

Aus dem Institut für Landwirtschaftliche Verfahrenstechnik  
der Christian-Albrechts-Universität zu Kiel

**Reduction of ammonia emissions by applying a urease inhibitor in dairy  
livestock systems**

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zur Erlangung des Doktorgrades  
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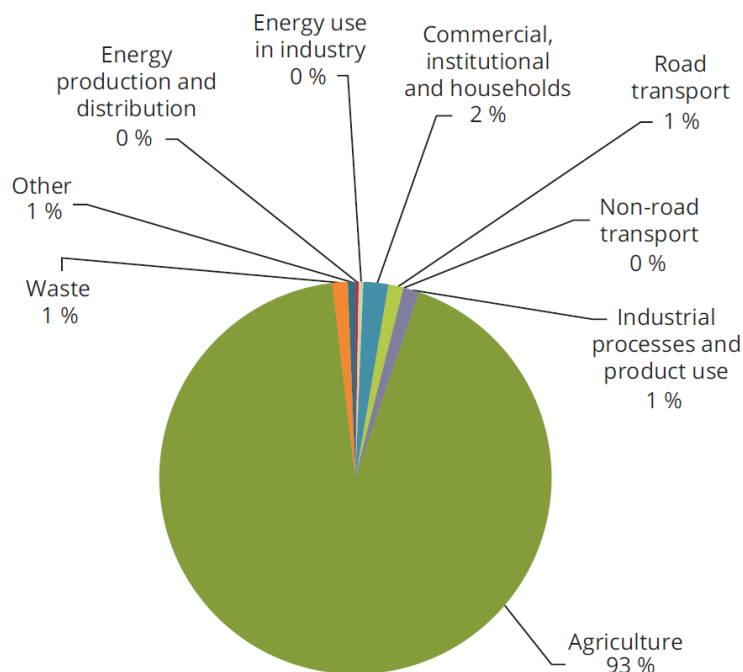
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## General introduction

Air pollution, which includes ammonia emissions, affects human and animal health (ATSDR, 2004). Moreover, these emissions cause long-term environmental harm due to eutrophication and acidification (EEA, 2019a; Erisman, Bleeker, Hensen, & Vermeulen, 2008). The gas ammonia contributes to the formation of secondary particles such as PM<sub>2.5</sub> (Backes, Aulinger, Bieser, Matthias, & Quante, 2016; EEA, 2019a). These particles can be related to respiratory health problems (Loftus et al., 2015). A reduction in ammonia emissions could result in a decrease in PM<sub>2.5</sub> particles (Backes et al., 2016). Therefore, Germany is aiming to improve air quality based on the Directive 2016/2284 (EC, 2016). This requires an annual decrease in ammonia emissions between 2020 and 2029 of 5 %, using the values from the year 2005 as a reference. Subsequent to 2030 and on an ongoing basis, ammonia emissions must be reduced by 29 % every year (EC, 2016). However, between 2014 and 2017, ammonia emissions increased by 2.5 %, resulting in an increase in European Union ammonia emissions for the fourth consecutive year. The main reason was considered to be a lack of reduction measures in the agricultural sector (Figure 1). Of all the member states, Germany was the largest ammonia emitter in 2017 (EEA, 2019b). In Germany, agriculture is responsible for almost 95 % of the total ammonia emissions (UBA, 2018).



**Figure 1** Ammonia emissions (2018) of the European Union share by sector (EEA, 2020)

Due to an improvement in animal welfare standards, most dairy cows are kept in freestalls (Statistisches Bundesamt, 2010). These livestock systems release ammonia directly into the

atmosphere. However, the amount of released ammonia is dependent on the interaction of multiple parameters. Emission levels can vary widely, depending e.g. on the season, housing, floor surface and management (Hristov et al., 2011). In order to improve and achieve the quotas established in the Directive 2016/2284 (EC, 2016), several mitigation measures have been implemented on dairy farms. The implementation of good agricultural practice measures (UNECE, 2015), such as the adjustment of crude protein in feed (Cole et al., 2005; Erickson & Klopfenstein, 2010; Frank, Persson, & Gustafsson, 2002; Todd, Cole, & Clark, 2006) prevents an oversupply and the resulting increase in ammonia emissions. The hydrolysis process, in particular, is limited by the available concentration of urea (up to 40–50 % of N, manures and faeces combined (van Horn, Newton, & Kunkle, 1996)) in urine. Therefore, the correct handling of manure is mandatory. Covering the manure pits (Berg, Brunsch, & Pazsiczki, 2006) prevents the uncontrolled volatilization of gas into the air. Furthermore, more specific mitigation measures could be the use of special floor types (Braam, Ketelaars, & Smits, 1997; Braam, Smits, Gunnink, & Swierstra, 1997; Braam & Swierstra, 1999; Snoek, Haesen, Groot Koerkamp, & Monteny, 2010; Swierstra, Braam, & Smits, 2001; Zähler & Schrade, 2020) and the use of additives (Kavanagh et al., 2019; Shi, Parker, Cole, Auvermann, & Mehlhorn, 2001). Further studies have revealed that emissions can be reduced by flushing with, for e.g., water (Kroodsma, Huis in 't Veld, & Scholtens, 1993; Ogink & Kroodsma, 1995). However, the previously mentioned measures might require excessive reorganisation and, especially when mixing any kind of acid with the liquid manure, safety precautions are needed. Moreover, the amount of flushing water required is incompatible with sustainability. Farming schedules are already limited and there is ongoing profitability pressure. Therefore, the implementation of these time-consuming and possible expensive methods are not considered practical.

