

Comparison of three different measuring devices of ammonia and evaluation of their suitability to assess animal welfare in pigs

Johanna Witt^{a,*}, Joachim Krieter^a, Kira Schröder^a, Kathrin Büttner^b, Christina Hölzel^a, Katja Krugmann^a, Irena Czycholl^c

^a Institute of Animal Breeding and Husbandry, Christian-Albrechts-University, Olshausenstraße 40, 24098 Kiel, Germany

^b Unit for Biomathematics and Data Processing, Faculty of Veterinary Medicine, Justus Liebig University, 35392 Giessen, Germany

^c Department of Veterinary and Animal Sciences, University of Copenhagen, 1870 Frederiksberg, Denmark

HIGHLIGHTS

- Comparisons of different ammonia measuring devices (hand-held vs. sensor).
- Ammonia concentration is influenced by farm, season, age of pigs and barn.
- No good agreement between all three measuring devices.
- Significant connection between ammonia and some on-farm/slaughterhouse indicators.

ARTICLE INFO

Keywords:

Ammonia
Animal welfare assessment
PLF
On-farm indicators
Slaughterhouse indicators

ABSTRACT

Since negative effects of ammonia (NH₃) on health and productivity are known NH₃ concentrations should be considered a possible indicator of animal welfare. Thus, a simple and reliable control of NH₃ concentrations is necessary. Therefore, this study aimed to investigate factors influencing two hand-held measuring devices - the 'Basic Ammonia Detector-NH3000' ammonia meter (AM) and the 'Dräger accuro®' gas detection pump (DA) - and the continuously measuring 'DOL53 Ammonia Sensor' (DOL) with regard to their practical use in pig houses and to compare them in terms of their reliability. Subsequently, the suitability of NH₃ as a welfare indicator was considered by analyzing the connections of NH₃ to different on-farm/slaughterhouse welfare indicators. The NH₃ concentration on three farms (farm A: organic; farms B, C: conventional) over three to four batches from farrowing to the end of finishing were measured weekly with the AM ($n = 284$) and the DA ($n = 142$). In addition, the DOL ($n = 77,170$) measured the NH₃ concentration every 15 min and revealed its daily course. The influence of the farm on NH₃, investigated with a linear mixed model, was significant ($P \leq 0.05$) for all three measuring devices. The freely ventilated farm A showed the lowest NH₃ of all farms (mean DOL 0.69 ppm), which could be caused by a lower animal density, litter and an outdoor arena, compared to the mechanically ventilated farms B (mean DOL 12.4 ppm) and C (mean DOL 10.6 ppm). The season was a further significantly ($P \leq 0.05$) influencing factor for the farms B and C, which was reflected in higher NH₃ in winter compared to summer. This could be due to a lower ventilation rate and different animal behavior, because of lower outdoor temperatures in winter. Continuous measurement with the DOL also showed influences of the production stage of the pigs, with highest values with increasing age and the barn. The comparison of the AM and DA with the DOL did not achieve acceptable reliability/agreement. Hand-held devices cannot record variations in the NH₃ concentration due to the non-continuous measurement, which may lead to wrong conclusions. A strong relationship between the NH₃ number ≥ 20 ppm per batch and the number of affected animals of the on-farm/slaughterhouse indicators determined by significant ($P < 0.05$) Chi² Tests and Cramér's $V \geq 0.60$ could be detected for coughing ($V = 0.89$), tail length ($V = 0.81$), tail lesions ($V = 0.60$) and M. hyopneumoniae ($V = 1.00$). These findings indicate NH₃ as a potential indicator of animal welfare.

* Corresponding author.

E-mail address: jwitt@tierzucht.uni-kiel.de (J. Witt).

<https://doi.org/10.1016/j.livsci.2023.105372>

Received 27 June 2023; Received in revised form 19 October 2023; Accepted 8 November 2023

Available online 10 November 2023

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1. Introduction

Pig production is often the subject of public debate due to environmental problems and rising concern about animal welfare (Fangmeier et al., 1994; Greef et al., 2011). In the future, one of the greatest challenges will be to meet these demands in terms of animal welfare and respect for the environment. In this context, monitoring the barn climate is of fundamental importance for animal welfare, health, productivity (Arango et al., 2021) and the environment (Krupa, 2003). Ammonia (NH₃) in particular, as a pollutant gas, is considered an important criterion for evaluating air quality (Bachmann et al., 2007). Basically, NH₃ is the product of the enzymatic reaction of urea, which derives from the urine of the animals, and the enzyme urease, which is contained in the feces. The amount of nitrogenous emissions depends on the husbandry system, the management and the environmental conditions, such as air speed, temperature and pH value (EFSA, 2022; Ni et al., 1999). Already known are the negative effects of NH₃ exposure on pig health and productivity (Banhazi et al., 2008). This is caused by the porous structure of the gas particles, allowing a continuous and slow release of the harmful gas and enabling it to reach the deeper parts of the lungs (Donham et al., 1977; Takai and Pedersen, 2002). A prolonged exposure to an NH₃ concentration above 400 ppm can destroy mucous membranes by dissolving keratin, fat and cholesterol. Acute exposure to NH₃ can damage the epithelial lining of the less resistant bronchioles (Stombaugh et al., 1969). However, levels as low as 20 ppm can influence the inflammatory response (Urbain et al., 1994), and concentrations of 50 to 75 ppm reduced the ability of young pigs to clear bacteria from their lungs (Gonyou et al., 2006). Effects on the respiratory system were confirmed in the study by Donham (1991) by a significant correlation in finishing pigs between NH₃ (23.6 ppm) and pleurisy lesions. According to various studies, pigs even avoid areas with air containing NH₃, provided the husbandry system allows it (Smith et al., 1996; Wathes et al., 2002). Due to these relationships, the monitoring of the NH₃ concentration could be a tool to determine animal welfare. Also Fablet et al. (2012) expected to find relationships between the climatic conditions and animal-based welfare indicators both on-farm and at the slaughterhouse. The advantages of using precision livestock farming systems to monitor climate and thus animal welfare compared to using animal-based animal welfare assessment protocols are the objectivity and feasibility of the measurement (Gómez et al., 2021). These circumstances underline the importance of measuring NH₃, moreover in Germany a maximum limit of 20 ppm is stipulated by law (Anonymous, 2006b). NH₃ can be detected by the human nose, but reliable and feasible measurement methods are needed to control limit value exceedances and to determine the NH₃ concentration (Timmer et al., 2005). As a result of technical development, more and more sensors and analytical technologies are available for the determination of NH₃, whereby the costs vary widely. The use of electrochemical sensors enables direct reading and continuous measurement, which allows concentration trends to be recorded and the ambient air to be constantly monitored (Ni and Heber, 2001).

