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Binnen- en tussendagvariaties in bacterie- en endotoxineconcentraties in stallen voor vleesvarkens en -kuikens

A.J.A. Aarnink, T.G. van Hattum and N.W.M. Ogink

LIVESTOCK RESEARCH
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In this study the diurnal variations in bacteria and endotoxin concentrations and emissions in houses for finishing pigs and broilers were determined.

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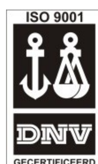

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Foreword

Over the last decade the scale of especially pig and poultry production facilities in rural areas in the Netherlands has drastically increased. Residents living in rural areas with high animal production densities have been alarmed by possible effects of large scale livestock production on their health and their quality of life. Public health concern is mainly related to expected increasing concentrations of fine dust and dust components (e.g. endotoxins) in the environment and possible transmission of airborne diseases from animals to humans (zoonotic diseases). There is a strong need for information that quantifies emissions of these so-called bio-aerosols from livestock operations based on representative measured values. Accurate emission data are indispensable in assessing the exceedance of critical concentration and related risks of bio-aerosol components in residential areas surrounding livestock facilities. Establishing reliable emission data requires sampling and measurement methods that take into account the variation characteristics in concentration and emission of bio-aerosol components. Data providing insight in these variation characteristics however are extremely scarce. This study was commissioned and financed by the Ministry of Economic Affairs to acquire information by monitoring diurnal and between-day variation patterns in a pig house with fatteners and in a broiler house. The results will be used to develop a standardized approach in measuring bio-aerosol emissions from livestock facilities.

Nico Ogink
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Samenvatting

De veehouderij stoot grote hoeveelheden bio-aerosolen uit. Deze bio-aerosolen bevatten micro-organismen, waaronder bacteriën, en componenten van bacteriën, met name endotoxinen. Voor het bepalen van concentraties en emissies van deze micro-organismenencomponenten van micro-organismenmoeten metingen worden gedaan. Om de concentraties en de uitgestoten hoeveelheden goed te kunnen bepalen en te kunnen vergelijken met andere waarden is een goed meetprotocol vereist. Om een meetprotocol te kunnen opstellen is het belangrijk dat er inzicht bestaat in de variaties die zich voor kunnen doen bij deze metingen. Uit eerder onderzoek is bekend dat zich grote variaties kunnen voordoen tussen metingen die gedaan zijn op verschillende dagen van de groeiperiode of tussen metingen die gedaan zijn onder verschillende weersomstandigheden. Er is echter nog weinig bekend over de variatie in bacterie- en endotoxineconcentraties gedurende de dag. Wanneer concentraties van levende bacteriën moeten worden vastgesteld kan een bemonsterings-periode geen 24 uur duren, aangezien de bacteriën dan tijdens de monstername al weer (voor een deel) afsterven. Om afsterving te voorkomen moet de monstername zich beperken tot minder dan één uur. Om representatieve monsternamemomenten tijdens een dag te kunnen vaststellen, moet inzicht bestaan in de variaties die zich tijdens een dag voordoen. De doelstelling van dit onderzoek is om de binnen-dag variatie in bacterie- en endotoxineconcentraties en –emissies vast te stellen. Dit is gedaan voor twee belangrijke diercategorieën, namelijk vleesvarkens en vleeskuikens. In deze studie is tevens vastgesteld wat de bijdrage is van de variatie tussen dagen en binnen dagen op de totale variatie. Tevens zijn onderlinge correlaties en correlaties met fijnstof (PM10), ammoniak (NH_3) en kooldioxide (CO_2) concentraties vastgesteld en zijn correlaties vastgesteld met de temperatuur, luchtvochtigheid en het ventilatiedebiet in de stal.

Deze studie is gedaan in een stal voor vleesvarkens en in een stal voor vleeskuikens in de periode november – december. Het volgende is gemeten tijdens drie perioden van 24 uur:

- Op 6 momenten tijdens de 24-uurs perioden werd de uitgaande lucht gedurende 20 min met impingers bemonsterd voor bepaling van bacterie- en endotoxineconcentraties.
- Continu werd de concentratie fijnstof (PM10) gemeten met een instrument gebaseerd op lichtverstrooiing.
- Continu werd de concentratie CO2 en NH3 gemeten met een foto-akoestisch infrarood multi-gas monitor.
- Continue metingen van de temperatuur en de RV in de stal met een T/RV gecombineerde sensor.
- Continue meting van het ventilatiedebiet met meetventilatoren in de ventilatiekokers.

Monsters in de impingers werden geanalyseerd op totaal bacteriegetal, *Enterobacteriaceae*, *Enterococcus* (alle bacterieconcentraties uitgedrukt in kolonievormende eenheden per m^3 lucht = kve/m^3) en endotoxine (uitgedrukt in endotoxine units per m^3 lucht = EU/m^3). In de data-analyse is het effect van bemonsteringsmoment tijdens de dag en het effect van licht/donker perioden in de stal op de bacterie- en endotoxineconcentraties bepaald. Daarnaast zijn de correlaties bepaald tussen de volgende variabelen: LOG_{10}van Totaal bacteriegetal, *Enterobacteriaceae*, *Enterococcus*, endotoxine, CO_2, en NH_3 concentraties, ventilatie debiet, temperatuur en relatieve luchtvochtigheid.

Bij vleesvarkens (64 – 75 kg) werd een duidelijk dagpatroon waargenomen voor concentraties bacteriën, endotoxine en PM10. Het gemiddeld verschil tussen de tijdstippen van maximum en minimum concentraties waren voor deze variabelen kleiner dan 0,6 LOG_{10}, met het grootste verschil voor totaal bacteriegetal (0,58; minimum = 26% van maximum) en het kleinste verschil voor PM10 (0,23; minimum = 59% van maximum). Voor *Enterococcus* en endotoxine concentratis waren deze verschillen 0,45 (minimum = 36% van maximum) en 0,41 (minimum = 39% van maximum). PM10 ($p<0.001$; medianen: 0,49 vs. 0,68 mg/m^3) en endotoxine ($p=0,02$; medianen: 741 vs. 1202 EU/m^3) concentraties waren significant lager gedurende de nacht dan gedurende de dag.Er was een tendens dat LOG_{10} *Enterococcus* concentratie (in kve/m^3) lager was tijdens de nacht dan tijdens de dag ($p=0,09$; 3.49 vs. 3.70). LOG_{10} totaal bacteriegetal was niet significant lager tijdens de nachtdan tijdens de dag ($p=0.46$; 4.54 vs. 4.63).

Ook bij vleeskuikens (0,75 – 2,52 kg) werd een duidelijk dagpatroon waargenomen voor concentraties bacteriën, endotoxine en PM10. Concentraties waren het laagst tijdens de donkerperioden in de stal. Het gemiddeld verschil tussen de tijdstippen van maximum en minimum (LOG_{10}) concentraties was voor totaal bacteriegetal 1,5 (minimum = 3,2% van maximum)), 0.71 voor *Enterococcus* (minimum = 20% van maximum), 0,53 voor endotoxine (minimum = 30% van maximum) en0,52 voor PM10 (minimum = 30% van maximum). LOG_{10} concentraties van totaal bacteriegetal (in kve/m^3) (p=0,05; 5,59 vs. 6,29), *Enterococcus* (p=0,004; 4,11 vs. 4,64) en concentraties endotoxine (p<0,001; medianen: 93 vs. 257 EU/m^3) en PM10 (p<0,001; medianen: 0,31 vs. 0,89 mg/m^3) waren significant lager tijdens de donkerperioden dan tijdens de lichtperioden in de stal.

Bij vleesvarkens werd een significante relatie gevonden tussen (LOG_{10}) PM10 concentratie en(LOG_{10}) endotoxine concentratie (p<0,01).Bij vleeskuikens werd een significantie relatie gevonden tussen (LOG_{10}) PM10 en(LOG_{10}) *Enterococcus* concentraties (p<0,05) en tussen(LOG_{10}) PM10 en(LOG_{10}) endotoxine concentraties (p<0,001).

De correlatiematrix voor (LOG_{10}) concentraties van totaal bacteriegetal, *Enterococcus*, endotoxine, PM10, NH_3 en CO_2, en temperatuur, RV, ventilatiedebieten diergewicht laten zien dat totaal bacteriegetal alleen was gecorreleerd (p<0,05) met endotoxine concentratie. *Enterococcus* concentratie was gecorreleerd met concentraties endotoxine (p<0.05), PM10 (p<0,001) en NH3 (p<0.001) en met de temperatuur (p<0,01), RV (p<0,001), ventilatiedebiet (p<0,001) en diergewicht(p<0,01). Endotoxine concentratie was gecorreleerd met concentraties PM10 (p<0,01) en CO_2 (p<0,05) en er was een tendens voor een correlatie met de ammoniakconcentratie (p<0,10).

