Determinants of endotoxin levels inside pig confinement buildings

Pilot study findings and recommendations for further research

Research Thesis Veterinary Medicine Utrecht University

R. J. Cummins, BSc.
Student no. 3402657

29th August 2018

Project supervisors

GD Animal Health — Manon Houben, DVM, Dipl. ECPHM
Institute for Risk Assessment Sciences — dr. ir. Inge Wouters

Abstract

There is a growing concern about the emission of endotoxins by farms into the surrounding environment. Endotoxins have been linked to respiratory disease in both humans and pigs. Endotoxin levels in stables may exceed 1000 EU/m^3 in a majority of pig farms. This pilot case-control study aims to determine the endotoxin levels inside pig confinement buildings and determine if there is are any associations between these levels, herd respiratory health and potential exposure determinants, such as ventilation and other farm management characteristics, as well as associations between herd respiratory health and stable management characteristics. Thirteen pig confinement rooms housing weaners aged 33 to 66 days were visited once during May and June 2018. Determinants were inventoried and an electrostatic dust collector (EDC) was left in the room for two weeks. High levels of endotoxins were found (GM 1016 x 10^4 EU/m^2). No significant association was found between endotoxin levels and farmers or veterinarians reporting a history of herd respiratory health problems ($p = 0.22$). Associations were found between a reported history of respiratory disease and building age (OR = 0.760; $p = 0.045$) and plastic slatted floors (OR = 30; $p = 0.027$). Feed protein content may be a possible determinant for endotoxin level ($b_1 = 0.025$; $p = 0.053$). We make recommendations for future research in this area, most importantly improving the generalizability of the sample population and investigating the spatial aspects of endotoxin detection using EDC's.

Table of contents

Introduction

Background

Endotoxins are lipopolysaccharides (LPS) that occur in the cell wall of gram-negative bacteria and are known to have a strong pro-inflammatory effect on both human and animal alike[1–6]. They can be found in organic dust in various (occupational) environments, such as inside and outside farms, but also in households[1,2]. Endotoxin levels are expressed in endotoxin units (EU) per m^2 in cases where surface areas are sampled and m^3 in cases of air volume[7].

There is a growing concern about the emission of endotoxins into the general environment by farms. Based on occupational exposure studies and experimental research, the Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council recommended an occupational exposure limit of 90 EU/m^3 [8]. For environmental exposure of the general population a limit of 30 EU/m^3 was advised [9]. This limit is not based on any direct evidence from environmental research, but by applying a standard factor 3 safety margin on the occupational exposure limit. Unfortunately, at the time there was not enough research available concerning the endotoxin levels in the areas surrounding farms and the dose-response relationship under these circumstances. It thus remains an area of ongoing research to identify if there is a truly safe level of environmental endotoxin exposure, and if so, which level marks the safety limit.

Since the publication of those recommendations, there has been a Dutch study called Livestock Farming and Neighbouring Residents' Health. Among other things, the study reports the endotoxin levels at residential areas surrounding livestock farms. Based on an annual average, endotoxin levels in PM10 within an area of 250 m around a livestock farm vary between 0.18 – 0.85 EU/m^3 [10]. However, this does not rule out that there are higher levels present at certain peak moments in time[11]. Much is still unknown considering the health risk to the public, warranting a certain level of caution is when it comes to endotoxin emissions. Considering the known risk to farmers and their animals and the potential risk to the public, further research into reducing endotoxin levels inside animal confinement buildings remains necessary in the interest of the farmers' health, animal health and as a precaution, for the public health in the area.

Occupational exposure & health effects

Various studies have shown endotoxin levels inside pig farms often exceed the aforementioned occupational exposure limit[12–15] . These studies show a large variation in endotoxin levels, ranging from zero up to 10,000 EU/m^3, often averaging in the hundreds of EU/m^3. A recent Dutch study reported levels exceeding 1000 EU/m^3 in a majority of pig and poultry farm stables investigated[10]. Specific activities, such as moving large groups of pigs or power-washing the stables may cause even higher levels going beyond 100,000 EU/m^3 [16].

These levels are cause for concern, because as mentioned earlier, endotoxins have been linked to adverse health effects. Endotoxins are associated with a decrease in lung function, marked by a decrease in FEV_1 (forced expiratory volume in 1 second), and increase in respiratory disease in humans. Symptoms include wheezing, coughing, fever, headache, shortness of breath, chronic bronchitis and many other signs of inflammation of the upper and lower airway. Some farmers experience a specific combination of these symptoms which is often referred to as organic dust toxic syndrome (ODTS)[1,2,5,17].

Though most endotoxins will be removed from the upper respiratory tract through mucociliary transportation, those that pass onto the lower airways will bind to macrophages,

triggering a cascade leading to the release of cytokines and pro-inflammatory proteins, most importantly IL-1β, TNF-α and IL-6[5,8,17].

Health effects in pigs

With respect to pigs, endotoxins have been demonstrated to play a role in the development of respiratory disease (e.g. Porcine respiratory and reproductive syndrome virus (PRRSV), *Pasteurella multocida*) among pigs[4,6,18–20]. Halloy et al., demonstrated that a combined exposure to endotoxins and Pasteurella multocida caused a subacute bronchopneumonia in piglets. They also found that both the piglets exposed to endotoxin and Pasteurella multocida (0.19 ± 0.07 kg), and the piglets exposed to endotoxin alone (0.24 ± 0.02 kg), showed a significant ($p < 0.05$) reduction in daily weight gain compared to the control group (0.45 ± 0.12 kg)[18]. Knetter et al. showed that in vitro, endotoxins enhanced the expression of a receptor for PPRSV[4]. Van Gught et al. demonstrated that in vivo exposure to PRRSV and endotoxins resulted in clinical disease, whereas exposure to the PPRSV or endotoxins separately only gave subclinical complaints[6,20]. Knetter et al. also demonstrated in vitro that porcine macrophages react to endotoxins by secreting cytokines [4], just as human macrophages do, but there is a long way to go before we will understand the porcine immune response to endotoxins as well as we do in humans, and they may still differ considerably.

Determinants

In light of the above, there is a clear need to reduce the endotoxin levels within pig confinement buildings: if not for the health of surrounding residents, then out of concern for the farmers' health and herd health. However, it is unclear which interventions farmers can use to reduce their emissions without negatively impacting their own health or that of their animals. A first step is to look at factors which correlate to higher levels of endotoxin being present. Obviously, there is a relationship with the presence of dust and as such, many determinants for dust will also be applicable to endotoxins. In general, these would be factors that cause materials such as feed, manure, urine, and the animals' skin to dry and become aerosolised.