The aim of this study was to investigate factors influencing the NH₃ measurement results of a continuously measuring sensor, the 'DOL53 Ammonia Sensor' (DOL) and the lower priced and more feasible alternative devices, the hand-held 'Basic Ammonia Detector-NH3000' (AM) and the 'Dräger accuro®' gas detection pump (DA) with regard to the practical use in pig houses and to compare them in terms of their reliability. In addition, possible connection of NH₃ with both animal-based on-farm and slaughterhouse indicators were considered in order to validate NH₃ as a possible animal welfare indicator.

2. Material and methods

2.1. Ethical statement

No animal experiments were carried out. By nature of welfare assessment protocols, the indicators used should not pose harm,

suffering, injury or stress to the animals. All procedures (e.g. marking of animals, routine health checks) were carried out primarily for the farmers' management purposes and not for scientific ones. The animals in the study were kept farm animals and were housed conventionally or according to the EU organic scheme (Anonymous, 2007). In both cases, the animals were kept according to EU and national law ('German Animal Welfare Act' (German designation: TierSchG) (Anonymous, 2006a) and the 'German Order for the Protection of Production Animals used for Farming Purposes and other Animals kept for the Production of Animal Products' (German designation: TierSchNutzTV) (Anonymous, 2006b)).

2.2. Farms involved

Data collection on three farms with a closed system in Schleswig-Holstein, northern Germany, was carried out between October 2020 to February 2022. The farms participated in this study on a voluntary basis and were not participants in specific health programs. However, the farms differed in terms of their production factors to increase the variance of the study. Table 1 shows the differences between their production factors.

Farm A was an organic farm where the animals were kept on concrete floors with straw and an outdoor arena in every production stage was available. The piglets from farm A remained with the lactating sow in the farrowing barn for three weeks and were then rearranged into a group suckling barn with two other sows and their litters in a community pen. On the 50th day of life the piglets from farm A were weaned and transferred to the piglet barn. In contrast, farms B and C were two conventionally managed farms where the animals were kept on a partially slatted floor. In these farms, the piglets were moved directly to the post-weaning barn after a four-week suckling period. Within the post-weaning period, the piglets of all batches were rehoused once. In all three farms, the animals were then transferred to the finishing barns at a live weight of approximately 30 kg. The animals of farm B, batch 3 spent the finishing period in a different finishing barn than the animals of batch 1 and 2 of farm B. A detailed overview of the barns (number/size of the pens, group size) of the individual farms is presented in Table 2.

In addition to the production factors described above, there were other criteria that distinguish the three farms. While the animals in farm A were fed by hand twice a day, feeding in farm B took place several times a day in the form of liquid feeding and automatic feeders (finishing barn 2, batch 3). Similar to farm A, feeding on farm C was also done by hand twice a day, but only in the post-weaning period, whereas feeding within the finishing period was ensured several times daily by automatic feeders.

Furthermore, the farms differed in terms of the ventilation systems (see Table 2). Farm A had free ventilation, with all pens in each barn having an exit to the outdoor arenas protected with plastic curtains. In

Table 1
Overview of the production factors of the three farms included in the present study (*d* = days; *n* = numbers).

Farm	A	B	C
Production type	Organic	Conventional	Conventional
Herd size, <i>n</i> sows	50	1400	60
Suckling period (d)	50	28	28
Pen floor	Concrete with straw	Partially slatted	Partially slatted
Outdoor arena	Yes	No	No
Feeding system	By hand plus roughage	Liquid feeding/automatic feeders ^b	By hand/automatic feeders ^a

^a : During the post-weaning period, the piglets were fed by hand, whereas during the finishing period, automatic feeders were available

^b : Post-weaning barn and finishing barn 1 liquid feeding; Finishing barn 2 automatic feeders.

addition, the pens were not divided into individual compartments; instead a large air space is created across all pens. In contrast, the barns of farms B and C were equipped with forced ventilation, where the air inlet was provided via pore ceilings/pore ceiling channels, except for finishing barn 2 of farm B, where ceiling valves were used. The exhaust air was discharged via overfloor ventilation, with the exhaust fans installed centrally in the compartments. In the finishing barn 2 of farm B, in contrast, there was a central extraction of the exhaust air at the front side of the building by a fan.

The piglets were crossbred, including Large White, Danish/German/Norwegian Landrace and Danish Yorkshire as sow genetic and Pietrain as boar. Sows on farms A and C were vaccinated against *Mycoplasma (M.) hyopneumoniae*, in contrast to sows on farm B.

2.3. Data collection NH₃-concentration

During the data collection, altogether three batches (batch: a group of pigs followed from birth to slaughter) for farm A and B and four batches for farm C were observed. This allowed measurement during the four seasons (spring (1): March, April, May; summer (2): June, July, August; autumn (3): September, October, November; winter (4): December, January, February). Due to the partial overlap of the batches, it was only possible to measure two batches per farm with the DOL (farm A, batch 2 and 3; farm B, batch 1 and 3; farm C, batch 1 and 4). For the measurement of the NH₃ concentration, the DOL's measuring equipment was placed approximately 1.5 m above a pen (if practicable, the middle pen of a compartment) at the beginning of a batch and followed the pigs included in the study through all production stages. This allowed the NH₃ concentration to be measured at 15-minute intervals with the integrated electrochemical sensor. Data was stored in a cloud. According to the manufacturer 'dol-sensors', the measuring range is between 0 ppm to 100 ppm and the accuracy is 1.5 ppm or $\pm 10\%$ of the measured value. The lifetime of the sensor is three years if the sensor element is installed by the stated expiry date (dol-sensors, 2021).

Measurements with the hand-held ammonia meters (AM, DA) were taken by one observer during the weekly assessment of the animals, in the same pen where the DOL was installed. The weekly assessment always started at 9 a.m. During this procedure the NH₃ concentration was measured at nose level of the animals (approximately 30 cm above the floor) with the AM and DA in the middle of the pen. The gas test tube (Ammoniak 5/a) was used for the measurement with the DA, which is equipped with a bellows pump. For each measurement, 10 manual strokes were performed, with 100 ml of air being sucked through the sample body for each stroke, resulting in a measuring range between 5

ppm and 70 ppm. According to the manufacturer 'Dräger', the standard deviation is $\pm 10\%$ to 15% . The NH₃ is indicated by a color scale, whereby the original color is yellow and a respective area of the yellow part changes to blue in dependence of the NH₃ concentration (Dräger, 2021). This was reported accordingly by the one observer. In contrast, the second hand-held ammonia meter, the Basic Ammonia Detector-NH3000 (AM) from 'Forensic Detectors', is equipped with an electrochemical sensor, which makes it possible to read the measured value easily. According to the manufacturer, this device measures NH₃ concentration in the range from 0 ppm to 1000 ppm and achieves an accuracy of ± 3 ppm. The error is specified as $\pm 10\%$ of the measured value. The lifetime of the sensor is specified as two years from the date of purchase (Forensic Detectors, 2020).