Het volgende kan geconcludeerd worden uit deze studie:

- In deze studie kon geen Enterobacteriaceae worden gedetecteerd in de monsters genomen in de uitgaande lucht van een vleesvarkensafdeling en ook niet in de uitgaande lucht van een vleeskuikenafdeling.
- Er werden duidelijke dagpatronen waargenomen voor concentraties en emissies van bacteriën (totaal bacteriën en Enterococcus), endotoxineen PM10 in afdelingen voor vleesvarkens en vleeskuikens.
- Concentraties waren het hoogst gedurende de licht perioden en het laagst tijdens de donkere perioden in de stal. Zoals voor stof is aangetoond, lijkt dit gerelateerd te zijn aan de activiteit van de dieren.
- In deze studie werd de meeste variatie in concentraties en emissies van totaal bacteriën en endotoxine verklaard door het meetmoment van de dag, terwijl weinig variatie werd verklaard door de meetdag.
- Voor Enterococcus werd de meeste variatie verklaard door de meetdag en veel minder door het meetmoment van de dag.
- Er werden lage correlaties gevonden tussen bacterie-concentraties (totaal bacteriën en Enterococcus) en PM10 concentraties, mogelijk veroorzaakt door de beperkte overlevingstijd van bacteriën in lucht.
- Er werd binnen beide stallen een duidelijke relatie gevonden tussen (LOG_{10}) PM10 en (LOG_{10}) endotoxine concentraties. In deze studie kon, via lineaire regressieanalyse, 53% van de variatie in (LOG_{10}) endotoxine concentratie bij vleesvarkens en 69% van de variatie in (LOG_{10}) endotoxine concentratie bij vleeskuikens worden verklaard door (LOG_{10}) PM10 concentratie.
- Een meetprotocol voor bepaling van representatieve concentraties en emissies van micro-organismen en microbiële componenten in stallen moet rekening houden met de binnen-dag variaties.

Summary

A measuring protocol is needed for determining concentrations and emissions of micro-organisms and health-affecting components from micro-organisms (e.g. endotoxins) in animal houses. Such a protocol needs knowledge about the variations in these concentrations and emissions. From previous studies it is known that large variations may exist between different measurements on different days of the growth cycle or between measurements taken in different seasons. Little is known about the variation in bacteria and endotoxin concentrations within a day. When concentrations of viable bacteria need to be determined in the air, sampling periods could not last for 24 h, but should belimited to less than one hour. To determine representative sampling moments during the day, variations in concentrations and emissions during the day should be known. The objective of this study was to determine the diurnal variations in bacteria and endotoxin concentrations and emissions in houses for finishing pigs and broilers. Furthermore, the contribution to the overall variance of measurements on different days and on measurements at different moments within a day were determined and compared with the residual variance.

The study has been performed in a house for fattening pigs and in a house for broilers in the period November – December 2011. The following was measured during three 24 h periods:

- At 6 moments during the 24 h periodsthe exhaust air was sampled for bacteria and endotoxins during 20 min with impingers.
- Continuous measurement of PM10 concentrations with a light-scattering system.
- Continuous measurements of carbon dioxide (CO2) and ammonia (NH3) concentrations with photo-acoustic infrared multi-gas monitor.
- Continuous measurements of temperature and relative humidity with a T/RH sensor.
- Continuous measurement of the ventilation rate with an anemometer.

Samples from the impingers were analysed for total bacteria count, *Enterobacteriaceae*, and *Enterococcus*(all three in colony forming units per m^3 air = cfu/m^3), and endotoxin (given in endotoxin units per m^3 air = EU/m^3). In the data analysis the effects of sampling time and whether it was light or dark on bacteria and endotoxin concentrations were determined. Correlations were estimated between the following variables: LOG_{10} of Total bacteria, *Enterobacteriaceae*, *Enterococcus*, endotoxin, CO_2, and NH_3 concentrations, ventilation rate, temperature, and humidity.

In fattening pigs clear diurnal patterns of bacteria, endotoxin and PM10 concentrations were found. Mean differences in concentrations between the times of maximum and minimum values were all smaller than 0.6 LOG_{10}, with the largest difference for total bacteria (0.58; minimum = 26% from maximum) and the smallest difference for PM10 (0.23; minimum = 59% from maximum). For *Enterococcus* and endotoxin concentrations these differences were 0.45 (minimum = 36% from maximum) and 0.41 (minimum = 39% from maximum).PM10 ($p<0.001$; geometric means (gmeans): 0.49 vs. 0.68 mg/m^3) and endotoxin ($p=0.02$; gmeans: 741 vs. 1202 EU/m^3) concentrations were significantly lower during the dark period than during the light period of the day. There was a tendency that LOG_{10}*Enterococcus* concentration (in cfu/m^3) was lower during the dark period than during the light period of the day ($p=0.09$; 3.49 vs. 3.70). LOG_{10} total bacteria ($p=0.46$; 4.54 vs. 4.63) was not significantly different between the dark and the light period of the day.

In broilers also clear diurnal patterns of bacteria, endotoxin and PM10 concentrations were found. Concentrations were lowest during the dark periods inside the broiler room. Mean differences between maximum and minimum (LOG_{10}) values were 1.5 for total bacteria (minimum = 3.2% from maximum), 0.71 for *Enterococcus* (minimum = 20% from maximum), 0.53 for endotoxin (minimum = 30% from maximum), and 0.52 for PM10 (minimum = 30% from maximum). LOG_{10}concentrations of total bacteria (in cfu/m^3) ($p=0.05$; 5.59 vs. 6.29), *Enterococcus* ($p=0.004$; 4.11 vs. 4.64) and concentrations of endotoxin ($p<0.001$; gmeans: 93 vs. 257 EU/m^3), and PM10 ($p<0.001$; gmeans: 0.31 vs. 0.89 mg/m^3) were significantly lower during the dark periods than during the light periods inside the broiler room.

In fattening pigs, there was a significant relationship between (LOG_{10}) PM10 concentration and (LOG_{10}) endotoxin concentration ($p<0.01$). In broilers there were significant relationships between (LOG_{10}) PM10 and (LOG_{10}) *Enterococcus*concentrations ($p<0.05$) and between (LOG_{10}) PM10 and (LOG_{10}) endotoxin concentrations ($p<0.001$).

The correlation matrix for (LOG_{10}) concentrations of total bacteria, *Enterococcus*, endotoxins, PM10, NH_3, and CO_2, and temperature, RH, ventilation, and animal weight shows that total bacteria was only correlated ($p<0.05$) with endotoxin concentration. *Enterococcus* was correlated with endotoxin ($p<0.05$), PM10 ($p<0.001$), NH3 ($p<0.001$), temperature ($p<0.01$), RH ($p<0.001$), ventilation ($p<0.001$), and animal weight ($p<0.01$). Endotoxin concentration was correlated with PM10 ($p<0.01$) and CO_2 ($p<0.05$), and there was a tendency for a correlation with ammonia ($p<0.10$).

The following could be concluded from this study:

- In this study no Enterobacteriaceae could be detected in the exhaust air of a room for fattening pigs nor in the exhaust air of a room for broilers.
- There are clear diurnal patterns for bacteria (total bacteria, Enterococcus), endotoxin and PM10 concentrations and emissions in (exhaust air of) rooms for fattening pigs and broilers.
- Concentrations are highest during the light periods and lowest during the dark periods inside the animal rooms. As for dust, this might be related to the activity of the animals.
- Within the measurement set-up of this study, most variance of total bacteria and endotoxin concentrations and emissions was accounted for by measurement time of the day, while little variance was accounted for by measurement date.
- For Enterococcus most variance within this study was accounted for by measurement date and much less by the time of the day.
- Low correlations were found between bacteria concentrations (total bacteria and Enterococcus) and PM10 concentration, probable caused by the limited survival time of bacteria in the air.
- A clear relationship was found between (LOG_{10}) PM10 concentration and (LOG_{10}) endotoxin concentration. Within this study, regression analysis showed that within both animal houses 53% of the variation in (LOG_{10}) endotoxin concentration in pigs and 69% of the variation in (LOG_{10}) endotoxin concentration in broilers could be explained by (LOG_{10}) PM10 concentration.
- A measurement strategy to determine representative concentration and emission levels of micro-organisms and microbial components in animal houses should consider the diurnal variation.

1 Introduction

The increasing scale of especially pig and poultry production in the Netherlands have alarmed neighbouring people by possible effects on their health and quality of life. Public health concern is mainly related to expected increasing concentrations of fine dust and dust components (e.g. endotoxins) in the environment and possible transmission of airborne diseases from animals to humans (zoonotic diseases). This became very actual after the outbreak of Q-fever some years ago.