Determinants for both have been extensively studied and a large literature review covering 3 decades' worth of research was recently published[5]. This review shows that endotoxin levels are highest in weaning and finishing stables, compared to other categories of pig stables. Season is a major factor, as most studies in the review found higher levels during winter, often attributed to lower ventilation rates. The presence of a convex floor and use of automated dry feeding appear to lower endotoxin exposure, whilst stable characteristics such as a fully slatted floor, use of floor heating increase exposure. Other determinants include floor dampness, ventilation and hygiene[5] as well as indoor and outdoor temperature (through their influence on ventilation rates and airflow) animal activity (often initiated by certain tasks performed by farmers, such as relocating entire groups of animals) and the number of animals present[13,15,21]. Manure storage conditions on the farm are also of interest as manure is a likely source of bioaerosols and the type of waste management may influence the level of endotoxins[14,22].

Task-related exposures are a major cause of variation in exposure levels within animal confinement buildings. Especially in the pig industry, farmers will execute a number of tasks per shift, each lasting only a short period of time and in various locations with different characteristics. The earlier mentioned study of O'Shaughnessy *et. al.* 2012[16] showed high pressure power-washing to be linked to an exposure of 40,000 EU/m3 (range: 5401–180,864 EU/m3) but this only related to personal exposure. Another study among dairy parlour workers showed that increased cleaning

frequencies may potentially decrease personal exposure, again suggesting a role for hygiene as part of a possible control strategy[5].

Goals & hypotheses

Although the literature reveals a great deal of determinants for endotoxin exposure levels, the most recent study of determinants based on measurements in Dutch pig farms dates back to 1995[23]. Accordingly, an update is needed, to determine if the aforementioned determinants are applicable to the modern pig farm in the Netherlands, before going ahead with any intervention study.

The current observational study will serve as a pilot, due to the short time span and therefor small scaled size, to inform a possible future intervention study. This study aims to quantify the concentration of endotoxins in the indoor air of pig farms via simple means and will investigate the relationship between this concentration and on-farm determinants, such as those mentioned above. The determinants will be investigated using a case-control study design, comparing farms which have or do not have, a history of herd respiratory complaints, because we expect that the endotoxin exposure will vary between these groups.

The goals of this study are to:

- determine the concentration of endotoxins in the air inside pig confinement buildings in farms with and without respiratory problems in weaned piglets.
- determine if there is an association between this concentration, respiratory problems and certain risk factors, such as ventilation and other farm management characteristics.
- Determine if there is an association between respiratory problems in pigs and aforementioned risk factors.

We hypothesize that there is a significant association between the concentration of endotoxins and some, if not all, determinants tested in this study (see Appendix A).

Materials and Methods

As sampling frame a case-control study design was applied, comparing the presence of determinants and endotoxin levels between farms with a history of respiratory complaints in the pig population. Cases are defined as herds where regular respiratory problems are present in the weaned piglets. Control farms are selected from herds with no respiratory problems. The farms were each visited once between the 16th May and 28th June 2018.

Selection of farms

12 farms were included in the study, six cases and six controls, and at each farm one weaner room was used for data collection. At one control farm using multiple housing systems, two rooms were sampled, leading to thirteen rooms sampled. Veterinarians with the professional network of the author and supervisor at GD Animal Health were approached if they knew of any farmers matching the following inclusion criteria and might be motivated to participate in the study:

- Conventional pig husbandry
- Using dry feed
- Weaners aged 6-8 weeks present

As we were interested whether farms with a history of respiratory problems are likely to have different endotoxin levels in the confinement buildings, we looked for an equal number of farms with and without a history of respiratory problems.

Endotoxin detection

Electrostatic dustfall collectors (EDC) as described in Noss *et. al.* 2008 were placed on top of a small stool or overturned bucket at the end of the feed path in the weaner room and left there for approximately 14 days, after which the farmer would close them and send them to the laboratory at the Institute for Risk Assessment Sciences in Utrecht. Upon arrival, each EDC was stored at -20°C. Once all EDCs had arrived, endotoxin levels were determined using a quantitative kinetic chromogenic Limulus amoebocyte lysate (LAL) assay[7,24]. Endotoxin levels are expressed in EU, endotoxin units, per m^2 [7]. The surface area of the EDC's used is 209 cm^2. One EDC was used per sampled weaner room.

Assessment of determinants

Based on the available literature on determinants of fine dust and endotoxins[5,13,14,21,25], a risk factor checklist was made up (see Appendix A). The checklist encompasses several questions about farm management and also involves collecting data on the respiratory health of the weaners present (based on the Welfare Quality protocol). Additionally, the climate in the room was tested for ammonia and carbon dioxide levels, temperature and relative humidity. For these purposes the following equipment was used:

- Kitagawa ammonia gas detector tubes & pump
- Testo instruments (probes) for the measurement of CO_2, relative humidity and temperature.
- A phone camera (minimum 13 megapixels) to take pictures of determinants that were subject to observer bias, such as the % of dirt on the floor and a visible dust check.

In order to correct results for abrupt changes in the weather, the inside and outside temperature for each farm during the 14-day measuring period was recorded. The inside temperature was based on actual on-farm measurements where possible, and otherwise on the previously set temperature in the farms' climate control system. The daily average outdoor temperature was based on the recordings of the Dutch Royal Meteorological Institute (www.knmi.nl) at survey station nearest to each farm.

Data analysis

The data was analysed using SPSS Statistics (version 24). For the descriptive statistics, several means were compared using an independent two-sample t-test. All data was tested for collinearity using Spearmann's correlation coefficient. Because sample size is small and many variables showed to be correlated, we restricted ourselves to univariable analyses where variables were entered into their respective regression models one at a time. The pursuing analyses can be grouped into three categories:

- Case-control status vs. determinants
- Endotoxin level vs. determinants
- Endotoxin level vs. case-control status

Logistic regression was used to analyse the relationship between case-control status and each individual potential determinant. The analyses involving endotoxin levels were analysed using linear regression. For the linear regression, frequency histograms were made for each variable and

inspected for normality. Those which were non-normal, were log10 transformed. In case neither normality or log normality could be ascertained, the dataset that most approximated normal distribution was used for further analysis. Consequently, the following variables entered into the linear regression as log transformed data: endotoxin levels, age building, number of fattening pigs, weaners per room, air volume ventilated per animal, size room, air replacement ratio, carbon dioxide level, and feed fat content. Residuals were tested for normality. Both statistically significant results ($p < 0.05$) and statistical trends ($p < 0.10$) are reported, as the trends may turn out to be statistically significant in a larger sample population.