Due to the previously listed challenges, the requirements of the mitigation measure should include a convenient handling, adaptability to new and old livestock housings and affordability. One solution could be the use of urease inhibitors. This mitigation approach appears to be an efficient and economical way to reduce ammonia emissions. The gas ammonia originates through the hydrolysis of urea. The enzyme urease is ubiquitous and works as a catalyst in this process. The inhibitor prevents the breakdown of urea by blocking the active centre of the enzyme urease (Leinker, 2007). Several research studies have tested different inhibitors and their potential over recent years (Hagenkamp-Korth, Haeussermann, & Hartung, 2015; Hagenkamp-Korth, Haeussermann, Hartung, & Reinhardt-Hanisch, 2015; Hagenkamp-Korth, Ohl, & Hartung, 2015; Leinker, 2007; Parker et al., 2005; Parker et al., 2016; Reinhardt-Hanisch, 2008; Varel, 1997; Varel, Nienaber, & Freetly, 1999). However, until this stage in the work, the urease

inhibitor has not been officially classified in a reduction category because the reliable data sources are still based on estimations (Eurich-Menden, Grimm, & Wulf, 2018), since the procedures concerning the working mechanisms of the inhibitor have still not been fully investigated. Most of the studies are based on laboratory experiments, and the transferability to investigations under practical conditions are difficult (Ndegwa, Hristov, Arogo, & Sheffield, 2008). Therefore, the aim of this thesis was to quantify the reduction potential for the inhibitor K under laboratory and field conditions and to investigate the chronological development of the reduction after the application of the inhibitor by using different statistical methods. In order to contribute to the general emissions measurement inventar, the field investigations were based on the international “Test Protocol for Livestock Housing and Management Systems; Version 3:2018-09” (International VERA Secretariat, 2018). The structure and content of the thesis is based on the following chapters:

Chapter 2 (Material & methods) provides a detailed description of the experimental setups, the measurement schedule, the measurement technique and the considerations relating to the pretests.

Chapter 3 (first publication) presents investigations of the inhibitor inside respiration chambers. This setup provided a controlled environment and the focus was solely on ammonia emissions. This chapter represents the first steps in the possible data selection and statistical analysis in order to estimate the chronological reduction development.

Chapter 4 (second publication) addresses the testing of the inhibitor in two small-scale, mechanically ventilated dairy farm units over all seasons. In this chapter, two different experimental approaches were investigated in order to estimate the reduction potential and the working mechanisms under field conditions.

Chapter 5 (third publication) addresses the inhibitor testing in two naturally ventilated dairy farms over all seasons. In addition to the calculated reduction potential, two different theoretical scenarios were created in order to provide information on the annual reduction pattern.

Chapter 6 (general discussion), discusses the experiences gained, classifies the experiments conducted and provides recommendations for upcoming studies. A general cost calculation of selected mitigation measures and a comparison to the current study could be provided.

Chapter 7 (general conclusion) outlines the gained experiences of the current study.

Chapter 8 (summary/english) summarizes the current study.

Chapter 9 (summary/german) summarizes the current study.

Chapter 10 (Appendix) represents additionally informations via tables and graphs.

## References

- ATSDR, US. [Agency for Toxic Substances and Disease Registry] (2004). Toxicological profile for ammonia. *US Department of Health and Human Services, Agency for Toxic Substances and Disease Registry*. Retrieved August 05, 2020, from <https://www.atsdr.cdc.gov/toxprofiles/tp126.pdf>.
- Backes, A. M., Aulinger, A., Bieser, J., Matthias, V., & Quante, M. (2016). Ammonia emissions in Europe, part II: How ammonia emission abatement strategies affect secondary aerosols. *Atmospheric Environment*, *126*, 153–161. <https://doi.org/10.1016/j.atmosenv.2015.11.039>
- Berg, W., Brunsch, R., & Pazsiczki, I. (2006). Greenhouse gas emissions from covered slurry compared with uncovered during storage. *Agriculture, Ecosystems & Environment*, *112*(2-3), 129–134. <https://doi.org/10.1016/j.agee.2005.08.031>
- Braam, C. R., Ketelaars, J. J. M. H., & Smits, M. C. J. (1997). Effects of floor design and floor cleaning on ammonia emission from cubicle houses for dairy cows. *Netherlands Journal of Agricultural Science*. (45), 49–64. <https://doi.org/10.18174/njas.v45i1.525>
- Braam, C. R., Smits M. C. J., Gunnink, H., & Swierstra, D. (1997). Ammonia Emission from a Double-Sloped Solid Floor in a Cubicle House for Dairy Cows. *Journal of Agricultural Engineering Research*. (68), 375–386. <https://doi.org/10.1006/jaer.1997.0215>
- Braam, C. R., & Swierstra, D. (1999). Volatilization of Ammonia from Dairy Housing Floors with Different Surface Characteristics. *Journal of Agricultural Engineering Research*. (72), 59–69. <https://doi.org/10.1006/jaer.1998.0345>
- Cole, N. A., Clark, R. N., Todd, R. W., Richardson, C. R., Gueye, A., Greene, L. W., & McBride, K. (2005). Influence of dietary crude protein concentration and source on potential ammonia emissions from beef cattle manure. *Journal of Animal Science*, *83*(3), 722–731. <https://doi.org/10.2527/2005.833722x>
- EC [European Commission]. (2016). Directive (EU) 2016/2284 of the European Parliament and of the Council of 14 December 2016 on the reduction of national emissions of certain atmospheric pollutants, amending Directive 2003/35/EC and repealing Directive 2001/81/EC. *OJL*, *344*, 1-31. Retrieved August 28, 2018 from <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L:2016:344:FULL&from=EN>