Although different researchers mention airborne transmission as a possible route of spread of a number of infectious animal diseases and zoonosis, the exact (quantitative) contribution of airborne transmission is still unknown. The formation and emission of these so-called bio-aerosols is a multi-factorial process. A bio-aerosol is defined as a suspension of airborne particles that contain living organisms or were released from living organisms (Cox & Wathes, 1995). Dust and micro-organisms can be carried with the airflow to the outside air. Emitted bio-aerosols are carried by the airflow over shorter or longer distances depending on their size and mass, the exhaust location, the ventilation rate, the exhaust airspeed, the meteorological and geographical circumstances. Depending on the half life time of a certain pathogen under the given circumstances, it can keep its infectivity during a shorter or longer period.

Endotoxin is a major health-affecting component in dust. Research over the last decades has shown that endotoxin exposure is related to deleterious respiratory health effects (Heederik et al., 2007). In the area around pig and poultry farms raised endotoxin concentrations have been measured (Heederik & IJzermans, 2011). In the same study more cases of pneumonia were found of people living in the vicinity of poultry farms.

To develop a measuring protocol for determining concentrations and emissions of micro-organisms and health-affecting components from micro-organisms (e.g. endotoxins), knowledge is needed about the variations in these concentrations and emissions. From previous studies it is known that large variations exist between different measurements on different days of the growing cycle or between measurements taken in different seasons (Chinivasagam et al., 2009; Gartner et al., 2009). Little is known about the variation in bacteria and endotoxin concentrations within a day. When concentrations of viable bacteria need to be determined in the air, sampling periods cannot last for 24 h, but are limited to less than one hour (Lai et al., 2012). To determine representative sampling moments during the day, variations in concentrations and emissions during the day should be known.

The objective of this study was to determine the diurnal variations in bacteria and endotoxin concentrations and emissions in houses for finishing pigs and broilers. Furthermore, the contribution to the overall variance of measurements on different days and on measurements at different moments within a day were determined and compared with the residual variance.

2 Material and methods

The study has been performed in a house for fattening pigs and in a house for broilers in the period November – December 2011.

Pig house
The measurements in the pig house were done at a commercial farm, in one of the rooms for fattening pigs (for age and weight see table 2). The room had 10 partially slatted pens containing 10 pigs each. The pigs had an area of 1.0 m^2/pig. The room was mechanically ventilated, with air inlet through the door and air outlet through an exhaust fan (diameter 56 cm) at the ceiling, which was controlled by the inside temperature. The fan was horizontally placed. See figure 1 for the air outlet with the installed measuring equipment. The pigs received ad libitum feed from a dry-feeder and ad-libitum water from a drinking nipple within the feeder. The room had natural light via windows in the side wall. No artificial light was used during the measurements. The sampling interval times in relation to light or dark inside the animal house is given in table 1.

Figure 1 *Air outlet in the room with fattening pigs and the installed measuring equipment.*

Broiler house
The measurements in the broiler house were done at the experimental farm Het Spelderholt in Lelystad, in one of the rooms for broilers. The room had two pens, one at each side of the alley. The birds were raised on a bedding of white wood shavings. There were 16 broilers per m^2. This is a normal density at the end of a growing period of 6 weeks, with max. broiler weights of 2.6 kg. According regulations the total broiler weight should be less than 42 kg/m^2. However, in practice, during the first 5 weeks a higher density is generally used (20 broilers per m^2). To fulfil regulations a part of the broilers is brought to slaughter at 5 weeks of age. The rooms were heated with plate radiators along the sidewalls underneath the air inlet valve. The air inlet was controlled per room with 12 turning valves, six on each side of the room. Each room had three exhaust fans (60 cm diameter). Each fan could be controlled over its full range (0 to 7000 m^3 h^{-1}); one fan was operated continuously, and the other two operated when needed (temperature controlled). The room was artificially lit. The light scheme including the measuring moments are given in table 1.

Table 1

Time intervals of sampling for bacteria and endotoxins inside the rooms for fattening pigs and broilers in relation to light or dark inside the room.

Animal type	Light scheme[1)]	Light / Dark	Start sampling
Fattening pigs	00:00 – 07:30	Dark	00:00
		Dark	04:00
	07:30 – 17:30	Light	08:00
		Light	12:00
		Light	16:00
	17:30 – 24:00	Dark	20:00
Broilers	07:00 – 10:00	Light	08:15
	10:00 – 11:00	Dark	
	11:00 – 14:00	Light	12:15
	14:00 – 15:00	Dark	
	15:00 – 18:00	Light	16:15
	18:00 – 19:00	Dark	
	19:00 – 23:00	Light	20:15
	23:00 – 03:00	Dark	00:15
	03:00 – 06:00	Light	
	06:00 – 07:00	Dark	06:15

[1)] The room for fattening pigs was naturally lit, while the room for broilers was artificially lit.

Measurements

The following was measured during three 24 h periods in the exhaust air of a room for fattening pigs and in a room for broilers:

- At 6 moments during the 24 h periods (see table 1) the air was sampled for bacteria and endotoxins during 20 min with impingers (AGI-30, 7540, Ace glass Inc., Vineland, USA). Impingers scrub airborne bacteria and other particles into 20 ml buffered peptone water (BPW, bioTRADING Benelux B.V., Mijdrecht, the Netherlands) with 0.005%silicone antifoam. The air flow of AGI-30 is 12.5 L min–1. Samples were taken in duplicate. At each measurement day two blank samples were included as well. These blank samples underwent the same procedure as the other samples, but they were not subjected to an airflow through the liquid. The flow rates of the impingers were checked with a flow meter beforehand and they were within 5% of the nominal value of 12.5 L min^{-1}. Before sampling the impingers were sterilised in an autoclave and transported in an air tight bag.
- Continuous measurement of PM10 concentrations with a light-scattering system (DustTrak, TSI, Inc., Shoreview, Minn.). Minute mean values were stored in the data logger. Two DustTraks were used and the mean values were used in the data analysis.
- Continuous measurements of carbon dioxide (CO_2) and ammonia (NH_3) concentrations with photo-acoustic infrared multi-gas monitor(INNOVA 1312-5, Lumasense Technologies A/S, Ballerup, Denmark). Five minute means were stored in the data logger.
- Continuous measurements of temperature and relative humidity with a T/RH sensor (HygroClip, Rotronic AG, Bassersdorf, Switzerland). Hourly means were stored in the data logger.
- Continuous measurement of the ventilation rate. The number of rotations of the ventilator were counted and a calibration line was used to determine the ventilation rate. Hourly means were stored in the data logger.

The air was sampled in the airflow to the ventilator at a distance from the ventilator shaft of approx. 0.5 m (figure 1). The measurement days in the pig house started at 31 October 16:00 h, 8 November 00:00 h, 15 November 08:00 h. The measurement days in the broiler house started at 29 November 08:15, 12 December 20:15, 20 December 16:15. Directly after each sampling, the impingers were stored at 4^oC in a transportable cooling box. The samplings started at different moments to prevent a confounding effect between time of sampling and storage period of the samples. The analysis of the samples started within 24 h after the last sample was taken. In the lab the volume of the samples was determined (approx. 14 to 15 ml; blanks 20 ml) and of this volume 4 ml was used for analysing the following bacteria:

- Total bacteria count;
- *Enterobacteriaceae*;
- *Enterococcus*.

For each analysis 1.0 ml sampling liquid was used and from this a decimal dilution series was made and plated on plates with certain agar media. Bacteria colonies were counted on those plates with a number of colonies between 15 and 150. When too few bacteria were present on the plate with the smallest dilution (1/10), the original liquid was plated, as well. This was always the case for the *Enterobacteriaceae*.

Total bacteria count
For determining the total bacteria count (colony forming units = cfu) 1 ml of each dilution was pipetted in an empty sterile petri-dish. Then ±20 ml Plate Count Agar (PCA) (temperature ± 40°C) was added and mixed with the bacteria solution. After clotting the plates were incubated for 72 h at 30°C and then the number of colonies were counted.

Enterobacteriaceae
For determining *Enterobacteriaceae*1 ml of each dilution was pipetted in an empty sterile petri-dish. Then 15 ml Violet Red Bile Glucose agar (VRBG)(± 40°C) was added and mixed with the bacteria solution. When the agar became solid, a second VRBG agar layer was poured to create an micro-anaerobe environment. After clotting the plates were incubated for 24 h at 37°C and then the number of colonies were counted.

Enterococcus
Of each dilution 0.1 ml was pipetted on a Slanetz & Bartley agar plate (SB) and with a sterile spatula divided over the media. The plates were incubated for 48 h at 35°C and then the number of colonies were counted.