Results

Descriptive statistics

An overview of the various characteristics of the farms is given in Table 1a and 1b. The 12 participating farms were fairly evenly spread throughout the livestock rearing areas of the Netherlands: 5 farms in the northeast, 5 in the southeast and 2 in the middle. The farms vary greatly in size, the smallest housing 90 sows and the largest housing 850; the average number of sows per farm was 455. Only four of the participating farms also housed fattening pigs on the same site. The farms housed an average 1930 weaners, ranging from 48 to 1300 weaners per room. On average, the farms weaned their piglets at 25.9 (range: 21-32.5) days of age and at the time of data collection the piglets had been weaned for 21.3 (range: 12-33) days. As shown in Table 1b, the means for days weaned and age weaned are not statistically different for the case and control groups. However, the control group does have a statistically higher mean age of the buildings.

All farms used manure pits to store the animals' waste, though there was some variability in how often these pits were emptied: most farmers will empty these once a year, but 2 farms explicitly mentioned emptying their pits more often: one every 2 weeks and the other once every 6 weeks. Most farms stock the feeders once daily. Most farms have automated this process but 2 of the farms still fill feeders by hand. Most of the farms feed pelleted feed (62%) especially in the control group (86%). In the case group crumble is more popular (50%) and only one farm uses meal. Only three farms provide enrichment material to the piglets, all of them in the control group. Of these three farms (4 rooms), two provide compressed straw and one scatters corn cob meal (CCM) into the pens once daily.

Only 6 of the farms use disinfectant but when they do, most of them use it in between each group of new weaners to enter the room. The other farms use detergents, sometimes in combination with leaving the room empty for a certain amount of days. All farms have some form of pest control: 9 out 12 farms hire professionals to do this, and all 12 farms use poison or pesticides, of which 5 also used traps. Three of the farms allowed pets (dogs) behind the hygiene barrier of the farm, but only on two of these farms were the dogs allowed inside the confinement buildings and could they have entered the weaner rooms.

Four out of the 13 sampled rooms did not have floor heating, but these were also the only rooms to have entirely slatted floors. If there were closed floors present, these were always made out of cement, though in the case of one farm, the closed floor was covered in tiles. Four of the farms had fully slatted floors. The slatted floors were made of either plastic or stainless steel and each group had a clear material preference, with 83% of the case group using plastic and 86% of control farms using stainless steel.

Table 1a. individual farm characteristics

Farm number	History of resp. illness?	No. of sows	No. of weaners	Weaners/room	Age confinement building (years)	Notes
1	Y	400	2000	200	12	
2	Y	700	3300	156	5	
3	Y	850	3200	210	12	
4	Y	500	2600	1300	8	
5	N	215	800	75	25	
6	N	650	2650	210	20	
7	Y	750	3000	450	8	Nebulizer present, active 5 x 20 s daily
8	Y	600	2000	216	9	
9	N	200	650	109	25	Tiled floor
10a	N	0	500	48	22	Manual feeding; fully slatted floor, multiple feeders
10b	N	0	500	48	4	Manual feeding; partially closed floor, single feeder
11	N	500	2200	240	18	Pit empty every 6 weeks
12	N	90	260	75	28	Manual feeding Pit empty every 14 days

10a and 10b refer to two rooms within the same farm, but with different housing characteristics (see notes).

Table 1b. farm characteristics overall and at group level

	all farms	**(n = 13)**	**case group**	**(n = 6)**	**control group**	**(n = 7)**
	Mean*	Range	Mean*	Range	Mean*	Range
Endotoxin level (EU/m^2 x 10^4)	1.016	*(53 – 326.421)*	428	*(53 – 1.789)*	2.131	*(150 – 326.421)*
Age at weaning (days)	26,0	*(21 - 32,5)*	25,9	*(21 - 32,5)*	26,1	*(23,5 – 29)*
Days weaned (days)	21,3	*(12 – 33)*	20,5	*(12 – 33)*	21,9	*(17-26)*
Age building (years)	16	*(4 – 28)*	9	*(5 – 12)*	20	*(4 – 28)*
Use of disinfectant (%)	46	*(n = 6)*	67	*(n = 4)*	29	*(n = 2)*
Pest control by professional (%)	69	*(n = 9)*	83	*(n = 5,5)*	43	*(n = 4)*
Pets present (%)	31	*(n = 4)*	17	*(n = 1)*	29	*(n = 2)*
Floor heating (%)	69	*(n = 9)*	67	*(n = 4)*	71	*(n = 5)*
Fully slatted floor (%)	31	*(n = 4)*	33	*(n = 2)*	29	*(n = 2)*
In case of closed floor, % total surface closed	47,3	*(n = 9)*	55	*(n = 4)*	41,2	*(n = 5)*
Type of feed used (%)						
Meal	8	*(n = 1)*	17	*(n = 1)*	0	*(n = 0)*
Crumble	31	*(n = 4)*	50	*(n = 3)*	14	*(n = 1)*
Pellets	62	*(n = 8)*	33	*(n = 2)*	86	*(n = 6)*
Enrichment material provided (%)						
None	8	*(n = 9)*	100	*(n = 6)*	0	*(n = 3)*
Compressed straw	31	*(n = 3)*	0	*(n = 0)*	14	*(n = 3)*
CCM	62	*(n = 1)*	0	*(n = 0)*	86	*(n = 1)*
Slatted floor material (%)						
Plastic	46	*(n = 6)*	83	*(n = 5)*	14	*(n = 1)*
Stainless steel	54	*(n = 7)*	17	*(n = 1)*	86	*(n = 6)*

**geometric mean for endotoxin levels, arithmetic mean for the other variables.*

Statistical analysis

Case-control status vs. determinants

Table 2a summarizes the results of the logistic regression analysis (see Appendix B for a complete overview of both the logistic and the linear regression analyses). Nine determinants were related to case-control status ($p < 0.1$). Older confinement buildings seem to have a protective effect for development of respiratory disease in the herd with an odds ratio of 0.76. However, it is unknown if this is a causal relationship. The use of plastic slatted floors appears to be strongly associated with herds having a history of respiratory disease; however, as with the previous determinant, there may be confounding factors at play due to the composition of the sample population. This will be reviewed under "Discussion".