Across farms, the number of measured values was higher for the DOL ($n = 77,170$) than for the AM ($n = 284$) and the DA ($n = 142$) due to the continuous measurement; see Table 3. The highest mean of the NH₃ concentration across all farms was measured with the DOL and amounted to 12.4 ppm (Farm B). This measuring device also had the highest maximum value of 60.8 ppm, also for farm B. The mean NH₃ values determined for farm A are <1 ppm for all three measuring devices, although the maximum values differ more significantly (AM: 4.0 ppm; DA: 3.0 ppm; DOL: 13.1 ppm). The measured values for farm B show more differences between the measuring devices than for farm A in the maximum values (AM: 59.0 ppm; DA: 15.0 ppm; DOL: 60.8 ppm). The measurement results for farm C show a similar pattern to those of

Table 3

Overview of the measurements of each farm and the descriptive statistics of the measuring devices (n = number; NH₃ = ammonia; ppm = parts per million; Min. = minimum, Max. = maximum; Std. = standard deviation; AM = Basic Ammonia Detector-NH300; DA = Dräger accuro® gas detection pump; DOL = DOL53 Ammonia Sensor).

Farm	Device	n NH ₃ ≥ 20 ppm*	n	Mean	Min.	Max.	Std.
A	AM	.	65	0.51	0.00	4.00	0.81
	DA	.	58	0.36	0.00	3.00	0.77
	DOL	0	29,730	0.69	0.20	13.10	0.70
B	AM	.	126	9.79	0.00	59.00	9.34
	DA	.	45	5.51	0.00	15.00	3.27
	DOL	5064	19,925	12.44	0.30	60.80	8.49
C	AM	.	93	4.60	0.00	22.00	3.22
	DA	.	39	6.77	2.00	30.00	4.84
	DOL	3730	27,515	10.61	0.30	58.50	7.32

* : The number of NH₃ concentration measurements per batch and farm was previously adjusted to 10,800 measurements per batch to allow comparability of the numbers of NH₃ concentrations higher than 20 ppm.

Table 2

Overview of the ventilation systems of the barns from the three farms included in the present study (n = numbers; m² = square meter).

Farm	Barn	Compartments/ barn	Pen/ compartment	Pen size (m ²)	Group size (n) ^c	Space per pig (m ²)	Air inlet/ air outlet
A	Farrowing barn	2	8	13.2 ^a	.	.	free/free
	Group suckling	1	6	34.6 ^a	33.0	1.05	free/free
	Post-weaning barn	1	6	18.0 ^a	26.0	1.20	free/free
B	Finishing barn	1	8	22.0 ^a	10.0	2.45	free/free
	Farrowing barn	10	12	7.00	.	.	Pore ceiling/overfloor ceiling fans
	Post-weaning barn	6	6	11.3/8.1 ^b	50.0/20.0 ^b	0.22/0.40 ^b	Pore ceiling/overfloor ceiling fans
	Finishing barn 1	12	8	10.0	12.0	1.10	Pore ceiling/overfloor ceiling fans
C	Finishing barn 2	1	20	10.0	12.0	1.10	Ceiling valves/overfloor central fan front side
	Farrowing barn	3	6	7.00	.	.	Pore ceiling channels/overfloor ceiling fans
	Post-weaning barn	4	10	2.60	10.0/5.00	0.26/0.52	Pore ceiling channels /overfloor ceiling fans
	Finishing barn	4	6	15.0	13.0	0.75	Pore ceiling channels /overfloor ceiling fans

^a : The outdoor arena is included.

^b : Two values result for this cell, as the piglets were rehoused within the post-weaning period.

^c : The values shown represent the average number of animals over all batches.

farm B with differences in maximum values (AM: 22.0 ppm; DA: 30.0 ppm; DOL: 58.5 ppm) between the measuring devices.

2.4. Data collection of the on-farm and slaughterhouse indicators

In addition to the NH₃ measurement, the housed animals of the farms over the ten batches in the same pens of the NH₃ measurement were assessed using on-farm animal health/welfare indicators. Moreover, these animals were evaluated using slaughterhouse indicators at the slaughterhouses. For this purpose, in total 548 individually marked pigs were assessed weekly directly after birth until slaughter by one trained observer using on-farm indicators. A total of five indicators, which could be related to the NH₃ in the barn, were selected from different sources, the Welfare Quality Animal Welfare Assessment Protocol for pigs, the KTBL animal welfare indicators database and the general clinical examination (Baumgartner, 2009; KTBL, 2016; Welfare Quality, 2009). The on-farm indicators were assessed at animal-level using a two-category scale (0 = not present throughout all weekly assessments until slaughter, 1 = present at least once during all weekly assessments), except the indicator coughing. Coughing was evaluated at pen-level in the pens of the animals of this study, whereas the number of coughs per pen were recorded after making the animals stand up. The prevalence per visit was calculated and an average prevalence $\geq 5\%$ per batch was assumed as 1 (= present) and an average prevalence $< 5\%$ per batch was assumed as 0 (= not present) for the further calculations. The exact definitions of these five indicators and the number of affected animals are shown in Table 4. The number of affected animals was highest for coughing with 301 animals (54.93 %), followed by tail length with 250 animals (45.62%). In contrast, the lowest numbers of affected animals were nine (1.64 %) and 14 (2.55 %) for eye alterations and pumping, respectively.

Due to the individual marking of the pigs, the slaughterhouse findings of the meat inspection, regulated by the General Administrative Regulation of Food Hygiene (AVV LmH), could be assigned to the corresponding 548 animals at the slaughterhouse (BMEL, 2009). The exact definition of the slaughterhouse indicators used for the evaluation in this study and the number of affected animals are presented in Table 4 (QS, 2020). Pneumonia with 28 animals had the highest number of affected animals (5.11 %), whereas pleuritis showed the lowest number with nine (1.64 %) affected animals. In addition to the slaughterhouse indicators, lung tissue samples were taken from the slaughter line to detect a possible infection at batch-level with *M. hyopneumoniae* by qPCR. For this purpose, small lung samples from the left or right apical lobe (regardless of whether there was a change in the tissue or not) of the pigs were collected and stored in sterile containers. After the collection the samples were immediately frozen and DNA was extracted in the laboratory using the Qiagen DNeasy Blood & Tissue Kit. The determination of the purity and the measurement of the nucleic acid content was

carried out with the Nano Drop Spectrometer. Amplification of the DNA was done with VetMax-Plus qPCR Kit in duplicates. Only double positive samples were considered as positive results, where the cut-off was 40 cycles. Runs were included only if linear correlation of standard dilution rows was ≥ 0.98 . The results were validated with a second kit, the BactoReal Kit *Mycoplasma hyopneumoniae*, Biozol. Again, an average prevalence $\geq 5\%$ per batch was assumed as 1 (= present) and an average prevalence $< 5\%$ was assumed as 0 (= not present) for further calculations. The determined number of affected animals for *M. hyopneumoniae* was 188 (34.31 %) (Table 4).