Endotoxin
Two ml of the rest of the original samples were stored in batches of 1 ml at -20°C for possible future analyses. The remaining part of the sample, approx. 8 ml was centrifuged at 1 000 g for 15 min at room temperature. The upper half of the supernatant per sample was stored at -20°C for analyses of endotoxins. Endotoxins were analysed with the Limulus Amebocyte Lysatetest (LAL-test). This is a quantitative test. A sample is mixed with the LAL in the test kit and incubated at 37°C (±1°C) for 10 minutes. A substrate sample is then mixed with the LAL sample and incubated at 37°C (±1°C) for another 6 minutes. The reaction is stopped with a ‘stop’ reagent. When there is endotoxin in the sample the mixture will get a yellow colour. The light absorption of this sample is then determined with a spectrophotometer at 405-410 nm. Because the absorption is linearly related to the amount of endotoxin in the sample, the endotoxin concentration can be determined with a standard curve. For more information about the LAL-test, see the guidebook of the company delivering this kit (Lonza, 2011).

Data analyses
Data were analysed with the REML procedure of Genstat. Data from the pig house and the broiler house were separately analysed. The effect of the factor sampling time on bacteria and endotoxin concentrations was determined with Date as random factor in the model. The same model was used for the factor light/dark instead of sampling time. The mean of the duplicate values were used in these analyses. For determining the different variance components duplicate values were both used in the analysis. The following variance components were estimated for bacteria and endotoxin concentrations: date, time, and residual . Correlations were estimated between the following variables: (LOG_{10}) Total bacteria, (LOG_{10}) *Enterobacteriaceae*, (LOG_{10}) *Enterococcus*, (LOG_{10}) endotoxin, (LOG_{10}) CO_2, and (LOG_{10}) NH_3 concentrations, ventilation rate, temperature, and humidity. Furthermore, linear regression analyses were performed of (LOG_{10}) bacteria and (LOG_{10}) endotoxin concentrations on (LOG_{10}) PM10, (LOG_{10}) CO_2, and (LOG_{10}) NH_3 concentrations. This analysis estimated parallel lines for the different measurement days. In the correlations and regression analyses the means of the duplicates were used.

3 Results

3.1 Means

In table 2 the means during the measurement days are given. From this table it can be seen that total bacteria concentrations did not change very much in pigs during the successive dates of measurements. In broilers total bacteria concentrations even decreased with age of the birds, probably caused by the increased ventilation rate with age. Total bacteria count was approx. 1 to 2 LOG_{10} higher in broilers than in fattening pigs. *Enterobacteriaceae* could not be detected in any of the samples from the houses for fattening pigs and broilers, therefore they were left out of further analysis. *Enterococcus* concentrations were 3 to 4 LOG_{10} in pigs and at the first measurements at 19 days of age of broilers. In broilers *Enterococcus* concentrations were clearly higher during the second and third measurements compared to the first one. Emissions of total bacteria were not very much influenced by the date of measurement; the same was true for *Enterococcus* emissions in pigs. Emissions of *Enterococcus*, however, were clearly higher during the second and third measurement than during the first measurement. Endotoxin concentrations and emissions were clearly higher in fattening pigs than in broilers. PM10, NH_3, and CO_2 concentrations increased in fattening pigs in the sequential weeks of measurements. This seemed mainly related to the ventilation rate (lower ventilation – higher concentrations).

Table 2
Background data during the measurements in a house for fattening pigs and in a house for broilers.

Animal type	Fattening pigs			Broilers		
Measuring date	31 Oct	8 Nov	15 Nov	29 Nov	12 Dec	20 Dec
Number of animals	100	100	100	1779	1767	1751
Days in room	48	56	63	19	32	40
Animal live weight, kg	64	70	75	0.75	1.82	2.52
Bacteria concentrations, $LOG_{10}(cfu^{1)}/m^3)$						
• Total bacteria	4.59	4.52	4.63	6.28	6.11	5.78
• *Enterobacteriaceae*	0	0	0	0	0	0
• *Enterococcus*	3.44	3.97	3.38	3.34	5.40	4.65
Bacteria emissions per animal, $LOG_{10}(cfu^{1)}/h)$						
• Total bacteria	5.90	5.78	5.72	6.28	6.38	6.11
• *Enterobacteriaceae*	0	0	0	0	0	0
• *Enterococcus*	4.74	5.22	4.46	3.34	5.67	4.99
Endotoxin concentration, $LOG_{10}(EU/m^3)$	2.84	3.04	3.05	2.20	2.41	2.18
Endotoxin emission per animal, $LOG_{10}(EU/h)$	4.14	4.29	4.13	2.20	2.69	2.52
PM10, mg/m^3	0.42	0.68	0.71	0.33	0.96	1.08
NH_3, ppm	18.3	22.8	29.5	2.7	5.8	3.7
CO_2, ppm	1593	2520	3532	1692	1698	1677
Temperature, °C	24.5	24.6	23.6	24.3	21.3	19.0
RH, %	58.0	59.3	60.4	50.7	58.5	61.1
Ventilation per animal, m^3/h	20.4	18.0	12.3	1.23	2.35	2.78

1) Colony forming units

In broilers PM10 concentrations increased towards the end of the growing period, NH_3 concentrations were highest at day 32 and CO_2 concentrations was similar at the different days. Ventilation rate was quite low in fattening pigs during the second and third measurement. At the third measurement day in fattening pigs the CO_2 concentration was even higher than the maximum advised limit (3000 ppm) in the Netherlands (Klimaatplatform Varkenshouderij, 2008).

3.2 Diurnal patterns

3.2.1 Fattening pigs

In figure 2 the diurnal patterns are given of (LOG_{10}) bacteria, endotoxin, and PM10 concentrations in fattening pigs. Concentrations were lowest in the early morning at 04:00, except for PM10, with lowest concentrations in the evening at 20:00. Concentrations of total bacteria and *Enterococcus* were highest in the afternoon at 16:00, while concentrations of endotoxin and PM10 were highest in the morning at 08:00. Mean differences in concentrations between the times of maximum and minimum values were all smaller than 0.6 LOG_{10}, with the largest difference for total bacteria (0.58; minimum = 26% from maximum) and the smallest difference for PM10 (0.23; minimum = 59% from maximum). For *Enterococcus* and endotoxin concentrations these differences were 0.45 (minimum = 36% from maximum) and 0.41 (minimum = 39% from maximum). The effect of measurement time, however, was only significant for LOG_{10} PM10 concentration (P=0,003), but not for LOG_{10} total bacteria (p=0.15), *Enterococcus* (p=0.25), and endotoxin (p=0.26) concentrations. PM10 (p<0.001; geometric means (gmeans): 0.49 vs. 0.68 mg/m^3) and endotoxin (p=0.02; gmeans: 741 vs. 1202 EU/m^3) concentrations were significantly lower during the dark period than during the light period of the day. There was a tendency that LOG_{10}*Enterococcus* concentration (in cfu/m^3) was lower during the dark period than during the light period of the day (p=0.09; 3.49 vs. 3.70). LOG_{10} total bacteria (p=0.46; 4.54 vs. 4.63) was not significantly different between the dark and the light period of the day.

In figure 3 the diurnal patterns are given of the emissions of (LOG_{10}) bacteria, endotoxin, and PM10 in fattening pigs. Emissions were lowest in the early morning at 04:00 and highest in the afternoon at 16:00. Mean differences between maximum and minimum values were all smaller than 0.8 LOG_{10}, with the largest difference for total bacteria (0.77; minimum = 17% from maximum) and the smallest difference for PM10 (0.27; minimum = 54% from maximum). For *Enterococcus* and endotoxin emissions these differences were 0.66 (minimum = 22% from maximum) and 0.47 (minimum = 34% from maximum). A significant effect was found of measurement time on (LOG_{10}) total bacteria (p=0.04) and PM10 (p=0.001) emissions, and there was a tendency towards an effect of measurement time on emission of (LOG_{10}) *Enterococcus* (p=0.08). (LOG_{10}) endotoxin emission was not significantly affected by measurement time (P=0.16). The dark and light periods of the day had no significant effect on (LOG_{10}) total bacteria emission (p=0.32). There was a tendency (p=0.08; 4.68 vs. 4.94) that (LOG_{10}) *Enterococcus* emission was lower during the dark period than during the light period of the day. Endotoxin (p=0.004; gmeans: 11 200 vs. 20 900 EU/h per pig) and PM10 (p<0.001; gmeans: 7.6 vs. 11.7 mg/h per pig) emissions were significantly lower during the dark period than during the light period of the day.