- EEA [European Environment Agency] (2019a). Air quality in Europe — 2019 report. (No 10/2019). Retrieved August 10, 2020 from <https://www.eea.europa.eu/publications/air-quality-in-europe-2019> DOI: 10.2800/822355
- EEA [European Environment Agency] (2019b) National Emission Ceilings (NEC) Directive reporting status 2019 Retrieved August 10, 2020 from <https://www.eea.europa.eu/themes/air/air-pollution-sources-1/national-emission-ceilings/nec-directive-reporting-status-2019/download.pdf.static> DOI: 10.2800/49299
- EEA [European Environment Agency] (2020). European Union emission inventory report 1990-2018 under the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP). (No 5/2020), Retrieved August 10, 2020 from <https://www.eea.europa.eu/publications/european-union-emission-inventory-report-1990-2018> DOI: 10.2800/233574
- Erickson, G., & Klopfenstein, T. (2010). Nutritional and management methods to decrease nitrogen losses from beef feedlots. *Journal of Animal Science*, 88(13 Suppl), E172-80. <https://doi.org/10.2527/jas.2009-2358>
- Erismann, J. W., Bleeker, A., Hensen, A., & Vermeulen, A. (2008). Agricultural air quality in Europe and the future perspectives. *Atmospheric Environment*, 42(14), 3209–3217. <https://doi.org/10.1016/j.atmosenv.2007.04.004>
- Eurich-Menden, B., Ewald Grimm, & Wulf, S. (2018). Emissionsminderung Rinderhaltung – Möglichkeiten und Grenzen. In Bayerische Landesanstalt für Landwirtschaft (Eds.), *Milchviehhaltung - Lösungen für die Zukunft Landtechnisch-bauliche Jahrestagung* (pp. 89-96). Retrieved August 12, 2020, from <https://www.lfl.bayern.de/mam/cms07/publikationen/daten/schriftenreihe/milchviehhaltung-ilt-jahrestagung-2018-lfl-schriftenreihe.pdf>
- Frank, B., Persson, M., & Gustafsson, G. (2002). Feeding dairy cows for decreased ammonia emission. *Livestock Production Science*, 76(1-2), 171–179. [https://doi.org/10.1016/S0301-6226\(02\)00021-0](https://doi.org/10.1016/S0301-6226(02)00021-0)
- Hagenkamp-Korth, F., Haeussermann, A., & Hartung, E. (2015). Effect of urease inhibitor application on urease activity in three different cubicle housing systems under practical conditions. *Agriculture, Ecosystems & Environment*, 202, 168–177. <https://doi.org/10.1016/j.agee.2015.01.010>
- Hagenkamp-Korth, F., Haeussermann, A., Hartung, E., & Reinhardt-Hanisch, A. (2015). Reduction of ammonia emissions from dairy manure using novel urease inhibitor formulations under laboratory conditions. *Biosystems Engineering*, 130, 43–51. <https://doi.org/10.1016/j.biosystemseng.2014.12.002>

- Hagenkamp-Korth, F., Ohl, S., & Hartung, E. (2015). Effects on the biogas and methane production of cattle manure treated with urease inhibitor. *Biomass and Bioenergy*, 75, 75–82. <https://doi.org/10.1016/j.biombioe.2015.02.014>
- Hristov, A. N., Hanigan, M., Cole, A., Todd, R., McAllister, T. A., Ndegwa, P. M., & Rotz, A. (2011). Review: Ammonia emissions from dairy farms and beef feedlots. *Canadian Journal of Animal Science*, 91(1), 1–35. <https://doi.org/10.4141/CJAS10034>
- International VERA Secretariat (2018). VERA test protocol for Livestock Housing and Management Systems: Version 3:2018-09, 1–60. Retrieved January 21, 2019 from [https://www.vera-verification.eu/app/uploads/sites/9/2019/05/VERA\\_Testprotocol\\_Housing\\_v3\\_2018.pdf](https://www.vera-verification.eu/app/uploads/sites/9/2019/05/VERA_Testprotocol_Housing_v3_2018.pdf)
- Kavanagh, I., Burchill, W., Healy, M. G., Fenton, O., Krol, D. J., & Lanigan, G. J. (2019). Mitigation of ammonia and greenhouse gas emissions from stored cattle slurry using acidifiers and chemical amendments. *Journal of Cleaner Production*, 237, 117822. <https://doi.org/10.1016/j.jclepro.2019.117822>
- Kroodsma, W., Huis in 't Veld, J.W.H., & Scholtens, R. (1993). Ammonia emission and its reduction from cubicle houses by flushing. *Livestock Production Science*, 35(3-4), 293–302. [https://doi.org/10.1016/0301-6226\(93\)90099-4](https://doi.org/10.1016/0301-6226(93)90099-4)
- Leinker, M. (2007). *Entwicklung einer Prinziplösung zur Senkung von Ammoniakemissionen aus Nutztierställen mit Hilfe von Ureaseinhibitoren*. Ph.D. Thesis. Germany: Martin-Luther-University. Forschungsbericht Agrartechnik VDI-MEG Nr. 462.
- Loftus, C., Yost, M., Sampson, P., Arias, G., Torres, E., Vasquez, V. B., . . . Karr, C. (2015). Regional PM2.5 and asthma morbidity in an agricultural community: A panel study. *Environmental Research*, 136, 505–512. <https://doi.org/10.1016/j.envres.2014.10.030>
- Ndegwa, P. M., Hristov, A. N., Arogo, J., & Sheffield, R. E. (2008). A review of ammonia emission mitigation techniques for concentrated animal feeding operations. *Biosystems Engineering*, 100(4), 453–469. <https://doi.org/10.1016/j.biosystemseng.2008.05.010>
- Ogink, N. W. M., & Kroodsma, W. (1995). Reduction of Ammonia Emission from a Cow Cubicle House by Flushing with Water or a Formalin Solution. *Journal of Agricultural Engineering Research*. (63), 197–204. <https://doi.org/10.1006/jaer.1996.0021>
- Parker, D. B., Pandrangi, S., Greene, L. W., Almas, L. K., Cole, N. A., Rhoades, M. B., & Koziel, J. A. (2005). Rate And Frequency Of Urease Inhibitor Application For Minimizing Ammonia Emissions From Beef Cattle Feedyards. *Transactions of the ASAE*, 48(2), 787–793. <https://doi.org/10.13031/2013.18321>