Table 2a. Results logistic regression

Determinant	Odds ratio	p =	95% - CI Odds ratio	
Age Building (year)	0.76	0.045	0.58	0.99
Slatted floor material (plastic vs steel)	30	0.027	1.47	611.80
Automatic lights (Y/N)	0.083	0.073	0.006	1.257
Light on outside checks (Y/N)	0.080	0.067	0.005	1.192
Number of sows	1.008	0.055	1.000	1.017
Number of weaners	1.002	0.061	1.000	1.005
Number of other pigs	1.022	0.092	0.997	1.047
Number of piglets	1.004	0.081	1.000	1.008
Size room (m^3)	1.023	0.076	0.998	1.049

Endotoxin level vs. determinants

Figure 1 provides a scatterplot of the endotoxin levels in case and control weaner chambers . One farm in the control group stands out as an outlier and considering the small number of farms in the study, this farm would have a disproportionate impact on the results for the control group, so this outlier was disregarded from any of the endotoxin-related analyses.

Figure 1. Scatterplot endotoxin level case/control groups

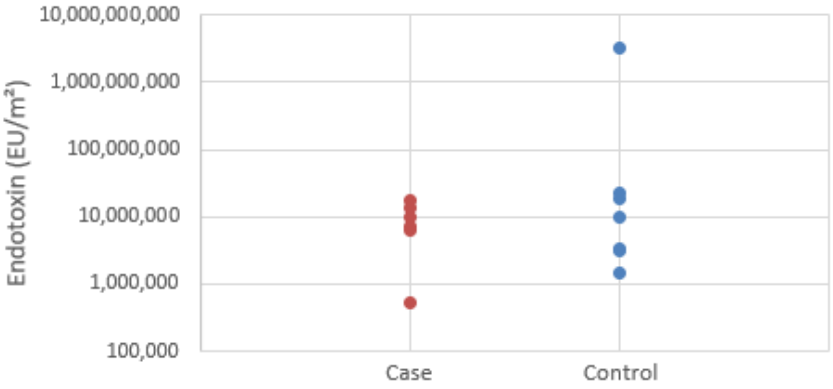

Table 2b summarizes the significant results of the linear regression analysis. There appears to be a slight (0.025) association between the protein content of the feed and an increased presence of

endotoxins. There is also a clear trend with feed fat content – as the fat content increases, the endotoxin levels increase almost by a factor of 4. This seems contradictory as oils are often added to feed to ensure pellets do not crumble into dust, but it may be that feed fat content has some effect on the gram negative bacteria present in the weaner room or in the pigs themselves, for example, by acting as a nutrient source or growth medium for the bacteria.

Table 2b. Results linear regression

Determinant	regression coefficient	p =	95% - CI		R^2
Protein content feed (g/kg)	0.025	0.053	0.000	0.050	0.355
Fat content feed (g/kg) (log10 transformed)	3.989	0.075	-0.498	8.476	0.310
History (case/control)	-0.333	0.221	-0.951	0.285	0.152

Endotoxin level vs. case-control status

As shown in Table 2b, endotoxin levels were not statistically significant (p = 0.221) different in farms with a history of porcine respiratory disease compared to those without a history of respiratory illness. The R-squared value also indicates a poor model fit.

Discussion

High levels of endotoxins were found (GM 1016 x 10^4 EU/m^2). No significant association was found between endotoxin levels and farmers or veterinarians reporting a history of herd respiratory health problems (p = 0.22). Associations were found between a reported history of respiratory disease and building age (OR = 0.760; p = 0.045) and plastic slatted floors (OR = 30; p = 0.027). Feed protein content may be a possible determinant for endotoxin level (b_1 = 0.025; p = 0.053).

Endotoxin levels

There are only a few studies to properly compare our results with, because the use of the EDC is relatively new. Noss et al. used the EDC method in homes, both urban and rural, and inside stables, though it is not specified what species of animal were housed inside the stables[24]. They reported a geometric mean endotoxin level of 103 x 10^4 EU/m^2 inside the stables, which is much lower compared to our geometric mean of 1.016 x 10^4 EU/m^2.

There a few factors to consider that may have possibly influenced our endotoxin levels: the position of the EDC within the room in relation to the ventilation system, the height at which it was placed, and the category of pigs housed in the rooms used for the measurements. First of all, the EDC was placed in the same place within each room sampled: at the end of the feed path, opposite the door. However, each farm has a different ventilation system and thus air enters and leaves each room in different places. For example: all the case farms applied ground canal ventilation, which means the fresh air enters from below the feed path, and possibly the freshest air enters from below the spot where the EDC was placed. Whilst on farms employing feed path ventilation, the freshest air enters below the door. Figures 2 and 3 show examples of these two ventilation systems. Notably, figure 2 shows a ground canal system where the freshest air enters from below the feed path nearest the door, but there are situations in which this is not the case, and the air travels underneath the closed floor towards the end of the feed path before entering the room through slits in the feed path floor. Also, in both figures the air exits through a ventilation pipe near the door, but in some farms this ventilation pipe is placed in the middle or at the end of the feed path. These variations in air

flow may have affected the amount of dust that settled in the EDC's and as such our endotoxin levels may be underestimated for the farms using ground canal ventilation.

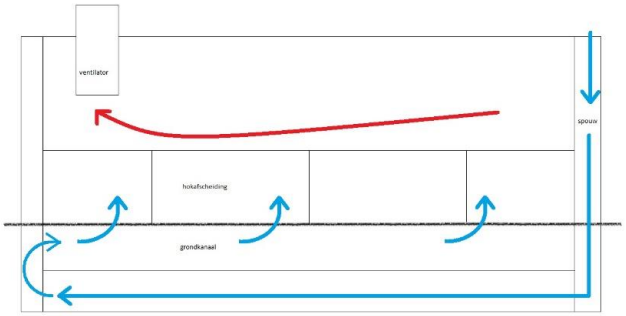

Figure 2. diagram of ground canal ventilation

Secondly, the EDC's were placed on upturned buckets or stools, placing them at "animal level" in order to reflect the exposure levels of the animals. However, in other studies, the EDC's were often placed at a higher height, on top of book cases and such. This height difference may partially explain why we found higher endotoxin levels than in Noss et al. At the moment, these are mere speculations, but it would a useful for further research to look into the effect of placement within the pig room on the found endotoxin levels.