3.2.2 Broilers

In figure 4 the diurnal patterns are given of (LOG_{10}) bacteria, endotoxin, and PM10 concentrations in broilers. Concentrations were lowest during the dark periods of the measurements, at 06:15 for total bacteria and endotoxin, and at 00:15 for *Enterococcus* and PM10. Concentrations were highest during the light periods, at 16:15 for total bacteria, at 12:15 for *Enterococcus* and PM10, and at 08:15 for endotoxin. Mean differences between maximum and minimum values were 1.5 for total bacteria (minimum = 3.2% from maximum), 0.71 for *Enterococcus* (minimum = 20% from maximum), 0.53 for endotoxin (minimum = 30% from maximum), and 0.52 for PM10 (minimum = 30% from maximum). Differences within the light periods were small for *Enterococcus* (max. 0.16; minimum = 69% from maximum), endotoxin (max. 0.14; minimum = 72% from maximum), and PM10 (max. 0.10; minimum = 79% from maximum).The effects of measurement time on (LOG_{10}) total bacteria (p=0.11) and *Enterococcus* (p=0.18) concentrations were not statistically significant. (LOG_{10})

endotoxin ($p=0.004$) and PM10 ($p<0.001$) concentrations, however, were significantly affected by measurement time. LOG_{10}concentrations of total bacteria (in cfu/m^3) ($p=0.05$; 5.59 vs. 6.29), *Enterococcus* ($p=0.004$; 4.11 vs. 4.64) and concentrations of endotoxin ($p<0.001$; gmeans: 93 vs. 257 EU/m^3), and PM10 ($p<0.001$; gmeans: 0.31 vs. 0.89 mg/m^3) were significantly lower during the dark periods than during the light periods of the day.

In figure 5 the diurnal patterns are given of the emissions of (LOG_{10}) bacteria, endotoxin, and PM10 in broilers. Emissions were lowest during the dark periods of the measurements, at 06:15 for total bacteria and endotoxin, and at 00:15 for *Enterococcus* and PM10. Emissions were highest during the light periods, at 16:15 for total bacteria, at 12:15 for *Enterococcus* and PM10, and at 08:15 for endotoxin. Mean differences between maximum and minimum values were 1.5 for total bacteria (minimum = 3.2% from maximum), 0.69 for *Enterococcus* (minimum = 20% from maximum), 0.54 for endotoxin (minimum = 29% from maximum), and 0.51 for PM10 (minimum = 31% from maximum). Differences within the light periods were small for *Enterococcus* (max. 0.15; minimum = 71% from maximum), endotoxin (max. 0.16; minimum = 69% from maximum), and PM10 (max. 0.08; minimum = 83% from maximum).A tendency was found for an effect of measurement time on emission of (LOG_{10}) total bacteria ($p=0.10$). (LOG_{10}) *Enterococcus* emission was not significantly affected by measurement time ($p=0.17$). (LOG_{10}) endotoxin ($p=0.003$) and PM10 ($p<0.001$) emissions were significantly affected by measurement time. The dark and light periods of the day had significant effects on (LOG_{10}) emissions (in cfu/h per broiler) of total bacteria ($p=0.04$; 5.59 vs. 6.29) and *Enterococcus* ($p=0.003$; 4.29 vs. 4.85), and emission of endotoxin ($p<0.001$; gmeans: 186 vs. 513 EU/h per broiler) andof PM10 ($p<0.001$; gmeans: 0.63 vs. 1.78 EU/h per broiler).

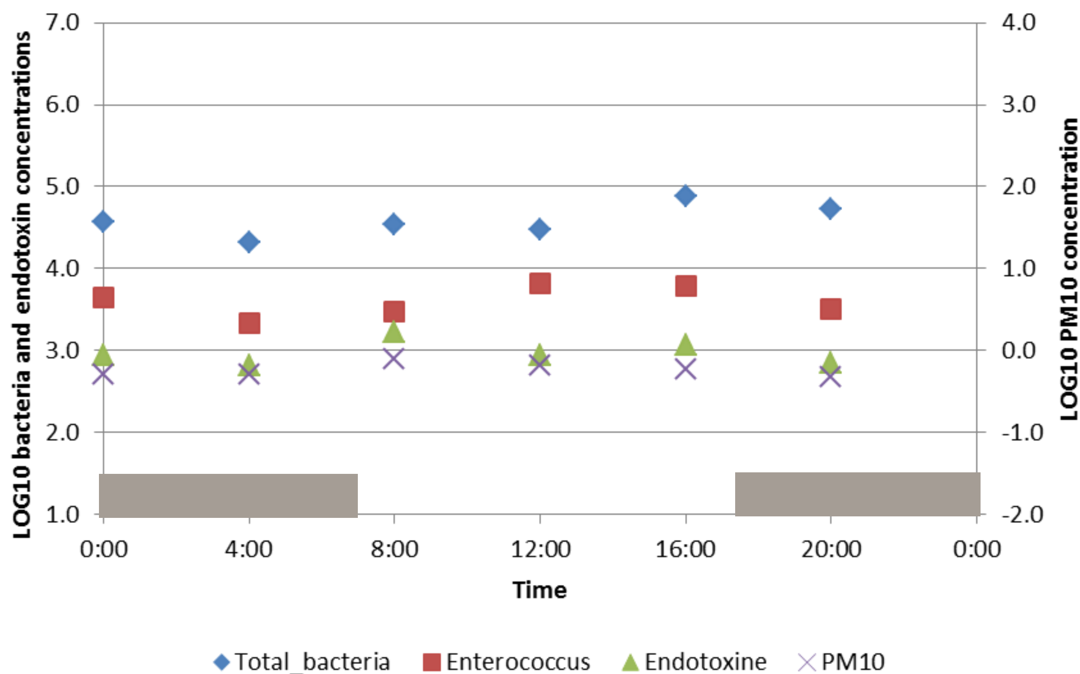


Figure 2 *Diurnal patterns of bacteria (in LOG_{10}(cfu/m^3)), endotoxin (in LOG_{10}(EU/m^3)), and PM10 (in LOG_{10}(mg/m^3)) concentrations in fattening pigs. The dark periods inside the animal house are shown in grey bars.*

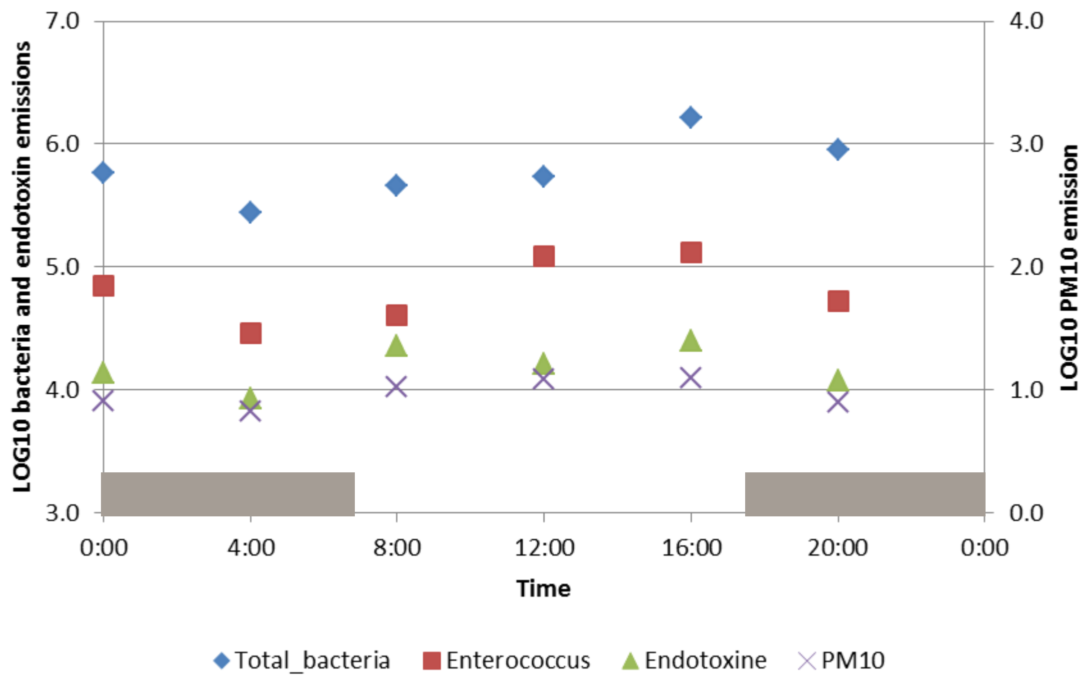


Figure 3 *Diurnal patterns of bacteria (in LOG_{10}(cfu/h)), endotoxin (in LOG_{10}(EU/h)), and PM10 (in LOG_{10}(mg/h)) emissions in fattening pigs.The dark periods inside the animal house are shown in grey bars.*