- Parker, D. B., Rhoades, M. B., Baek, B. H., Koziel, J. A., Waldrip H. M., & Todd, R. W. (2016). Urease Inhibitor for Reducing Ammonia Emissions from an Open-Lot Beef Cattle Feedyard in the Texas High Plains. *Applied Engineering in Agriculture*, 32(6), 823–832. <https://doi.org/10.13031/aea.32.11897>
- Reinhardt-Hanisch, A. (2008). *Grundlagenuntersuchungen zur Wirkung neuartiger Ureaseinhibitoren in der Nutztierhaltung*. Ph.D. Thesis. Germany: University of Hohenheim. Forschungsbericht Agrartechnik VDI-MEG Nr. 471.
- Shi, Y., Parker, D. B., Cole, N. A., Auvermann, B. W., & Mehlhorn, J. E. (2001). Surface amendments to minimize ammonia emissions from beef cattle feedlots. *American Society of Agricultural Engineers*. (Vol. 44 (3)), 677–682. <https://doi.org/10.13031/2013.6105>
- Snoek J. W., Haesen G. P. M. J., Groot Koerkamp P. W. G., & Monteny G. J. (2010). Effect of floor design in a dairy cow house on ammonia emission - Design, test and preliminary results with an experimental set-up for run off experiments. *International Conference on Agricultural Engineering. Cemagref, Clermont-Ferrand*. p. 1-10. <https://doi.org/10.13140/RG.2.1.1957.0168>
- Statistisches Bundesamt (2010). Wirtschaftsdünger, Stallhaltung, Weidehaltung - Landwirtschaftszählung/Agrarstrukturerhebung - 2010. Fachserie 3, Heft 6. Wiesbaden. Retrieved December 06, 2019, from [https://www.destatis.de/DE/Themen/Branchen-Unternehmen/Landwirtschaft-Forstwirtschaft-Fischerei/Produktionsmethoden/Publikationen/Downloads-Produktionsmethoden/stallhaltung-weidehaltung-2032806109004.pdf?\\_\\_blob=publicationFile](https://www.destatis.de/DE/Themen/Branchen-Unternehmen/Landwirtschaft-Forstwirtschaft-Fischerei/Produktionsmethoden/Publikationen/Downloads-Produktionsmethoden/stallhaltung-weidehaltung-2032806109004.pdf?__blob=publicationFile)
- Swierstra, D., Braam, C. R., & Smits, M. C. (2001). Grooved Floor System For Cattle Housing: Ammonia Emission Reduction And Good Slip Resistance. *Applied Engineering in Agriculture*. (17), 85–90. <https://doi.org/10.13031/2013.1929>
- Todd, R. W., Cole, N. A., & Clark, R. N. (2006). Reducing crude protein in beef cattle diet reduces ammonia emissions from artificial feedyard surfaces. *Journal of Environmental Quality*, 35(2), 404–411. <https://doi.org/10.2134/jeq2005.0045>
- UBA [Umweltbundesamt] (2018). Ammoniak-Emissionen. Umweltbundesamt. Berlin, Germany. Retrieved January 22, 2019, from <https://www.umweltbundesamt.de/daten/luft/luftschadstoff-emissionen-in-deutschland/ammoniak-emissionen#textpart-1>

- UNECE [United Nations Economic Commission for Europe] (2013). *Protocol to Abate Acidification, Eutrophication and Ground-Level Ozone to the Convention on Long-range Transboundary Air Pollution (1999), as amended on 4 May 2012* (No. ECE/EB.AIR/114). Retrieved August 28, 2018 from [https://www.unece.org/fileadmin/DAM/env/documents/2013/air/eb/ECE.EB.AIR.114\\_ENG.pdf](https://www.unece.org/fileadmin/DAM/env/documents/2013/air/eb/ECE.EB.AIR.114_ENG.pdf)
- Van Horn, H. H., Newton, G. L., & Kunkle, W. E. (1996). Ruminant nutrition from an environmental perspective: factors affecting whole-farm nutrient balance. *Journal of Animal Science*, 74(12), 3082. <https://doi.org/10.2527/1996.74123082x>
- Varel, V. H. (1997). Use of urease inhibitors to control nitrogen loss from livestock waste. *Bioresource Technology*, 62(1-2), 11–17. [https://doi.org/10.1016/S0960-8524\(97\)00130-2](https://doi.org/10.1016/S0960-8524(97)00130-2)
- Varel, V. H., Nienaber, J. A., & Freetly, H. C. (1999). Conservation of nitrogen in cattle feedlot waste with urease inhibitors. *Journal of Animal Science*, 77(5), 1162. <https://doi.org/10.2527/1999.7751162x>
- Zähner, M., & Schrade, S. (2020). Laufflächen mit 3 % Quergefälle und Harnsammelrinne in Laufställen für Milchkühe. *Agroscope Merkblatt*, Nr. 80/2020. Retrieved April 24, 2020, from <https://www.bauernzeitung.ch/media/113005382/lauffla-chen-ammoniak.pdf>

## Material & methods

### *Aim and content*

The aim of this thesis was to quantify the reduction potential and present information on the chronological response of the urease inhibitor type K. The research supports the intended commercial use of the inhibitor as a reliable mitigation method for reducing ammonia emissions and supporting the goals defined in the directive 2016/2284 (EC, 2016). Although the main Chapters 3, 4 and 5 (publications) also include a Material & methods section, more comprehensive information is provided in this chapter. A short summary of each paper is given in the following:

Chapter 3: Investigating the chronological reduction potential of a urease inhibitor in respiration chambers. The urease inhibitor was tested under laboratory conditions. The chronological reduction potential of the inhibitor was investigated in respiration chambers. In order to analyse and estimate the reduction of the ammonia emissions, the dataset was split into four-hour time slots.