2.5. Statistical analysis

The statistical software SAS® 9.4 (SAS Institute Inc., 2017) was used for the statistical analyses. First, possible influencing factors on the different measuring devices were investigated, and secondly, reliability and agreement parameters were determined to assess the comparability of the three measuring devices. Finally, the connection between NH₃ and the indicators were evaluated via Chi² Test and Cramér's V to determine the potential of NH₃ measurements as replacement for some on-farm indicators in welfare assessment schemes.

2.5.1. Linear mixed models

The measured NH₃ was approximately normally distributed, allowing linear mixed models to be used for each device (AM, DA, DOL) to determine the influencing factors. For this purpose, the models were created step by step and selected using the Akaike's information criteria corrected (AICC) and the Bayesian information criterion (BIC). One possible influencing factor was the season at the time of the measurement. For the integration of the season effect into the models, four classes were used (spring (1): March, April, May; summer (2): June, July, August; autumn (3): September, October, November; winter (4): December, January, February). The statistical significance value was set at $P < 0.05$.

Measuring devices AM, DA and DOL

The measured NH₃ concentration of the respective measuring device (AM, DA, DOL) was selected as the dependent variable. The final models for the AM, DA and DOL, respectively, included the fixed effects farm (A, B, C) and season (1, 2, 3, 4), while nesting the season within the farm.

2.5.2. Reliability and agreement parameters

In order to evaluate the comparability between the measuring devices a combination of different reliability and agreement parameters were calculated, which are described in detail below. All three

Table 4

Definition of category 1 of the on-farm/slaughterhouse indicators, the number (n) and proportion of affected animals (category 1) of all animals (548) in this study as well as the achieved P -value of the Chi² Test (bold = significant ($P < 0.05$)) and the Cramér's V for the connection of the indicators (category 1) with the NH₃ number ≥ 20 ppm. A connection was interpreted as meaningful if the values of the Cramér's V were ≥ 0.4 (italic type) and as strong if the values were ≥ 0.6 (bold type) and P -values were ≤ 0.05 (Baumgartner, 2009; Welfare Quality, 2009; KTBL, 2016; QS, 2020).

Indicator	Definition	n category 1	% category 1	P -Value	Cramér's V
<i>On-farm indicators</i>					
Coughing ^a	Number of coughs per 5 min. per pen	301/548	54.9	<0.0001	0.89
Eye alterations	At least one eye with alterations (swelling, discharge)	9/548	1.64	0.31	0.09
Pumping	Evidence of labored breathing	14/548	2.55	0.04	0.14
Tail length	Docked tails or partial/full loss of the tail (blunt)	250/548	45.6	<0.0001	0.81
Tail lesions	Visible lesions	206/548	37.6	<0.0001	0.60
<i>Slaughterhouse indicators</i>					
<i>M. hyopneumoniae</i> ¹	Detection of <i>Mycoplasma hyopneumoniae</i> on lung samples by PCR	188/548	34.3	<0.0001	1.00
Pericarditis	Altered	16/548	2.92	0.05	0.13
Pleuritis	>10 % of the pleura altered	9/548	1.64	0.42	0.08
Pneumonia	>10 % of the lung altered	28/548	5.11	<0.0001	0.22

^a : evaluation at pen-level.

measuring devices were compared with each other, therefore only measured values were retained, which were measured at the same date and time ($n = 125$). Each comparison was carried out separately for each farm as well as across all farms.

Pearson's correlation coefficient (r_p). The r_p is a parametric method for representing the relationship between two variables. The values can range from -1 to 1, with values closer to 1 indicating higher correlation and a greater confidence for reliability (Martin and Bateson, 1994). According to Sprinthal (1987) a $r_p \geq 0.40$ was interpreted as acceptable reliability and a $r_p \geq 0.70$ as good reliability.

Intraclass correlation coefficient (ICC). The ICC relates the variance between study objects to the variance between study objects plus measurement error (de Vet et al., 2006). According to Shrout and Fleiss (1979), a two-way model was taken and values between 0 and 1 could be obtained from the correlation coefficient. An ICC ≥ 0.40 was considered as acceptable and an ICC ≥ 0.70 as good reliability (McGraw and Wong, 1996).

Limits of agreement (LoA). The differences between two sets of measurement values and the standard deviations of these differences were estimated by the LoA (Bland and Altman, 1986). It indicated the differences between the compared measured values in the present study. The range of LoA values was from -60.8 to 60.8. Values towards -60.8 indicated higher readings by the first named device, while values towards 60.8 indicated higher readings by the second named device. The interpretation was based on the simple agreement coefficient by de Vet et al. (2006), an interval ≤ -6.0 to 6.0 implied acceptable agreement and an interval ≤ -3.0 to 3.0 implied good agreement (equaling a deviation of 10 % and 5 %, respectively).

2.5.3. Relationship between NH_3 and welfare indicators

To analyze the dependence between NH_3 and health status/animal welfare indicators on-farm and at the slaughterhouse, a Chi^2 Test was performed and Cramér's V was used to calculate the effect strength. A connection was interpreted as meaningful if the values of the Cramér's V were ≥ 0.4 and as strong if the values were ≥ 0.6 and P -values were ≤ 0.05 . For the calculation for each batch, the NH_3 values ≥ 20 ppm measured by the DOL were counted and the total number of NH_3 values ≥ 20 ppm were assigned to the animals of the corresponding batch. The number of the NH_3 concentration measurements per batch was previously adjusted to 10,800 measurements per batch and farm to allow comparability of the number of NH_3 concentrations ≥ 20 ppm. The highest number of measurements with a NH_3 concentration ≥ 20 ppm were obtained for farm B (5064), followed by farm C (3730), see Table 4. In addition to the NH_3 number ≥ 20 ppm, the binary values of the assessment of the on-farm/slaughterhouse indicators for each animal ($n = 548$) were used in the calculation.