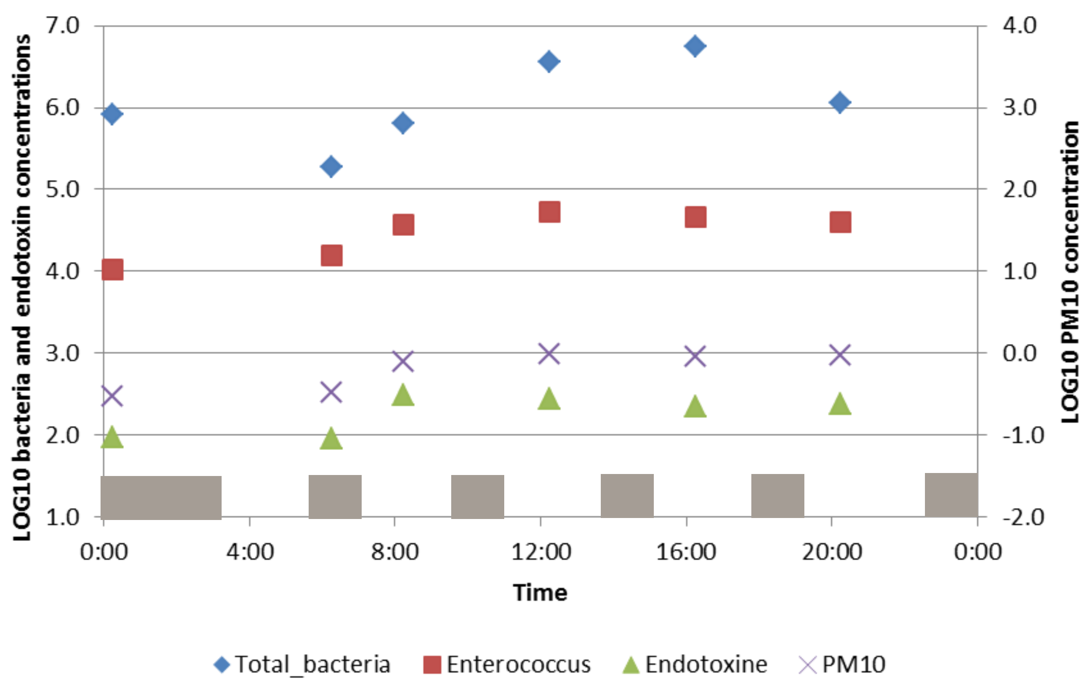


Figure 4 *Diurnal patterns of bacteria (in LOG_{10}(cfu/m^3)), endotoxin (in LOG_{10}(EU/m^3)), and PM10 (in LOG_{10}(mg/m^3)) concentrations in broilers. The dark periods inside the animal house are shown in grey bars.*

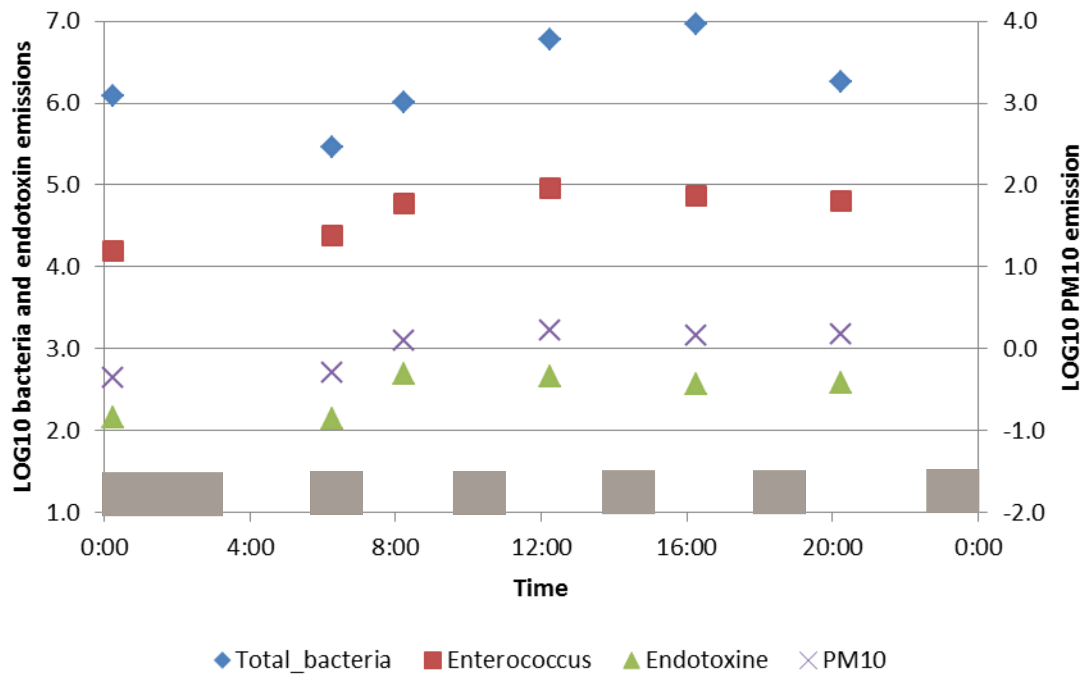


Figure 5 *Diurnal patterns of bacteria (in LOG_{10}(cfu/h)), endotoxin (in LOG_{10}(EU/h)), and PM10 (in LOG_{10}(mg/h)) emissions in broilers.The dark periods inside the animal house are shown in grey bars.*

3.3 Variance components

In tables 3 and 4 the variance component analysis are given for (LOG_{10}) concentrations and (LOG_{10}) emissions of total bacteria, *Enterococcus*, and endotoxin in a house for fattening pigs (table 3) and in a house for broilers (table 4). The tables show that the contribution to the overall variance is different for the different variables. The variance for total bacteria is mainly caused by sampling time and residual error, while variance for *Enterococcus* is mainly caused by measurements on different dates. In fattening pigs a part of the variance of *Enterococcus* is also caused by Time and Residual error. For endotoxin variance is mainly accounted for by Time. For PM10 variance is mainly accounted for by Date, although Time also accounts for a relatively high proportion of the variance. The variance accounted for by Residual error is small for PM10 concentrations and emissions. From the tables it can also be seen that the variance components have a large variation.

Table 3
Variance accounted for by Date and Time to (LOG_{10}) concentrations of bacteria, endotoxins, and PM10 for fattening pigs. The mean, the standard error (s.e.) and the relative variance accounted for (sum=1) are given. The Residual variance includes the residual variance of Date and Time and the variance caused by duplicate measurements.

	Variable		Date[1)]	Time	Residual
Concentration	• Total bacteria				
		○ Component	-0.007	0.037	0.036
		○ s.e.	0.004	0.022	0.013
		○ Relative	-0.11	0.56	0.55
	• *Enterococcus*				
		○ Component	0.088	0.026	0.070
		○ s.e.	0.098	0.025	0.023
		○ Relative	0.48	0.14	0.38
	• Endotoxin				
		○ Component	0.007	0.038	0.006
		○ s.e.	0.014	0.015	0.002
		○ Relative	0.14	0.74	0.12
	• PM10				
		○ Component	0.013	0.008	0.0007
		○ s.e.	0.015	0.003	0.0002
		○ Relative	0.60	0.37	0.03
Emission	• Total bacteria				
		○ Component	-0.008	0.066	0.037
		○ s.e.	0.008	0.032	0.013
		○ Relative	-0.08	0.69	0.39
	• *Enterococcus*				
		○ Component	0.119	0.057	0.070
		○ s.e.	0.134	0.035	0.023
		○ Relative	0.48	0.23	0.28
	• Endotoxin				
		○ Component	0.000	0.046	0.0060
		○ s.e.	0.008	0.018	0.0020
		○ Relative	-0.01	0.89	0.12
	• PM10				
		○ Component	0.0046	0.0128	0.0007
		○ s.e.	0.0069	0.0048	0.0002
		○ Relative	0.26	0.71	0.04

[1)] *In fact the variance accounted for cannot be negative, but this is caused by the way of calculation. A negative value means that the accounted variance is very small for this factor.*

Table 4
Variance accounted for by Date and Time to (LOG_{10}) concentrations of bacteria, endotoxins, and PM10 for broilers. The mean, the standard error (s.e.) and the relative variance accounted for (sum=1) are given. The Residual variance includes the residual variance of Date and Time and the variance caused by duplicate measurements.