Thirdly, in this study the EDC's where placed in weaner confinement rooms. Weaner and fattening pig confinement buildings are known to have relatively high endotoxin levels[5], which is why we chose weaner rooms for this study in the first place. As it is not reported which species of animal is kept in the farm stables sampled in Noss et al., we can only speculate that differences in age and species in the stable may explain some of the difference in findings between our study and Noss et al. (2008).

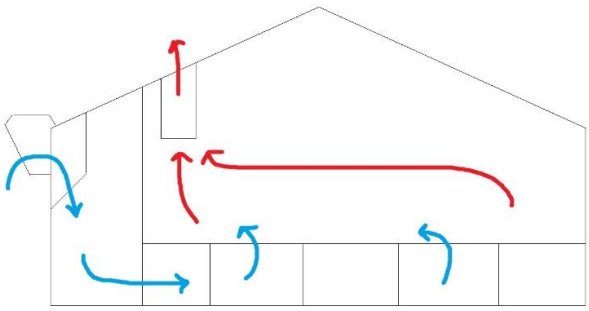

Figure 3. diagram of feed path ventilation

Finally, it would be useful to know how these results relate to the advised exposure limits. The exposure limits are based on air concentrations of endotoxin, whereas with EDCs another measure of exposure is captured, namely the endotoxins in precipitated dust over a certain surface area. EDC endotoxin levels cannot be precisely arithmetically converted from EU/m^2 to EU/m^3. A rough estimate can be made, as previous studies into the EDC method have established that the endotoxin levels found by EDC correlated to the endotoxin levels found with the more traditional method of active air sampling, as shown in figure 4 out of Noss et al., 2008. Based on this correlation, one might estimate that our geometric mean of 1016 x 10^4 EU/m^2 roughly corresponds to 1000 EU/m^3, much higher than the recommended occupational exposure limit of 90 EU/m^3. This is in line with previously discussed studies that measured endotoxin exposure in pig confinement buildings[10,12–16].

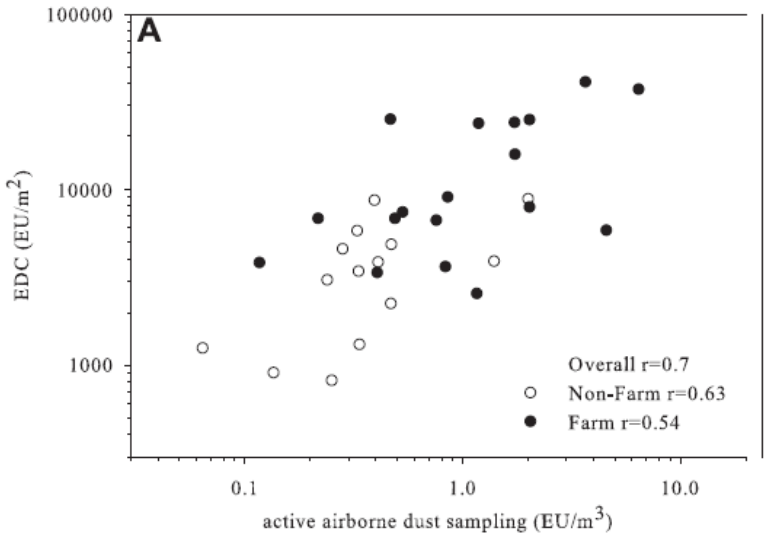

Figure 4. Correlation between EDC and active air sampling results.
Source: Noss et al., 2008

Associations found

The apparent associations between a herd's history of respiratory disease, building age and slatted floor material, are prime examples of the difficulty taking place in this study. As shown in the descriptive statistics, the case and control groups are too dissimilar in certain aspects, that the effects cannot be disentangled. For instance case farms tend to be modern, relatively new, larger farms, whilst the control group contains predominantly farms which are smaller and older. This may suggest a relation with age of building, however other factors may also explain the difference in endotoxin levels between the case and control groups. Similarly, it cannot be ruled out that the case farms all happen to use plastic slatted floor because this is the more modern way of housing piglets, but it is not known whether this is truly associated with respiratory disease.

Not finding any association between history of respiratory disease and endotoxin level does not correspond with the current literature on this topic. This suggests that "history of respiratory disease" may not be a suitable characteristic to use as a variable for studies such as this one. During the execution of this study, it became clear that the definition of "history of respiratory disease" was too vague and may mean different things for different veterinarians and farmers – it is possible that certain farmers may be more alert to signs of respiratory disease in their pigs, or that they consider these signs a problem sooner than other farmers. So, there is not only a certain amount of selection bias (specifically sampling bias) in this study, but also form of response bias. This raises questions about the generalizability of the results from the study population to the pig industry as a whole. As such, future research projects should take extra precautions to avoid these forms of bias (see "Recommendations for further research" for explicit suggestions how to avoid this). One way to avoid such bias is to more clearly define cases, for example, by using more objective measurements. As we have found no association between the health parameters used in this study (coughing, sneezing, tear stripes and red eyes) and endotoxin levels, these parameters seem insufficient. Lung lesions at slaughter and antibiotic use related to respiratory disease may be better indicators of herd respiratory health. Another option might be to measure the inflammatory response to endotoxins by measuring pro-inflammatory proteins and/or macrophages in blood samples.

The association between endotoxin levels and feed protein content, as well as feed fat content may be interesting to follow up in further research. Previous studies have found an association between declining respiratory function and endotoxin levels among animal feed workers[26,27], which is why we looked into various determinants related to feed. However, these studies have not looked into which components of the feed affect endotoxin levels, so further research will be needed to confirm whether feed protein and/or fat content truly affects endotoxin levels. It may be that these nutrients have some sort of effect on the piglets' physiology (e.g. gut flora) which in turn influences the endotoxin level in the surrounding area.

Recommendations for further research

One of the goals of this study was to function as a feasibility study for future research into determinants of endotoxins in pig farms. The most important recommendations are related to the issues with bias and generalizability that became apparent during data analysis, but some recommendations with respect to basic setup will also be made.

As previously discussed, the current study was affected by selection bias and a weak case definition. If a future study was to use a case-control design, it is recommended that the case definition not be dependent on subjective observations, but rather on objective data, for example, the level of antibiotic use due to respiratory problems. However, considering that endotoxins are not a rare occurrence on pig farms, and any clinical consequences they may have on farm data (antibiotic use, lung lesion upon slaughter, etc) are subject to interference by other factors (most obviously infectious diseases that cause similar outcomes) a cross sectional cohort study seems more appropriate. This will reduce the risk of selection bias compared to a case-control setup, though cohort studies are vulnerable to loss to follow-up.

