lower than that in previous studies (Curtis, Drummond, Grunloh, Lynch, & Jensen, 1975; Pavičić et al., 2007). It is possible that the prevalence in the animal population of our study was lower, considering HS are commensals and, thus, highly associated with the animals (Murphy et al., 2012; Palzer et al., 2008). The lack of significant differences shows, at minimum, that close social contact among sows (GH) does not affect airborne HS concentrations. The finding that the concentration of HS on day 5 was significantly lower than on day 33 could be related to the fact that HS were highly present in pig feces (Murphy et al., 2012) and, thus, concentration increased parallel to the soiling, as did the TAB concentration.

4.3. Fungi (molds and yeasts)

The concentrations of molds and yeasts were higher than reported by Cormier et al. (1990) but comparable to findings by Seedorf et al. (1998) and Kim et al. (2008), although the latter two failed to differentiate between molds and yeasts. We did not find any significant differences between the systems with regard to these two parameters either, which, as previously stated, is likely due to similarities in efficient ventilation systems and hygiene routines. The fact that concentrations of molds were significantly higher on day 5 than on day 19 suggests a facility-related influence, such as the contamination of ventilation equipment or feed (Kristiansen, Saunders, Hansen, Nielsen & Nielsen, 2012). Moreover, yeasts deviated highly between batches, indicating an environmental influence such as the load of outside air. Although no differentiation was considered in this study-except between molds and yeasts-factors other than animal- or husbandry related are known to strongly affect differences in fungal genera (Chang, Chung, Huang & Su, 2001). The concentration of yeasts had not increased or decreased over time, reinforcing the assumptions that nonanimal factors, such as fluctuations in the barn's humidity and temperature (Radon et al., 2002), are also relevant. This shows that the means for preventing allergic or pathogenic reactions caused by fungi lie outside the design of housing systems.

4.4. Correlations

LH appears to be closer to FC than to GH in terms of magnitude and orientation of correlation coefficients. This finding indicates that the conditions of group housing changed the interaction between the air parameters compared to individual husbandry. Correlations found between TAB and HS were to be explained by the fact that HS represent a fraction of TAB, although this was only significant in LH and FC and not in GH. It is not surprising that significant correlations between TAB and carbon dioxide were found, as carbon dioxide is a known indicator of the ratio of air exchange (Pedersen et al., 1998). This also shows that the parameters discussed here can be subject to seasonal effects, since ventilation rates are usually reduced to save energy in the cold season. However, this effect is unlikely to play a role in this study, as all systems were examined in parallel over a whole year. But this context could explain differences to findings of other studies.

Thus, these correlations show that TAB concentrations depend at least in part on ventilation but here, too, this connection is much weaker in GH and only significant in LH and FC. High correlations between dust and TAB are often reported (Kim et al., 2005), which results from airborne bacteria binding to dust particles (Seedorf et al., 1998). However, a significant correlation between TAB and dust concentration was only detectable for FC. It cannot be ruled out that the sows' freedom of movement in GH and LH accounted for this result. The significant correlation with total animal weight may be explained by the assumption that the release of TAB follows the animals' weight gain. However, this assumption seems to apply only in GH, in which the correlation was significant. Since the animal's weight depends on the age of the piglets, this could also point to the increasing activity behavior of the piglets as a contributing factor, whereby the exercise of the playing behavior was more spatially restricted in LH and FC.

The correlations between TAB and the microclimate parameters are generally low and quite unevenly distributed in both height and orientation of the values. This is most evident in FC with the humidity parameter: for humidity measured over 120 h a significant correlation coefficient was determined but for the punctual measured humidity no correlation was found. Other studies also come to differing conclusions on correlations between microclimate parameters and air contaminants (Cormier et al., 1990; Hadina, Pinter, Uhitil, Vucemilo & Jaksic, 2009; Kim et al., 2005; Sowiak et al., 2012; Yao, Choi, Lee, Suresh & Zhu, 2010). The changes in the microclimate parameters may be too small to affect the concentration of TAB in these systems, which possess largely constant conditions throughout the year using automated air conditioning technology. However, we previously observed correlations with ammonia concentrations—which is possibly due to its increased volatility-but not with dust concentrations (Lühken et al., 2019). For this reason, we have doubts as to whether there is a corresponding correlation for TAB with microclimate parameters in climate-regulated farrowing houses or whether these simply fail to represent the decisive variables for microbiological air contamination.

5. Conclusions

No significant differences in microbiological air quality between the analyzed housing systems for sows were found. Concerns may be raised that the lack of functional areas, the husbandry in groups, or increased activity of the animals could lead to poorer air quality. A conversion of the husbandry systems to free-farrowing must not necessarily be accompanied by an increase in airborne germ contamination. From an animal hygiene perspective, free-farrowing systems can replace conventional pens with crates without the need for special adaptation of existing ventilation systems or hygiene routines.

Data for reference

The datasets used in the study are available from the corresponding author upon request.

Declaration of Competing interest

The authors declare no conflict of interest.

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