Chapter 4: Reduction of ammonia emissions by using a urease inhibitor in a mechanically ventilated dairy housing system. The urease inhibitor was tested in a mechanically ventilated housing system. The small-scale setup in two identical housing units provided the option for conducting an “in time approach” and a “case-control approach”. The reduction potential could be calculated and the chronological effect could be estimated.

Chapter 5: Reduction of ammonia emissions by applying a urease inhibitor in naturally ventilated dairy barns. The urease inhibitor was investigated in two naturally ventilated dairy cow housings. The measurement was conducted over a period of one year in order to include seasonal effects. A seasonal and annual reduction potential could be calculated.

### **General material and methods**

The main objective of this thesis was to investigate the reduction potential of the inhibitor. Therefore, the inhibitor type K with a concentration of 2.5 mg m<sup>-2</sup> was used consistently in all experiments. The inhibitor was applied using a hand sprayer in the laboratory investigations (Chapter 3) and a modified backpack system under field conditions (Chapters 4 and 5). In order to avoid unnecessary repetitions, the material and methods which were similar in the three main chapters are presented below in five subsections.

1. Urease inhibitor type K
2. Application technique
3. Urease activity measurement
4. Verification of Environmental Technologies for Agricultural Production test protocol
5. Side parameters

### **1. Urease inhibitor type K**

The same inhibitor type K was used for all experiments (Chapters 3 to 5). The inhibitor, a phosphorodiamidate ready-made by a liquid chemical formulation based on pyrrolidone was supplied by the company Stickstoffwerke Piesteritz GmbH (2020) (SKWP). In this form, the inhibitor K was easily dispensable and mixable with water. The optimized synthesis process and urease inhibitor formulations with the necessary application and stability properties were developed by the SKWP and are protected via patents (two registrations). The selected concentration of 2.5 mg m<sup>-2</sup> was based on a previously conducted study by Hagenkamp-Korth, Haeussermann, & Hartung, (2015) and Leinker (2007). In order to achieve an even distribution on the floor surface, the liquid formulated inhibitor K was mixed with 100 ml m<sup>-2</sup> water for the laboratory experiments (Chapter 3) and with 50 ml m<sup>-2</sup> water for the applications under practical conditions (Chapters 4 and 5). The inhibitor, was added to the required volume of water and homogenized.

### **2. Application technique**

#### *Laboratory investigations*

The aim of the application technique in Chapter 3 was to apply the urease inhibitor to the floor surface areas with the highest emissions. However, contact with the feed, water and the sensitive parts of the animal (eyes, mouth, etc.) in the front of the chamber was to be avoided. The cow was unable to turn around inside the chamber and the urine and faeces could only be secreted at the rear of the chamber. Therefore, the end of the chamber was regarded as the area with the highest emission potential. The space for the staff working inside the chamber was limited. Only a small path at the end of the stanchion could be used to apply the inhibitor. Due to these boundary conditions and the defined emissions area, the application technique had to meet certain requirements in order to be useful inside the chambers. Therefore, a commercial hand sprayer (GLORIA Haus- und Gartengeräte GmbH) was used with a maximum pressure of three bar. The hand sprayer was filled with the inhibitor-water solution outside the chambers prior to the application process. To prevent any air leakages, the application took place with all

chamber doors closed. The inhibitor was applied to half of the lower end of the rubber mattress surface and to the three foils attached to the stanchion (see Figure 7, red line). A measurement protocol was created to document the process, including cow number, the beginning and end of the application process and any comments relating to exceptional events (*Appendix*; Table A1).

### *Field investigations*

The studies in Chapters 4 and 5 were carried out under practical conditions in different dairy cow housings. In order to avoid separate application techniques for each housing, the same general application system was used for all field tests. The general aim was to retrofit the application technique to the newer and older buildings. Only minor changes were implemented (nozzle sizes, adjustment of extra tank) between the different barns. A previous inhibitor investigation used a manual backpack sprayer in a cubicle cow housing system (Hagenkamp-Korth et al., 2015). The current study extended this approach by using a modified automatic backpack sprayer (SOLO® Kleinmotoren GmbH) and an extra 20 L tank. However, the extra tank was used only in the larger barn (Chapter 5).



**Figure 1** Application system with the automatic backpack sprayer in a newly built metal cage and a front extension for the spray bar and the two nozzles.