In order to make a future study more generalizable, ideally, one would use a random sample. However, this may not be feasible as this kind of research relies on farmers volunteering to participate. As was our experience in this study, farmers with strict biosecurity (often the larger, more modern farms) appear more motivated to participate in a research project like the current one if they feel it might help them solve the problems they are having with respiratory herd health. Some sampling bias may be unavoidable because of this. It may be possible to find other ways of motivating farmers to volunteer for a larger research project, for example by compensating them financially for their time or by working together with sector representatives, thus ensuring they feel they have some form of influence in the project. If this makes the sample population more representative, this is worth looking into. Alternatively, one could use a survey or similar methods to gain insight into the composition of the pig industry in the Netherlands, compare this to the group of participating farms and "fill in the blanks" with experimental studies, in farms experimentally applying the housing or other characteristics that are missing from the sample population.

In the current study, there were too few farms and there was too much correspondence in occurrence between potential determinants to apply multivariate analysis. A multivariate analysis may provide additional insights into the interaction between determinants. Therefore, it is recommended to use a larger sample population.

As for the more practical aspects of study design go, the current study shows that weaner rooms are suitable for this kind of research, as the circumstances within the weaner room can be relatively stable throughout a period of 2-4 weeks (little farmers activity, perhaps 1 or 2 changes in feed) and these rooms are known to contain high levels of endotoxins[5].

As for the measurement of endotoxin levels and potential determinants the are a few remarks regarding spatial and temporal aspects. First of all, the endotoxin levels in the current study were measured during two weeks, whilst the determinants were based on a one to three measurements on the first day of this two-week period. Though some determinants will not have changed in this period (e.g. housing), others may have changed on a daily or hourly basis (e.g. carbon dioxide levels, relative humidity, animal activity). It may be worth investigating the temporal relationship between such determinants and endotoxin levels through the use of sensors in the weaner rooms. Secondly, the spatial aspect: as mentioned earlier, the placement of the EDC in relation to air flow and height should be further investigated.

Conclusion

In conclusion, this study has found high levels of endotoxins in weaner rooms (GM 1016 x 10^4 EU/m^2). This may even be an underestimation due to the placement of the EDC's in relation to the air flow in farms using ground canal ventilation. No significant association was found between endotoxin levels and farmers or veterinarians reporting a history of herd respiratory health problems. Feed protein content may be a possible determinant for endotoxin level. The current study was possibly hampered by sampling and response bias, and the potential determinants showed much colinearity.
We make the following recommendations for future research in this area:

- Apply a cross sectional cohort study design with a large, preferably random, sample population
- Look into ways of motivating farmers to volunteer for research
- Weaner rooms are suitable for this kind of research and season does not have to factor into the planning of such projects.
- Investigate the spatial aspects of endotoxin detection with EDC's, especially with respect to airflow and height
- Investigate the temporal relationship between endotoxin levels and possible determinants that may vary throughout the two-week EDC sampling period (e.g. carbon dioxide, animal activity, and relative humidity).
- Use of EDC's is recommended, as they are a cost-effective and convenient way of measuring endotoxin levels in comparison with active air sampling, and they give an acceptable indication of the levels per air volume.

References

1. Douwes J, Thorne P, Pearce N, Heederik D. Bioaerosol health effects and exposure assessment: Progress and prospects. In: *Annals of Occupational Hygiene*. ; 2003. doi:10.1093/annhyg/meg032
2. Kim KH, Kabir E, Jahan SA. Airborne bioaerosols and their impact on human health. *J Environ Sci (China)*. 2018. doi:10.1016/j.jes.2017.08.027
3. Liebers V, Brüning T, Raulf-Heimsoth M. Occupational endotoxin-exposure and possible health effects on humans. *Am J Ind Med*. 2006. doi:10.1002/ajim.20310
4. Knetter SM, Tuggle CK, Wannemuehler MJ, Ramer-Tait AE. Organic barn dust extract exposure impairs porcine macrophage function in vitro: Implications for respiratory health. *Vet Immunol Immunopathol*. 2014. doi:10.1016/j.vetimm.2013.10.019
5. Basinas I, Sigsgaard T, Kromhout H, Heederik D, Wouters IM, Schlünssen V. A comprehensive review of levels and determinants of personal exposure to dust and endotoxin in livestock farming. *J Expo Sci Environ Epidemiol*. 2015. doi:10.1038/jes.2013.83
6. Van Gucht S, Labarque G, Van Reeth K. The combination of PRRS virus and bacterial endotoxin as a model for multifactorial respiratory disease in pigs. *Vet Immunol Immunopathol*. 2004. doi:10.1016/j.vetimm.2004.09.006
7. Paba E, Tranfo G, Corsetti F, Marcelloni AM, Iavicoli S. Indoor Exposure to Airborne Endotoxin: A Review of the Literature on Sampling and Analysis Methods. *Ind Health*. 2013;(51):237-255.
8. Health Council of the Netherlands. *Endotoxins. Health-Based Recommended Occupational Exposure Limit*. The Hague: Health Council of the Netherlands, 2010; publication no. 2010/04OSH
9. Health Council of the Netherlands. *Health Risks Associated with Livestock Farms.* The Hague: Health Council of the Netherlands, 2012; publication no. 2012/27.
10. National Institute for Public Health and the Environment. *Livestock Farming and Neighbouring Residents' Health*. De Bilt; 2016. Report no. 2016-0058
11. Wageningen University & Research. *Emissions of Endotoxins from Animal Production: Emission Measurements and Dispersion Modelling*. Wageningen: Wageningen University & Research, 2016. Livestock Research Report no. 959
12. Schierl R, Heise A, Egger U, et al. Endotoxin concentration in modern animal houses in southern Bavaria. *Ann Agric Env Med*. 2007;(14):129-136.
13. Duchaine C, Grimard Y, Cormier Y. Influence of Building Maintenance, Environmental Factors, and Seasons on Airborne Contaminants of Swine Confinement Buildings. *AIHAJ -American Ind Hyg Assoc*. 2000;61(1):56-63. doi:10.1080/15298660008984515
14. Ko G, Simmons, III OD, Likirdopulos CA, Worley-Davis L, Williams CM, Sobsey MD. Endotoxin Levels at Swine Farms Using Different Waste Treatment and Management Technologies. *Environ Sci Technol*. 2010;44(9):3442-3448. doi:10.1021/es9026024
15. O'Shaughnessy PT, Donham KJ, Peters TM, Taylor C, Altmaier R, Kelly KM. A task-specific assessment of swine worker exposure to airborne dust. *J Occup Environ Hyg*. 2010. doi:10.1080/15459620903327970
16. O'Shaughnessy P, Peters T, Donham K, Taylor C, Altmaier R, Kelly K. Assessment of swine worker exposures to dust and endotoxin during hog load-out and power washing. *Ann Occup Hyg*. 2012. doi:10.1093/annhyg/mes013
17. Liebers V, Raulf-Heimsoth M, Brüning T. Health effects due to endotoxin inhalation (review). *Arch Toxicol*. 2008. doi:10.1007/s00204-008-0290-1
18. Halloy DJ, Bouhet S, Oswald IP, et al. Pathophysiological changes occuring during Escherichia coli endotoxin and Pasteurella multocida challenge in piglets: Relationship with cough and temperature and predicitive value for intensity of lesions. *Vet Res*. 2004. doi:10.1051/vetres:2004012
19. Donham KJ. Association of environmental air contaminants with disease and productivity in swine. *Am J Vet Res*. 1991;(52):1723-1730.