3. Results

3.1. Linear mixed models

3.1.1. Basic ammonia detector-NH3000 (AM)

The results of the linear mixed model in Fig. 1a show a significant effect of farm and season. However, within farm A, no significant difference was observed between seasons. Whereas in farm B, the LS-mean of the NH_3 was estimated significantly higher in season 1 (15.6 ppm) compared to seasons 2 (2.80 ppm), 3 (5.80 ppm) and 4 (8.60 ppm). Furthermore, the estimates of the NH_3 were significantly higher in farm B for season 4 compared to season 2. Farm C showed no significant differences between seasons. Within the season, season 1 (0.60 ppm) and season 4 (0.30 ppm) in farm A had significantly lower estimated NH_3 than in farm B. In addition, farm B had significantly higher NH_3 in season 1 than farm C.

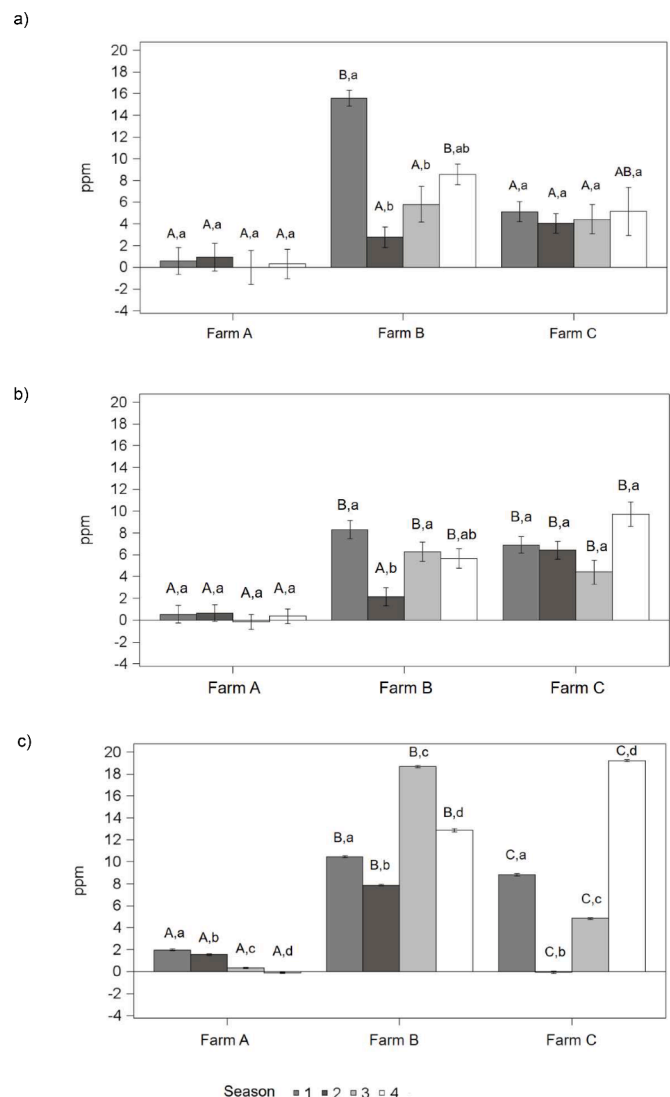


Fig. 1. a, b, c. Least-square means and standard error of the measured NH_3 concentration a) by Basic Ammonia Detector-NH3000; b) by Dräger accuro® gas detection pump; c) by DOL53 Ammonia Sensor over all farms and seasons. A, B, C: significant differences between farms within seasons ($P < 0.05$). a, b, c, d significant differences between seasons within farms ($P < 0.05$)

3.1.2. Dräger accuro® gas detection pump (DA)

The results of the linear mixed model in Fig. 1b indicate that farm and season also had a significant influence on the NH_3 measured by DA. However, again in farm A, the seasons did not differ significantly from each other. In contrast, farm B recorded significantly lower LS-means of the NH_3 in season 2 (2.10 ppm) than in seasons 1 (8.30 ppm) and 3 (6.30 ppm). As with the results from the AM, there was no difference between seasons within farm C. Within the seasons, NH_3 were estimated significantly lower in farm A compared to farm B (except season 2) and farm C. Furthermore, season 2 also showed significantly lower NH_3 in farm B than in farm C.

3.1.3. DOL53 ammonia sensor (DOL)

The results of the linear mixed model in Fig. 1c show the significant effect of farm and season on the NH_3 measured by the DOL. In this case, all seasons of farm A differed significantly from each other, the same applies to farm B and C. Also within the seasons, the estimated values of the four seasons differed significantly between the farms. Within season 4, farm C had significantly higher NH_3 than the other farms. Estimates were particularly high for season 3, farm B (18.7 ppm) and for season 4,

farm C (19.2 ppm).

3.2. DOL data – daily patterns

When considering the daily patterns of the average NH_3 concentration measured with the DOL, a variation between the farms as well as the respective barns can be recognized (see Fig. 2). In farm A (Fig. 2a), the NH_3 concentration in the farrowing barn varied around an average of 0.37 ppm and in the post-weaning barn around 0.33 ppm. In the group suckling barn as well as in the finishing barn, the NH_3 concentration was higher than that in the farrowing and post-weaning barns, with an average of 0.88 ppm in the group suckling barn and 0.75 ppm in the finishing barn. Common to all barns was the increase in the variation of the NH_3 concentration during the day and the decrease during the night.

In contrast to farm A, the NH_3 concentration of farm B in the post-weaning barn (average 6.30 ppm) decreased during the day and increased at night (Fig. 2b). The graph of the finishing barn of batches 1 and 2 shows a similar pattern, but at a higher level (average 9.32 ppm). The average NH_3 concentration varied markedly between the two

finishing barns, with 9.32 ppm for finishing barn 1 (batches 1 and 2), which was below the average NH_3 concentration of 20.0 ppm in finishing barn 2 (batch 3). In the second finishing barn, peaks were between 8–10 a.m. and from 6 p.m. onwards. No sensor data from the farrowing barn were available for farm B.

Fig. 2c also shows a decrease in the NH_3 concentration during the day and an increase at night for farm C in the farrowing and post-weaning barn. The NH_3 concentration varied around an average of 9.60 ppm in the farrowing barn and 7.40 ppm in the post-weaning barn. In contrast, the NH_3 concentration in the finishing barn was higher during the day than at night. The average NH_3 concentration in the finishing barn was 12.1 ppm for farm C.

3.3. Reliability and agreement parameters

Table 5 shows the results of the reliability and agreement parameters of the comparisons of the NH_3 concentration from all three measuring devices with each other ($n = 125$). The first comparison is between the AM and the DOL. On farm A, good agreement between the measuring devices (AM - DOL) was achieved with an LoA of $\epsilon [-0.77; -0.18]$. For farms B and C, as well as across all farms, neither acceptable/good results for the reliability nor for the agreement parameters could be determined.