	Variable	Date	Time	Residual
Concentration	• Total bacteria			
	○ Component	0.12	0.19	0.37
	○ s.e.	0.18	0.16	0.13
	○ Relative	0.17	0.28	0.54
	• *Enterococcus*			
	○ Component	0.95	0.10	0.09
	○ s.e.	0.97	0.06	0.03
	○ Relative	0.83	0.09	0.08
	• Endotoxin			
	○ Component	0.001	0.057	0.018
	○ s.e.	0.012	0.024	0.006
	○ Relative	0.01	0.75	0.24
	• PM10			
	○ Component	0.068	0.059	0.004
	○ s.e.	0.079	0.022	0.001
	○ Relative	0.52	0.45	0.03
Emission	• Total bacteria			
	○ Component	0.03	0.21	0.37
	○ s.e.	0.10	0.16	0.13
	○ Relative	0.05	0.34	0.61
	• *Enterococcus*			
	○ Component	1.27	0.11	0.09
	○ s.e.	1.30	0.06	0.03
	○ Relative	0.86	0.07	0.06
	• Endotoxin			
	○ Component	0.032	0.065	0.018
	○ s.e.	0.045	0.028	0.006
	○ Relative	0.28	0.57	0.16
	• PM10			
	○ Component	0.195	0.068	0.004
	○ s.e.	0.207	0.026	0.001
	○ Relative	0.73	0.25	0.02

3.4 Relationships

3.4.1 Fattening pigs

Table 5 shows, for fattening pigs, the correlation matrix for (LOG_{10}) concentrations of total bacteria, *Enterococcus*, endotoxins, PM10, NH_3, and CO_2, and temperature, RH, ventilation, and animal weight. This table shows that for total bacteria there was only a tendency for a correlation ($p<0.10$) with RH. *Enterococcus* was correlated with temperature ($p<0.01$), and there was a tendency for a correlation with ventilation ($p<0.10$). Endotoxin concentration was strongly correlated with PM10 ($p<0.001$), and to a lesser extent with RH ($p<0.05$). There was a tendency for a correlation with CO_2 and animal weight ($p<0.10$).

Regression analysis only showed a significant effect of (LOG_{10}) PM10 concentration on (LOG_{10}) endotoxin concentration (estimated regression coefficient = 1.56 (s.e. 0.41); $p<0.01$). These parallel regression lines for the different dates explained 53% of the variation in (LOG_{10}) endotoxin concentration. (LOG_{10}) NH_3 and (LOG_{10}) CO_2 concentrations did not significantly contribute to explanation of variations in (LOG_{10}) total bacteria, *Enterococcus*, and endotoxin concentrations.

3.4.2 Broilers

Table 6 shows, for broilers, the correlation matrix for (LOG_{10}) concentrations of total bacteria, *Enterococcus*, endotoxins, PM10, NH_3, and CO_2, and temperature, RH, ventilation, and animal weight. This table shows that total bacteria was only significantly correlated ($p<0.05$) with endotoxin concentration. *Enterococcus* was correlated with endotoxin ($p<0.05$), PM10 ($p<0.001$), NH3 ($p<0.001$), temperature ($p<0.01$), RH ($p<0.001$), ventilation ($p<0.001$), and animal weight ($p<0.01$).

Endotoxin concentration was correlated with PM10 ($p<0.01$) and CO_2 ($p<0.05$), and there was a tendency for a correlation with ammonia ($p<0.10$).

Regression analysis showed significant effects of (LOG_{10}) PM10 concentration on (LOG_{10}) *Enterococcus* (estimated regression coefficient = 1.03 (s.e. 0.35); $p<0.05$) and endotoxin (estimated regression coefficient = 0.90 (s.e. 0.16); $p<0.001$) concentrations. These parallel regression lines for the different dates explained 88% of the variation in (LOG_{10}) *Enterococcus* concentration and 69% of the variation in (LOG_{10}) endotoxin concentration. (LOG_{10}) NH_3 concentration did not significantly contribute to explanation of variations in (LOG_{10}) concentrations of total bacteria, *Enterococcus*, and endotoxin.(LOG_{10}) CO_2 concentration significantly contributed to explanation of variations in (LOG_{10}) *Enterococcus* (estimated regression coefficient = 8.06 (s.e. 2.8); $p<0.05$; 88% variance accounted for) and endotoxin (estimated regression coefficient = 5.17 (s.e. 1.8); $p<0.05$; 35% variance accounted for) concentrations, but not to (LOG_{10}) total bacteria concentration. It should be noted that the different concentration levels between different dates accounted for most of the variance in these analyses.

Table 5

Correlation matrix, for fattening pigs, for (LOG_{10}) concentrations of total bacteria, Enterococcus*, endotoxins, PM10, NH_3, and CO_2, and temperature, RH, ventilation, and animal weight.*

		1	2	3	4	5	6	7	8	9	10
Total bacteria	1	-									
Enterococcus	2	0.17	-								
Endotoxin	3	0.36	0.30	-							
PM10	4	0.00	0.35	0.76***	-						
NH3	5	-0.13	-0.20	0.38	0.67**	-					
CO2	6	0.14	0.05	0.45’	0.69**	0.86***	-				
Temperature	7	0.36	0.67**	-0.10	-0.33	-0.74***	-0.46’	-			
RH	8	0.45’	0.23	0.58*	0.48*	0.04	0.26	0.22	-		
Ventilation	9	0.38	0.46’	-0.19	-0.48*	-0.90***	-0.66**	0.94***	0.19	-	
Animal weight	10	0.06	-0.02	0.42’	0.71***	0.90***	0.97***	-0.55*	0.25	-0.73***	-

*Superscript signs indicate significant difference from zero: ‘ = $p<0.10$; * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$*

Table 6

Correlation matrix, for broilers, for (LOG_{10}) concentrations of total bacteria, Enterococcus*, endotoxins, PM10, NH_3, and CO_2, and temperature, RH, ventilation, and animal weight.*

		1	2	3	4	5	6	7	8	9	10
Total bacteria	1	-									
Enterococcus	2	0.07	-								
Endotoxin	3	0.50*	0.54*	-							
PM10	4	0.12	0.76***	0.64**	-						
NH3	5	0.06	0.89***	0.46’	0.65**	-					
CO2	6	0.31	0.23	0.57*	0.49*	0.24	-				
Temperature	7	0.27	-0.62**	0.00	-0.65**	-0.46’	0.09	-			
RH	8	-0.01	0.79***	0.29	0.81***	0.62**	0.27	-0.82***	-		
Ventilation	9	-0.21	0.78***	0.18	0.78***	0.65**	0.05	-0.95***	0.91***	-	
Animal weight	10	-0.29	0.66**	0.03	0.68**	0.49*	-0.06	-0.99***	0.86***	0.97***	-

*Superscript signs indicate significant difference from zero: ‘ = $p<0.10$; * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$*

4 Discussion

To develop a measuring protocol for determining concentrations and emissions of micro-organisms and health-affecting components from micro-organisms (e.g. endotoxins), knowledge is needed about the variations in these concentrations and emissions. In this study we could not detect any *Enterobacteriaceae*, not in fattening pigs and not in broilers. This is remarkable while in other studies a lot of *Enterobacteriaceae* were found in animal houses. In a study of Seedorf et al. (1998) this was on average more than 10^3 in broilers and more than 10^4 in fattening pigs. The calculated detection limit in our study for *Enterobacteriaceae* was 56 cfu/m^3, so far lower than the concentrations found in the study of Seedorf et al. (1998).

Within this study the diurnal variations in bacteria and endotoxin concentrations and emissions in houses for finishing pigs and broilers was determined. The study showed that there was a clear diurnal pattern in both animal houses for bacteria, endotoxin and PM10 concentrations and emissions. Concentrations were highest during the light periods and lowest during the dark periods of the day. In broilers for all these concentrations a significant effect was found of the dark and light periods. In pigs, however, differences between dark and light periods were only significant for endotoxin and PM10. The higher values during the light periods seem to be mainly related to the higher activity of the animals during these periods. For dust this relationship with animal activity has been shown before (Pedersen, 1993; Pedersen & Takai, 1999). As bacteria and endotoxins are part of the dust, it seems logical that concentrations of these variables are also related to animal activity. In broilers differences in concentrations and emissions between the dark and light periods were more pronounced than in fattening pigs. This might also be related to differences in animal activity. Broilers are all resting during the dark periods, while pigs still keep some activity during the night. Despite the clear diurnal pattern, concentrations of bacteria and endotoxin are still high during the dark periods of the day, e.g. in fattening pigs, total bacteria concentrations were 4.54 LOG_{10} in the dark compared to 4.63 LOG_{10} in the light; in broilers, total bacteria concentrations were 5.59 LOG_{10} in the dark compared to 6.29 LOG_{10} in the light. For LOG_{10} endotoxin this was 2.87 vs. 3.08 in fattening pigs and 1.97 vs. 2.41 in broilers.