In order to provide more mobility, the backpack sprayer was placed inside a metal cage on a commercial rollator (Figure 1). Two anti-drift fan nozzles were mounted to a spray bar in front

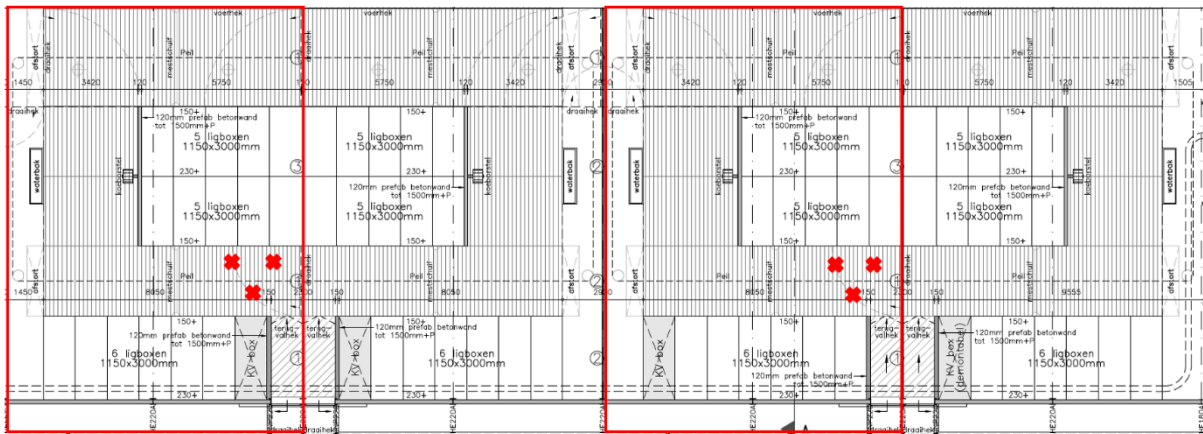
of the rollator. A ball-shaved valve (mesh size 0.35 mm) was used in front of each nozzle. The applications in Chapter 4 were carried out with 110° Hypro F04 nozzles and in Chapter 5 with 120° Lechler AD04 nozzles. The manual speed of the rollator was based on the pressure of the system and size of the barn. The whole system was previously tested in detail at the Institute of Agricultural Engineering (University of Kiel) and during pretests at Farm A in Chapter 4 and at both units in Chapter 5. In the pretests, different spray bars and pressures were tested and the speed of the application was adapted. The application times were adjusted to the farm schedules and the system was tested on the different floor surface (slatted/solid) conditions. In order to provide an even and full spraying pattern from the beginning, the pressure of the system was increased and the first inhibitor-water solution spray amount was caught in a measuring cup and returned to the tank. The pressure of the system was then sufficient to ensure an even and consistent spraying pattern would be supplied from the beginning. During the field experiments, the application of the inhibitor took place, in general, at the morning milking between 5 a.m. and 7 a.m. One exception was made during the application process at Farm B in Chapter 5. The help of an additional co-worker was needed since the cows never left the barn due to an integrated automatic milking system. The start and end times of the whole application processes were recorded in a protocol (*Appendix*, Table A2). After the completion of each application, the tanks were controlled for remaining liquids. Due to technical reasons, the system was not able to fully empty the tanks. However, the unused liquid was included in the calculation of the necessary amount before proceeding. The working stability of the whole system was checked multiple times during the pretests and both before and after the field tests.

### **3. Urease activity measurement**

Measurement of urease activity should confirm the reduction effect of the urease inhibitor. The urease activity measurement results were also used as an accompanying factor. The experimental setup was carried out according to a previous study and can be found in Hagenkamp-Korth et al. (2015). However, due to the laboratory circumstances, the urease activity measurements inside the respiration chambers (Chapter 3) were conducted as unpublished pretests. Before a new experiment began, the chambers were cleaned under high pressure, eliminating most of the urease enzyme. Due to safety reasons, the pretests could only be conducted inside empty chambers. The existence of an environment almost free of urease activity could be confirmed. However, confirmation of the urease inhibitor effect after the application with the animal inside the chamber was not possible due to the limited space and the inherent safety aspects. Therefore, urease activity measurements were conducted and used only under practical conditions in the

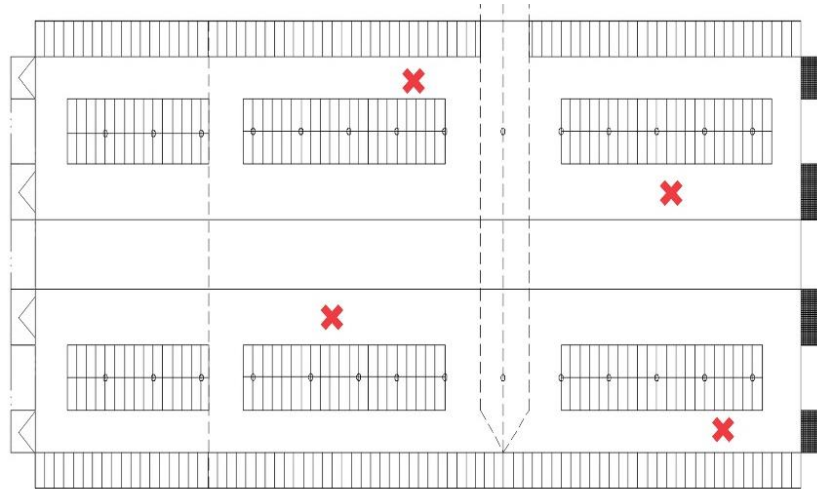


Chapters 4 and 5. The sample point locations were marked on a measurement scheme beforehand. The selection criterion for the sample locations in Chapter 4 were based on the unit's structure and equipment. The urease activity was measured when all cows were present. The location of the sample points was based on several assumptions. The front of the unit was located parallel to the feeding alley, where a sample point mixing with feed would impact the results. The water trough was placed on the side of a small corridor inside the unit. Due to the high frequency of water trough use and no additional co-worker to help shield the samples, this area was deemed to be inappropriate. Therefore, the end of the unit was selected as a suitable location for the three measurements and the three sample points were located at the entrance/exit of the units (Figure 2).