The second comparison is between the DA and DOL. The results of the reliability and agreement parameters of these two measuring devices achieved acceptable results (≤ 0.40) for the reliability parameter (r_p) at farm B and C. This led to an acceptable value of 0.58 for the r_p across all farms, but the other two parameters did not achieve acceptable/good results.

The third comparison of the two hand-held devices DA and AM showed acceptable to good results for the reliability parameters within all three farms. The agreement parameter LoA, in contrast, achieved only good results on farm A (LoA of $\epsilon [-1.01; 1.05]$). Considering the measured values of all farms, there was an acceptable result for the correlation coefficient ($r_p = 0.64$) and a good result for the statistical parameter ICC (ICC = 0.71).

3.4. Relationship between NH_3 and welfare indicators – Chi² Test and Cramér's V

Table 4 presents the P -values of the Chi² Test and the values of the Cramér's V of the NH_3 number ≥ 20 ppm with the on-farm/slaughterhouse indicators. As shown from the five on-farm indicators, a significant and a strong connection with the NH_3 number ≥ 20 ppm was determined for coughing ($V = 0.89$; $P < 0.0001$), tail length ($V =$

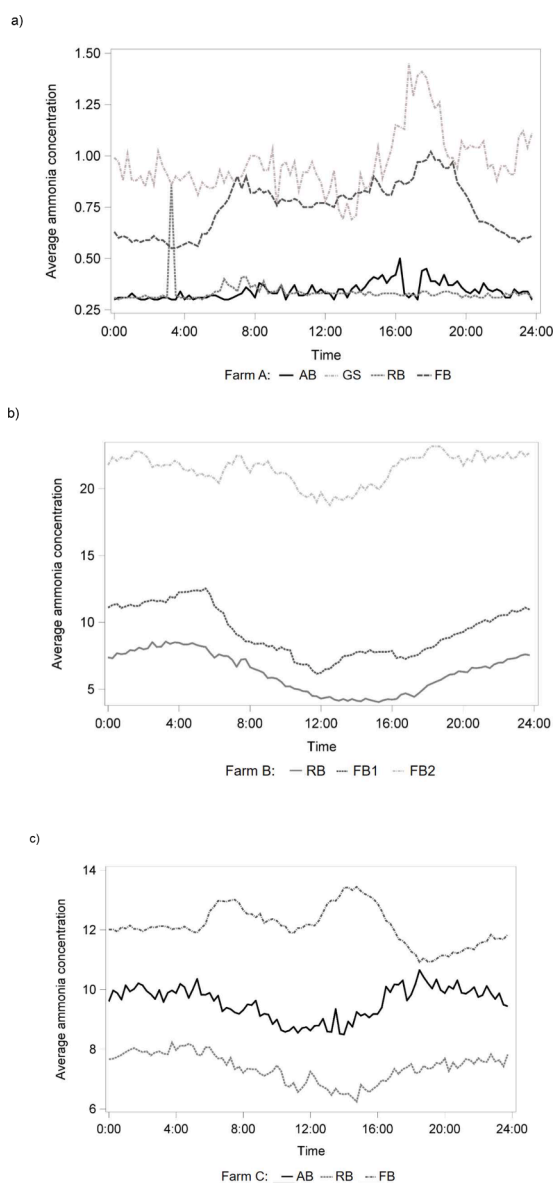


Fig. 2. a, b, c. Daily pattern of the average NH_3 concentration measured by DOL53 ammonia sensor for the barns of farm A (a), B (b) and C (c). (AB = farrowing barn; GS = group suckling barn; RB = post-weaning barn; FB = finishing barn; FB1 = finishing barn 1; FB2 = finishing barn 2).

Table 5

Statistical parameters (Pearson's correlation coefficient: r_p , intraclass correlation coefficient: ICC, limits of agreement: LoA) for the comparisons of the ammonia concentration ($n = 125$) measured at the same time and date by the DOL53 Ammonia Sensor (DOL) continuous measurement sensor and the hand-held Basic Ammonia Detector-NH3000 (AM) as well as the hand-held Dräger accuro® gas detection pump (DA) indicating poor (normal type), acceptable (italic type) and good agreement (bold type).

Devices	Farm	Batch	r_p	ICC	LoA
AM - DOL	A	2; 3	.	0.00	-0.77 to -0.18
	B	1; 3	0.05	0.05	-36.7 to 25.0
	C	1; 4	0.17	0.12	-16.0 to 6.22
	all	all	0.17	0.16	-29.3 to 19.1
	all	all	0.17	0.16	-29.3 to 19.1
DA - DOL	A	2; 3	.	0.00	-0.77 to -0.18
	B	1; 3	0.60	0.28	-33.0 to 14.6
	C	1; 4	0.60	0.57	-10.7 to 4.61
	all	all	0.58	0.34	-25.9 to 13.2
	all	all	0.58	0.34	-25.9 to 13.2
DA - AM	A	1; 2; 3	0.78	0.78	-1.01 to 1.05
	B	1; 2; 3	0.53	0.4	-11.3 to 15.4
	C	1; 2; 4	0.80	0.65	-7.44 to 4.97
	all	all	0.64	0.71	-8.63 to 9.66
	all	all	0.64	0.71	-8.63 to 9.66

0.81; $P < 0.0001$) and tail lesions ($V = 0.60$; $P < 0.0001$). Moreover, significant and strong connection were calculated for the slaughterhouse indicator *M. hyopneumoniae* ($V = 1.00$; $P < 0.0001$).

4. Discussion

The aim of this study was to investigate factors influencing the handheld ammonia meters (AM, DA) and the ammonia sensor (DOL) with regard to the practical use in pig houses and to compare them in terms of their reliability. The NH_3 concentration was also considered as a possible animal welfare indicator.

4.1. Linear mixed models

Regardless of the measuring device, both season and farm had a significant influence on the measured NH_3 concentration. The significant effect of the farm, which is noticeable in significantly lower NH_3 for farm A, could be explained by the different requirements of the organic compared to the conventional husbandry systems (floor, litter, outdoor arena, space per pig).