20. Van Gucht S, Van Reeth K, Pensaert M. Interaction between porcine reproductive-respiratory syndrome virus and bacterial endotoxin in the lungs of pigs: Potentiation of cytokine production and respiratory disease. *J Clin Microbiol*. 2003. doi:10.1128/JCM.41.3.960-966.2003
21. O'Shaughnessy PT, Achutan C, Karsten AW. Temporal variation of indoor air quality in an enclosed swine confinement building. *J Agric Saf Health*. 2002.
22. Nehme B, Létourneau V, Forster RJ, Veillette M, Duchaine C. Culture-independent approach of the bacterial bioaerosol diversity in the standard swine confinement buildings, and assessment of the seasonal effect. *Environ Microbiol*. 2008. doi:10.1111/j.1462-2920.2007.01489.x
23. Preller L, Heederik D, Kromhout H, Boleij JSM, Tielen MJM. DETERMINANTS OF DUST AND ENDOTOXIN EXPOSURE OF PIG FARMERS: DEVELOPMENT OF A CONTROL STRATEGY USING EMPIRICAL MODELLING. *Ann occup Hyg*. 1995;39(5):545-557.
24. Noss I, Wouters IM, Visser M, et al. Evaluation of a low-cost electrostatic dust fall collector for indoor air endotoxin exposure assessment. *Appl Environ Microbiol*. 2008. doi:10.1128/AEM.00619-08
25. Heinrich J, Gehring U, Douwes J, et al. Pets and vermin are associated with high endotoxin levels in house dust. *Clin Exp Allergy*. 2001. doi:10.1046/j.1365-2222.2001.01220.x
26. Post W, Heederik D, Houba R, Post W, Heederik D, Houba R. Decline in lung function related to exposure and selection processes among workers in the grain processing and animal feed industry. *Occup Env Med*. 1998;55(September 2006):349-355.
27. Smid T, Heederik D, Houba R, Quanjer PH. Dust-and endotoxin-related respiratory effects in the animal feed industry. *Am J Respir Crit Care Med*. 1992;146(6):1474-1479.

Appendix A

Risk factor checklist

Datum: _____

Naam bedrijf/veehouder: _____

Adres: _____

_____

Telefoonnummer: _____

Graag terugkoppeling resultaten? Ja/Nee eventueel e-mailadres: _____

Afdelingsnummer _____

Oplegdatum _____

Oplegleeftijd _____ dagen

Opleggewicht (indien bekend) _____ kg

Vragen:

Leeftijd gebouw _____ jaar / bouwjaar: _____

Desinfectie voor opleg gespeende biggen? Ja/Nee

Frequentie reiniging en desinfectie gesp. biggen afdelingen? _____ x per _____

Desinfectiemiddel: _____

Ongediertebestrijding? Ja/Nee indien ja, hoe? _____

Huisdieren aanwezig? Ja/Nee Indien ja, welke en hoeveel? _____

Vloerverwarming aanwezig? Ja/Nee Indien ja, hoe ingezet? _____

Heeft de te bemonsteren afdeling diarree gehad na spenen? Ja/Nee

Opmerkingen behandelkaart: _____

Aanzuren water? Ja/Nee

Aantal voermomenten per dag? _____

Samenstelling voer → foto voerbon/etiket indien zakgoed) + foto afdeling met flits!

Vragen (vervolg)

Aantal dieren		Zeugen	totaal op locatie
		Gespeende biggen	
		Vleesvarkens	
		Opfokgelten	
		Beren	
		Zogende biggen?	
Aantal dieren		Gespeende biggen	totaal afdeling
Mestopslag	dagontmesting / mestput / mestpannen		
Lichtschema	Licht: Donker: Automatisch / handmatig	Uren	

Klimaatkast

Ventilatie hoeveelheid		m^3/u en/of %
Temperatuurcurve	Dag	Temp

Metingen aan het dier

Hoesten		Aantal keer hoesten in 5 min
Niezen		Aantal keer niezen in 5 min
Rode ogen		% dieren met rode ogen, in stappen van 5%. (e.g. 20%, 25%, 30% etc)
Traanstrepen		% dieren met traanstepen, in stappen van 5%. (e.g. 20%, 25%, 30% etc)

Metingen klimaat

Type ventilatie	Plafond / voergang / grondkanaal / combinatie / inlaatventielen			
	Hok 1	Hok 2	Hok 3	
CO_2				ppm
NH_3				ppm
Relatieve luchtvochtigheid				%
Afdelingstemperatuur				°C

Huisvesting

Afmetingen afdeling		L x b x h (m), m^3 NB. Bij bepalen nok hoogte ook afstand van minstens 1 zijwand tot aan nok meten! (m.n. als de nok niet in het midden van de afdeling ligt)
Aantal voeruitlaten per afdeling		

Stortoppervlak voerbak		L x b x d (cm)
Voertype	Meel / kruimel / brok	
Afleidingsmateriaal	Geen / stro / zaagsel / luzerne Samengeperst / los	
Aanblik voer in trog (stevigheid pellet)	Wel / niet uiteengevallen Drinknippel in voerbak? Ja/nee	**Foto!**
Huisvestingsmateriaal	cement / kunststof / gietijzer	
Vloer		% dichte vloer
Hokbevuiling	Nat / droog	**Foto!** % dichte vloer vuil
Vloerverloop	Bol / hol / vlak	