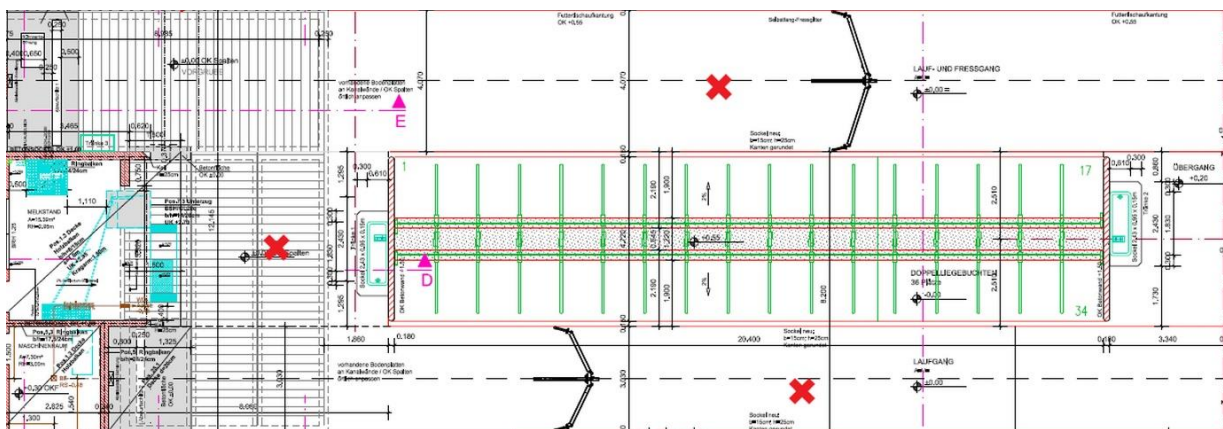
**Figure 2** Sketch of Unit A and Unit B (Chapter 4). The urease activity sample points are marked by the red crosses.

In Chapter 5, the urease activity measurement was conducted on two different farms. Farm A consisted of four different groups and approximately 374 cows. In order to cover every group and every walking area, the four sample points were arranged in a “Z” pattern (Figure 3). A second co-worker assisted in guarding the experimental setup inside the large barn. Hagenkamp-Korth et al. (2015) investigated the inhibitor type K under field conditions inside cubicle housing systems. The general working effect had therefore been previously confirmed. For this reason, a small sample size could be used for larger dairy cow housings.



**Figure 3** Sketch of Farm A (Chapter 5) and the urease activity sample points (red crosses).

The three measurement points on Farm B in chapter five were evenly distributed in the housing (Figure 4). The two solid surface walkways and the slatted area in front of the automatic milking system were covered. An accumulation of cows in front of the automatic milking system could result in critical conditions, however, the urease activity measurement could be successfully undertaken.



**Figure 4** Sketch of Farm B (Chapter 4) and the urease activity measurement points (red crosses).

In the experiments, it was observed that the cows walked through the milking system before entering the next walkway, rather than turning around towards the measurement points. In order to confirm the inhibitor effect, urease activity was measured one day before the inhibitor was applied and on the last (third) application day of the inhibitor. The measurements were carried out over all seasons. A measurement protocol (*Appendix*, Table A2) was created in order to record additional parameters such as date, time, pH value, surface temperature and the height and distribution of the faeces, following the example of Hagenkamp-Korth et al. (2015). The

samples were analysed at a laboratory according to Leinker (2007) and Hagenkamp-Korth et al. (2015).

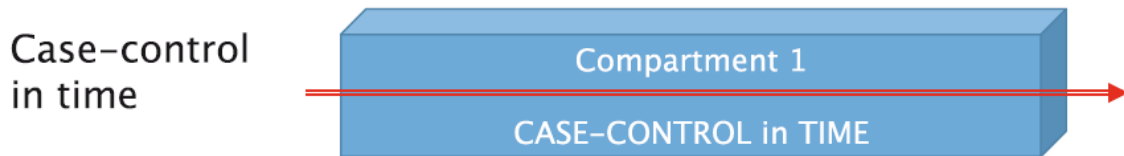
#### 4. Verification of Environmental Technologies for Agricultural Production test protocol

The experiments under practical conditions (Chapters 4 and 5) were based on the Verification of Environmental Technologies for Agricultural Production (VERA) test protocol “for Livestock Housing and Management Systems Version 3:2018-09” (International VERA Secretariat, 2018). The requirements of the protocol include test design, housing, number of animals, sampling strategy and side parameters. The advantage of the international VERA protocol is the opportunity to standardise emission measurements, since there is still no standard emission measurement setup (as reported by Hristov et al., 2011). The test design used was based on two case-control approaches in order to investigate the potential of the inhibitor (Figure 5 and Figure 6). The experimental setup in Chapter 4 was based on both case-control test designs. (1) Two similarly-equipped units were used and the control unit (without inhibitor) and the case unit (with urease inhibitor) were switched (Figure 5) during the experimental course. (2) Each unit could be also tested separately by using the case-control in time approach (also referred to as in time approach). The days before the application were used as the control and the days of the inhibitor application were used as the case (Figure 6).



**Figure 5** General case-control approach (International VERA Secretariat, 2018).

For the experiments in Chapter 5, two commercial dairy farms were used with no option for providing two similar units inside. Hence, the case-control in time was used. First, the ammonia emissions of the selected farm were measured without the inhibitor (control) and the inhibitor (case in time) was subsequently applied (Figure 6).