For NH_3 emissions, the size of the emitting surface plays a significant role (Aarnink et al., 1996; Ni et al., 1999), so that the consideration of the floor of the barns from the different husbandry systems is relevant. Various studies have shown that the use of partially slatted floors led to lower NH_3 emissions compared to fully slatted floors (Groot Koerkamp et al., 1998; Sun et al., 2008; Ye et al., 2009). Sun et al. (2008) explained this result by the fact that the open slurry area is reduced by partially slatted floors. Slurry storage below the slatted floor for months can account for 70–80 % of total barn emissions (Monteny, 1996). This may explain the higher NH_3 in the barns of farm B and C, where the slurry was stored below the partial slatted floors, whereas on farm A the outdoor areas were mucked weekly. However, when comparing deep litter systems with conventional systems, significantly higher NH_3 emissions were found in the deep litter system by Dewes (1996), whereas Kavelis (2006) and Groenestein et al. (2007) determined contrary results. The literature review of Philippe et al. (2011) confirmed these contrasting results and assumed strong influences of the management (amount of litter, removal of manure), the physiological state of the animals and the choice of litter. In general, the litter in farm A was obviously beneficial with regard to the measured NH_3 concentration. In addition to the factors influencing the litter, it must also be considered that the animals on farm A had access to an outdoor area. The observer could recognize that the defecation behavior of the animals mainly took place in the outdoor arena. This observation was also made by Olsen et al. (2001), where more than 75 % of the pigs, given free choice, defecated in the outdoor arena. This is due to the fact that pigs prefer cooler, brighter and more humid areas for defecation (Hoy, 2009). The frequent use of the outdoor area for defecation most probably added to good air quality inside the barn (Moeller and Olsen, 1999) where the sensor was positioned in this study.

Another important difference between organic farm A and conventional farms B and C is the space per pig and pen. Hacker et al. (1994), for example, recorded a higher level of pen fouling with a higher animal density at the same time. As a result of increased pen fouling the emitting surface extended and thus contributes to the higher release of NH_3 (Aarnink et al., 1997). Furthermore, more animals in the same space have more excretions, which can lead to an increase in the NH_3 concentration. However, it must also be noted that a low animal density can lead to increased NH_3 due to the resulting of a large defecation area (no passage of excrements through the slatted floor) (Krieter, 2016).

Another significant influence on the NH_3 concentration measured with all three measuring devices was, in addition to the farm (husbandry system), the season. The main influencing factors were probably the ventilation rate and the pig behavior, which is influenced by temperature. Thus, for the mechanically ventilated farms B and C, particularly a high NH_3 concentration could be measured with all three measuring

devices, especially in seasons 1 (spring) and 4 (winter), which coincides with the results of Harper et al. (2004) and Granier et al. (1996). The authors explained these results with the increasing temperatures during the summer period and the associated higher ventilation, resulting in lower NH_3 in the barns in summer compared to winter. An exception to the previously made statement were significantly higher NH_3 in season 3 (summer) than in season 4 (winter) measured with the DOL at farm B. However, during the third batch (season 3) of farm B, the animals were fattened in a different barn than the batches 1 and 2, so this effect is probably because of the barn. Due to the fact that in the model used the season were nested within the farm and the barn was not included, it is not possible to clearly separate the influences of these factors.

4.2. Daily ammonia patterns

The daily patterns of the NH_3 concentration measured by DOL are intended to clarify the question of how reliable a single measurement is with regard to determining the NH_3 concentration of a barn. As can be seen in the graphs, the daily NH_3 concentration in the single barns of the different farms varies greatly. The main influencing factors would be the behavior of the animals, the barn itself (ventilation system, compartment size/group size) and the age of the animals.

The daily patterns of the different barns from the three farms showed contradictory results regarding the NH_3 peak (day or night). Thus, for all three farms, the NH_3 peak during the day could only be determined for the finishing barns, with the exception of finishing barn 1, farm B, whereas in all other barns the NH_3 peak occurred during the night (with exception of the group suckling barn, farm A). These inconsistent results are similar to the literature (Aarnink et al., 1995; Banhazi, 2015), but the highest peaks tend to be during night (Rodriguez et al., 2020). Highest NH_3 peaks during night could be explained by the low ventilation rates overnight due to low animal activities (Calvet et al., 2011). However, the higher NH_3 concentration during the day for the finishing barn of farms B (finishing barn 2) and C were probably related to the activity peaks of the animals in the morning and late afternoon hours (de Sousa and Pedersen, 2004; Sambras, 1991). In contrast to finishing barn 1 of farm B, no long troughs were used in finishing barn 2 of farm B, but instead automatic feeders, which increased the activity of the animals due to a lower animal-feeding place-ratio. Also Hansen et al. (1982) confirmed that fewer feeding places than animals can lead to competition (more activity) for access to the feeder. According to de Sousa and Pedersen (2004) 15 % to 56 % of the diurnal variation in NH_3 emissions can be explained by animal activity behavior.

The different NH_3 concentration between the finishing barns on farm B may be due to different ventilation technology on the one side, but also to the size of the compartments on the other. While finishing barn 1 had eight pens of 20 animals per compartment, finishing barn 2 had 40 pens of 20 animals per compartment. As a result, there were significantly more animals and correspondingly more emissions per compartment in finishing barn 2, and the installed ventilation technology reached its limits at activity times.

In addition, it is also noticeable that the measured NH_3 concentration increased with the advancing production stage (increasing age of the pigs). These findings are in line with the results of Philippe et al. (2007) and Ni et al. (1999). This connection is justified by Philippe et al. (2011) with the increased feed intake and manure production of the growing pig.

Due to the varying NH_3 concentration during the day, a measurement once a week cannot completely capture these variations. Therefore, repeated measurements during the day and night over a long period of time are required to control the air quality (Ni and Heber, 2001) and make adjustments accordingly.

4.3. Reliability and agreement parameters

The evaluation of the reliability and agreement of the different

measurement devices was carried out using two reliability parameters (r_p , ICC) and one agreement parameter (LoA), which were interpreted together in order to balance the respective strengths and weaknesses of the individual parameters and thus prevent misinterpretations (de Vet et al., 2006). The reliability parameters are dependent on the variance of the examined objects, which is not the case for agreement parameters (Müller and Büttner, 1994). However, interpretation of agreement parameters is based on subjective interpretation (Bland and Altman, 1986; de Vet et al., 2006).

The comparison of the AM/DA with the DOL did not show good results for the statistical parameters with the exception of the LoA for farm A. The insufficient reliability but good agreement can be explained by the lack of variance (almost no NH_3 measurable at farm A) (Müller and Büttner, 1994). At the level of farm A, the same result was also found when comparing the measured values of the DA and the DOL. Thus, there is a consistency of the devices when no NH_3 is present. In contrast, the comparison of DA and DOL showed acceptable results for the reliability parameters at farm B (r_p) and farm C (r_p and ICC) as well as across all three farms (r_p), while not reaching sufficient agreement. Based on these different results between DOL and AM (low reliability) as well as DA (acceptable reliability), it can be concluded that the AM reacts more quickly to small changes in the NH_3 concentration due to environmental effects than the DA and DOL, such as opening the slurry channels. This assumption is confirmed by the study of Wheeler et al. (2000) in poultry.