Tekening hokindeling
(i.v.m. % dichte vloer + in kaart brengen afmetingen)

Tekening afdeling
(i.v.m. ventilatie + in kaart brengen afmetingen)

Appendix B

Codes used in regression analysis

	Explanation
LA	light automated
LH	light hours
LO	light outside checks
ND	number of drinkers
NF	number of fattening pigs
NH	ammonia
NO	number of other pigs
NP	number of piglets
NS	number of sows
NT	total number of animals
NW	number of weaners
PC1	pest control
PC2	pest control type
PP	pets present
RV	relative humidity
SF	size feeder
SN	sneezing
SR	size room
SS	size farm
TP	temperature
TR	treatments
TS	tear stripes
VA	ventilation per animal
VE	fat content feed
VT	ventilation type
WD	wet dirt
WR	weaners per room

	Explanation
AB	age building
AR	air replacement
AV	air ventilated
AW	age at weaning
CA	current age
CF	closed floor percentage
CO	carbon dioxide
CP	coughing present
CU	Coughing
DD	dry dirt
DI	disinfection
DW	days weaned
EI	protein content feed
EM	enrichment material
EM2	enrichment material
FC	closed floor present
FC2	curvature solid floor
FD	drinker in feeder
FF	feed stocking frequency
FH	floor heating
FI	feed intact
FL	lid on feeder
FM	floor material
FM2	slatted floor material
FP	number of feed pipes
FS	feed type

Overview logistic regression analysis

case vs. 0-1 parameters	ExpB	Sig.
AB	0.000	0.999
DI	0.200	0.181
PC1	0.000	0.999
PC2	1.500	0.725
PP	3.750	0.322
FH	1.250	0.853
TR	0.200	0.181
SS	4846424182.000	0.999
LA	0.083	0.073
LO	0.080	0.067
VT (ground canal as control)		1.000
ceiling	0.000	1.000
Floor path	0.000	0.999
FS (pellet as control)		0.299
meal	4846424529.000	1.000
crumble	9.000	0.120
EM (none as control)		1.000
CCM	0.000	1.000
straw	0.000	0.999
FI	2.667	0.396
FD	0.667	0.725
FM2	30.000	0.027
FC	1.250	0.853
DD	0.800	0.853
WD	1938569925.000	0.999
FC2	2.667	0.396
CP	0.750	0.797
TS	6.000	0.186
FL	0.000	1.000

case vs. continous parameters	ExpB	Sig.
DW	0.948	0.636
AW	0.980	0.919
CA	0.959	0.654
AB	0.760	0.045
FF	2827080921.000	0.999
NS	1.008	0.055
NW	1.002	0.061
NF	1.000	0.396
NO	1.022	0.092
NP	1.004	0.081
NT	1.003	0.199
WR	1.021	0.104
LH	1.054	0.832
AV	1.000	0.802
VA	0.904	0.117
SR	1.023	0.076
AR	0.711	0.223
NH	1.137	0.366
CO	1.001	0.558
RV	1.057	0.529
TP	1.456	0.359
FP	1.085	0.379
SF	1.000	0.218
ND	3.620	0.313
CF	1.111	0.144
DD	1.013	0.727
WD	0.891	0.324
CU	1.417	0.291
SN	1.024	0.239
TS	0.636	0.101
EI	0.877	0.128
VE	0.858	0.166

66 ## Overview linear regression analysis

ET vs. 0-1 and categorical parameters	unstandardized B	R^2	sig
AB	0.150	0.025	0.623
DI	0.282	0.088	0.350
PC1	0.486	0.238	0.108
PC2	0.063	0.018	0.682
PP	-0.062	0.004	0.848
FH	-0.268	0.072	0.398
TR	0.180	0.037	0.551
SS	-0.486	0.238	0.108
LA	0.468	0.242	0.104
LO	0.447	0.221	0.123
VT (baseline is "feedpath")		0.238	
ground canal	-0.486	0.238	0.108
ceiling (only 1, so left out of model)			
FS (baseline is "pellet")		0.257	0.263
meal	-0.508	0.257	0.336
crumble	-0.475	0.257	0.139
EM (baseline is "none")		0.287	0.000
CCM	0.504	0.287	0.326
Straw	0.542	0.287	0.114
FM2 (baseline is "stainless steel")		0.335	
Plastic	-0.543	0.335	0.049
cement left out of model because no farms had this			
FC2 (baseline is "flat")		0.003	0.864
Convex	0.053	0.003	0.864
concave left out of model because no farms had this			
EM2 (loose = control)	0.037	0.007	0.916
FI	-0.365	0.147	0.219
FD	0.198	0.040	0.534
FC	-0.268	0.072	0.781
DD	0.147	0.018	0.674
WD	-0.611	0.129	0.251
CP	-0.425	0.205	0.140
TS	-0.464	0.217	0.127
FL	-0.202	0.014	0.713
HI (case/control status)	**-0.333**	**0.152**	**0.211**

67
68

ET vs. continous parameters	unstandardized B	R^2	sig
DW	-0.018	0.039	0.539
AW	-0.120	0.523	0.007
CA	-0.039	0.258	0.091
AB*	-0.179	0.010	0.755
FF	0.384	0.093	0.335
NS	-0.001	0.107	0.298
NW	0.000	0.077	0.384
NF*	1.164	0.218	0.691
NO	-0.002	0.095	0.330
NP	0.000	0.172	0.180
NT	0.000	0.134	0.241
WR*	-0.300	0.064	0.428
LH	-0.039	0.075	0.554
AV	0.000	0.123	0.264
VA*	0.532	0.210	0.134
SR*	-0.026	0.001	0.944
AR*	0.278	0.056	0.459
NH	0.002	0.000	0.968
CO*	-1.494	0.162	0.194
RV	-0.038	0.236	0.109
TP	0.013	0.002	0.899
FP	0.018	0.067	0.416
SF	0.000	0.037	0.548
ND	0.128	0.026	0.703
CF	-0.029	0.499	0.050
DD	-0.024	0.441	0.019
WD	0.018	0.088	0.348
CU	0.049	0.048	0.495
SN	-0.004	0.087	0.353
TS	0.074	0.223	0.121
EI	0.025	0.355	0.053
VE*	3.989	0.310	0.075
***log10 transformed**			

69