Estimations were made of the variances accounted for by measurement date and measurement time of the day on concentrations and emissions of bacteria and endotoxin. Most variance of total bacteria and endotoxin concentrations and emissions was accounted for by measurement time of the day, while little variance was accounted for by date. This was valid for fattening pigs and for broilers. It should be noted, however, that this conclusion is only valid for the time frame of this study. In fattening pigs we only measured during the period 31 October until 15 November at days 48, 56, and 63 of the fattening period. In broilers we measured from 29 November until 20 December at days 19, 32, and 40 of the growing period. When we would have measured at different days during the whole growing periods of fattening pigs and broilers and during different seasons, the contribution of date to the total variance might have been (a lot) higher. It is remarkable that for *Enterococcus* most variance was accounted for by measurement date and much less by the time of the day. This was also valid for both fattening pigs and broilers. At this moment we cannot give a clear explanation for this difference. It might have something to do with the environmental conditions for survival of *Enterococcus*.

The correlation matrix showed very low correlations between the bacteria concentrations (total and *Enterococcus*) and PM10 concentration. This might be caused by the fact that bacteria have a limited survival time in the air. Dust is often accumulated in time and becomes airborne with animal activity. In 'older' dust probably less viable bacteria will be present. The viable bacteria will be mainly present in 'fresh' dust. The fact that a clear relationship was found between PM10 concentration and endotoxin concentration seems to support this hypothesis. Endotoxin is a cell wall compound from gram-negative bacteria and its concentration is not depending on weather the bacteria are alive or dead. Regression analysis showed (LOG_{10}) PM10 concentrations could explain 53% in pigs and 69% in broilers of the variation in (LOG_{10}) endotoxin concentration. It should be noted that we only measured at one farm

for fattening pigs and one farm for broilers. At other farms relationships and correlations might be different. With respect to concentration and emission differences between fattening pigs and broilers the same should be noted: because of the limited number of measured farms (one per category) we cannot conclude about systematic differences between these types of farms. This study only gives indications in this respect.

From this study it can be concluded that with respect to a measuring strategy for micro-organisms and endotoxins it is important to consider the diurnal variations in concentrations. In the report of Aarnink et al. (2015) the following sampling strategy within a sampling day is proposed for micro-organisms: 'Within a measuring day samplings should especially be done at moments of expected high emission levels, so during light hours with high activity levels within the animal house. Additional measurements should be done to get insight in the diurnal variations.' For microbial components like endotoxins the following sampling strategy within a sampling day is proposed in former mentioned report: 'Binnen dagen; monsters moeten bij voorkeur worden genomen gedurende 24 uur of een meervoud hiervan. Wanneer de monsterperioden korter zijn dan moet een goed inzicht worden verkregen in de binnen-dag variatie, b.v. door het continu meten van de stof (PM10) concentratie gedurende 24 uur.' Bij beide meetstrategieën wordt dus rekening gehouden met de binnen-dag variatie in emissies. Het onderzoek beschreven in dit rapport bewijst de noodzaak van deze strategie.

5 Conclusions

The objective of this study was to determine the diurnal variations in bacteria and endotoxin concentrations and emissions and their correlations with dust concentrations and emissions in houses for finishing pigs and broilers. Furthermore, the contribution to the overall variance of measurements on different days and on measurements at different moments within a day were determined and compared with the residual variance.

The following could be concluded from this study:

- In this study no *Enterobacteriaceae* could be detected in the exhaust air of a room for fattening pigs nor in the exhaust air of a room for broilers.
- There are clear diurnal patterns for bacteria (total bacteria, *Enterococcus*), endotoxin and PM10 concentrations and emissions in (exhaust air of) rooms for fattening pigs and broilers.
- Concentrations are highest during the light periods and lowest during the dark periods of the day. As for dust, this might be related to the activity of the animals.
- Within the measurement set-up of this study, most variance of total bacteria and endotoxin concentrations and emissions was accounted for by measurement time of the day, while little variance was accounted for by measurement date.
- For *Enterococcus* most variance within this study was accounted for by measurement date and much less by the time of the day.
- Low correlations were found between bacteria concentrations (total bacteria and *Enterococcus*) and PM10 concentration, probably caused by the limited survival time of bacteria in the air.
- A clear relationship was found between (LOG10) PM10 concentration and (LOG10) endotoxin concentration. Within this study, regression analysis showed that 53% of the variation in (LOG10) endotoxin concentration in pigs and 69% of the variation in (LOG10) endotoxin concentration in broilers could be explained by (LOG10) PM10 concentration.
- A measurement strategy to determine representative concentration and emission levels of micro-organisms and microbial components in animal houses should consider the diurnal variation.

Literature

Aarnink, A.J.A., Zhao, Y., Ogink, N.W.M. 2015. Measurement protocol for emissions of bio-aerosols from animal houses. Wageningen UR (University & Research centre), concept rapport

Chinivasagam, H.N., Tran, T., Maddock, L., Gale, A., Blackall, P.J. 2009. Mechanically Ventilated Broiler Sheds: a Possible Source of Aerosolized Salmonella, Campylobacter, and Escherichia coli. *Applied and Environmental Microbiology*, **75**(23), 7417-7425.

Cox, C.S., Wathes, C.M. 1995. *Bioaerosols Handbook*. CRC Press, Boca Raton, Florida.

Gartner, A., Gessner, A., Jackel, U. 2009. Determination of microorganism emissions from a poultry house. *Gefahrstoffe Reinhaltung der Luft*, **69**(9), 359-362.

Heederik, D., Sigsgaard, T., Thorne, P.S., Kline, J.N., Avery, R., Bonlokke, J.H., Chrischilles, E.A., Dosman, J.A., Duchaine, C., Kirkhorn, S.R., Kulhankova, K., Merchant, J.A. 2007. Health effects of airborne exposures from concentrated animal feeding operations. *Environmental Health Perspectives*, **115**(2), 298-302.

Heederik, D.J.J., IJzermans, C.J. 2011. Mogelijke effecten van intensieve-veehouderij op de gezondheid van omwonenden: onderzoek naar potentiële blootstelling en gezondheidsproblemen. IRAS Universiteit Utrecht, NIVEL, RIVM, 7 juni 2011.

Klimaatplatform Varkenshouderij. 2008. Richtlijnen klimaatinstellingen., http://www.wageningenur.nl/upload_mm/c/c/a/79a3b1e7-b7cc-4e3d-b0ad-5c84d43bc48b_Richtlijnen_klimaatinstellingen juni2008.pdf.

Lai, H.T.L., Nieuwland, M.G.B., Aarnink, A.J.A., Kemp, B., Parmentier, H.K. 2012. Effects of 2 size classes of intratracheally administered airborne dust particles on primary and secondary specific antibody responses and body weight gain of broilers: A pilot study on the effects of naturally occurring dust. *Poultry Science*, **91**(3), 604-615.

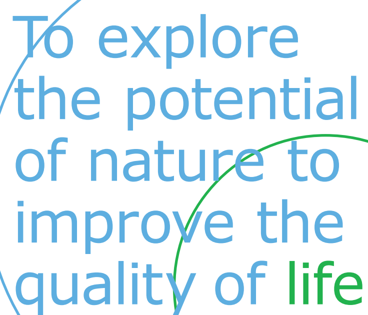
Lonza. 2011. Limulus Amebocyte Lysate (LAL); QCL-1000™. Lonza. Catalog Number: 50-647U, 50-648U; Certificate of Analysis at www.lonza.com/coa.

Pedersen, S. 1993. Time based variation in airborne dust in respect to animal activity. *Proceedings Fourth International Livestock Environment Symposium, Warwick-England, 6-9 July. p. 718-725*.

Pedersen, S., Takai, H. 1999. Dust response to animal activity. *Proceedings Int. Symp. on Dust Control in Animal Production Facilities, 30 May - 2 June.*, Aarhus, Denmark. Danish Institute of Agricultural Sciences, Horsens, Denmark. pp. 306-309.

Seedorf, J., Hartung, J., Schroder, M., Linkert, K.H., Phillips, V.R., Holden, M.R., Sneath, R.W., Short, J.L., White, R.P., Pedersen, S., Takai, H., Johnsen, J.O., Metz, J.H.M., Groot Koerkamp, P.W.G., Uenk, G.H., Wathes, C.M. 1998. Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings in Northern Europe. *Journal of Agricultural Engineering Research*, **70**(1), 97-109.

Smit, L.A.M., Wouters, I.M., Heederik, D., Douwes, J. 2009. Health effects of occupational endotoxin exposure: a review and relevance to veterinary practice. *Tijdschrift voor Diergeneeskunde*, **134**(20), 840-846.



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