Likewise, the comparison of the two hand-held measuring devices (AM, DA) led to acceptable to good results for the reliability parameters, whereas no acceptable/good results were obtained for the agreement parameter, except for farm A. These quite good reliability results of the two hand-held measuring devices are probably due to the identical measurement procedure. The NH_3 concentration was measured with both measuring devices directly after each other at the nose level of the animals (approximately 30 cm), whereas the sensor above the pen was suspended out of the reach of the animals. The question is whether an exact reliability/agreement is needed or not in order to replace one sensor with another. According to Bland and Altman (1986), two measurement methods can only be used substitutionally if they do not diverge in a manner that influences management decisions or interpretations of results. Generally, similar results can be obtained with both hand-held devices, giving the farmer an indication of the NH_3 concentration. In summary, it can be said that good reliability is sufficient for farm comparisons (e.g. certification purposes), since the order of the farms remains identical. However, if an evaluation or management decision is planned, a good agreement should also be reached and, in this case, continuous recording would be preferable so that all fluctuations can be detected. Nevertheless, with the DOL, the shelf life is significant and regular calibration must be carried out for valid results. Also, a power supply (and internet) is necessary, so the hand-held meters could be advantageous for hard-to-reach areas or for checking different spots of a barn. They are also user-friendly and not very cost-intensive (Ni and Heber, 2001; Wheeler et al., 2000).

4.4. Relationship – NH_3 x on-farm indicators

The maximum NH_3 concentration measured indicates that the legally prescribed value of 20 ppm (Anonymous, 2006b) for the protection of animals was exceeded for short time intervals in farms B and C during data collection. Hinz and Linke (1998) also found NH_3 of up to 35 ppm in German pig barns. Due to the known negative effects on health, the last part of the study examined the suitability of NH_3 as an indicator of animal welfare by analyzing the connection between animal-based on-farm indicators (which are often quite time-consuming to observe) and the NH_3 concentration. Out of five on-farm indicators, a significant Chi² Test and a strong connection could be determined for tail length, tail lesions and coughing. Thereby it should be noted that the prevalence of the on-farm indicators with no significant result was below fifteen affected animals out of 548, while the significant indicators had affected

animals of 200 and more. The reason for the high number of affected animals for tail length can be related to the docking of tails on farm B. Without the data of farm B no strong connection could be found. Coughing was considered at pen level due to feasibility considerations. Pessoa et al. (2022) and Stombaugh et al. (1969) reported similar results between the occurrence of coughing (evaluated at pen level) and a continuous measurement of NH_3 . Whereas Vermeer and Hopster (2018) in their study did not find a significant correlation between coughing at pen level and NH_3 . In comparisons with the present study they reported lower prevalence for coughing/sneezing (weaners: mean 4.13 %; finishing pigs 0.71 %), so coughing may not have been a clinical symptom in their study. The third on-farm indicator with a higher number of affected animals and a significant and strong connection to the NH_3 concentration in the present study was tail lesions. These results could be confirmed by Scollo et al. (2017). They showed that NH_3 above 28 ppm was an important factor in the prevalence of tail biting. Also other authors have described ammonia as the primary gas, which induces stress, including tail biting (Smith et al., 1996; Wathes et al., 2002).

Accordingly, the results confirm the statements of Vermeer and Hopster (2018), that an improved barn climate provides the animals a higher standard of animal welfare. However, it is difficult to identify negative effects of NH_3 <20 ppm in pigs using animal-based on-farm indicators. In order to increase the prevalence of the on-farm indicators, it would be useful in future studies to increase the variance of the data through farms that differ more in their technical equipment (ventilation system) and management (manure systems, manure removal).

4.5. Relationship – NH_3 x slaughterhouse indicators

The analysis between the NH_3 concentration and the slaughterhouse indicators showed only for the indicator *M. hyopneumoniae* a significant and strong connection to the NH_3 concentration. In contrast, Donham (1991) found a connection between NH_3 (above 24.6 ppm) and pleuritis. These results are also consistent with the findings of Michiels et al. (2015), where increasing NH_3 resulted in higher odds of pleuritis lesions and a higher number of qPCR positive *M. hyopneumoniae* nasal samples. However, it should be noted that the authors deliberately chose a farm where clinical problems due to *M. hyopneumoniae* and high NH_3 concentrations were known. In the present study, the prevalence for *M. hyopneumoniae* was significantly lower, but hardly much lower for the batches of farm B, where the animals were not vaccinated against *M. hyopneumoniae*. Thus, a prevalence of up to 20% for the batches of farm B was found, whereas the prevalence of the batches of farm A and C was below 1%. In general, the lung lesions heal twelve weeks after the infection (Sørensen et al., 1997). It is possible that the ability of the lung tissue to heal influenced the detection of significant connections. Again, increasing the variance by including farms with different health management would be useful for future studies.

Based on the determined significant connections between NH_3 and *M. hyopneumoniae*, it is possible that air quality can have an impact on health status/animal welfare throughout the production period.

5. Conclusion

The aim of this study was to evaluate the hand-held ammonia meters compared to the ammonia sensor with regard to the practical use in pig houses and to investigate their influencing factors. The NH_3 concentration was also considered as a possible animal welfare indicator, so that animal-related on-farm indicators, where problems with the reliability may have occurred, could be replaced. It could be demonstrated that NH_3 concentrations strongly depend on the farm (floor, litter, outdoor arena, space per pig) and the season (ventilation rate, animal behavior). In addition, there were strong diurnal fluctuations, which are probably due to the behavior of the animals (activity phases), the husbandry system (ventilation rate, compartment size) and the production stage (age of the animals). These various influencing factors lead to

constantly changing NH_3 concentrations, so a continuous measurement of NH_3 would be able to record these variations more accurately. This is also demonstrated by the unsatisfactory results of the reliability parameters. The influence of NH_3 on animal health and welfare has also been evidenced. Thus, connections between both on-farm indicators (coughing, tail length, tail lesions) and a slaughterhouse indicator (*M. hyopneumoniae*) could be demonstrated. In conclusion, it can be said that continuous control of NH_3 concentrations enable the detection of variability and thus allow for informed management decisions that can increase animal welfare. Additionally a continuous measurement could probably replace/add to some health/welfare indicators.

CRedit authorship contribution statement

Johanna Witt: Methodology, Investigation, Data curation, Writing – original draft, Visualization. **Joachim Krieter:** Conceptualization, Writing – review & editing, Supervision, Project administration. **Kira Schröder:** Methodology, Data curation. **Kathrin Büttner:** Software, Resources, Methodology. **Christina Hölzel:** Validation, Resources. **Katja Krugmann:** Writing – review & editing. **Irena Czycholl:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare no conflict of interest. The founding sponsors had no role in the literature research, in the writing of the manuscript and in the decision to publish the results.

Acknowledgements

This work was financially supported by the H.W. Schaumann Foundation and the QS research fund.

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