**Figure 6** General case-control in time approach (International VERA Secretariat, 2018).

## 5. Side parameters

The experimental setups of the field investigations (Chapters 4 and 5) were based on two measurement periods per season. Ammonia emissions can be influenced by several parameters, including inside/outside air temperature, inside/outside humidity, feed intake, liquid manure composition, etc (e.g. Hristov et al., 2011). However, only the feed and liquid manure samples were referred to in this subsection. The measurement setup of the other previously listed parameters is presented in the individual following chapters. The feed samples were taken once each season (except for Chapter 4) and liquid manure samples were collected at the beginning and end of each season (Table 1).

**Table 1** Number of feed and liquid manure samples collected during the practical investigations.

Chapter	Sample	Farm	Season	Measurement period (n)	Number of samples for both measurement periods
4	Liquid manure	Unit A	Winter	2	2
			Spring	2	2
			Summer	2	2
	Feed	Unit A	Winter	2	2
			Spring	2	2
			Summer	2	2
4	Liquid Manure	Unit B	Winter	2	2
			Spring	2	2
			Summer	2	2
	Feed	Unit B	Winter	2	2
			Spring	2	2
			Summer	2	2
5	Liquid manure	Farm A	Winter	2	2
			Spring	2	2
			Summer	2	2
	Feed	Farm A	Winter	2	1
			Spring	2	1
			Summer	2	1
5	Liquid manure	Farm B	Winter	2	2
			Spring	2	2
			Summer	2	2
	Feed	Farm B	Winter	2	1
			Spring	2	1
			Summer	2	1

The samples were used in order to confirm feed supply according to good practices, and the liquid manure samples were used in order to link the amount of measured ammonia emissions to the liquid manure parameters. All samples were immediately frozen and shipped to laboratories for standard analysis. The analysis of the liquid manure samples was done by the AGRO-LAB Agrar und Umwelt GmbH, Kiel. The methods DIN EN 12880 (S 2a), (2001); DIN EN 13342, (2001) and DIN EN 38406-5-2 (E-2), (1983) were used. The analysis of the feed samples was carried out by the Institute of Animal Nutrition and Physiology, Kiel. The methods used can be found in VDLUFA (2007). Dry matter ( $\text{g kg}^{-1}$ ) was calculated using Method No. 3.1, crude protein ( $\text{g kg}^{-1}$ ), or Kjeldahl, was analysed using Method No. 4.1.1. For net energy ( $\text{MJ kg}^{-1}$  DM), was estimated according to Menke and Steingass (1987). The used formula is provided in the *Appendix* (Eq 1).

**Table 2** Analysed side parameters based on the VERA test protocol (International VERA Secretariat, 2018).

Side parameters	Parameter
Feed	Dry matter ( $\text{g kg}^{-1}$ )
	Crude protein ( $\text{g kg}^{-1}$ )
	Net energy ( $\text{MJ kg}^{-1} \text{DM}^{-1}$ )
Manure	Dry matter (%)
	Nitrogen ( $\text{kg m}^{-3}$ )
	$\text{NH}_4\text{-N}$ ( $\text{kg m}^{-3}$ )

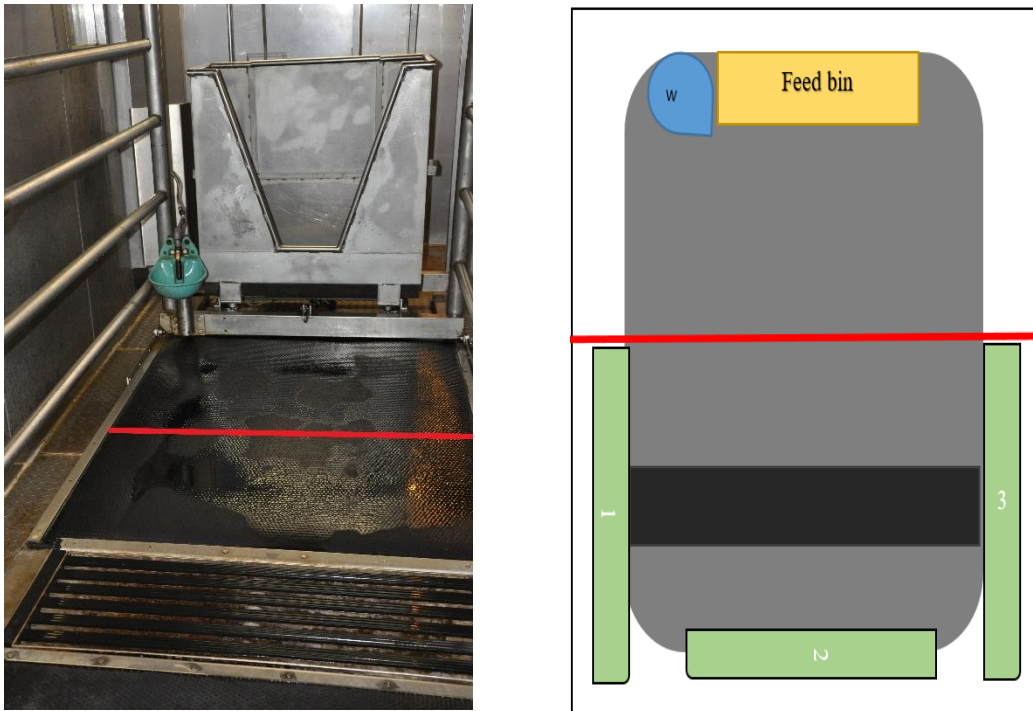
### Chapter 3: Investigating the chronological reduction potential of a urease inhibitor in respiration chambers

- Test facility
- Experimental design
- Urease inhibitor (see General material and methods)
- Application technique (see General material and methods, *Laboratory investigations*)
- Data processing and statistical analysis

#### *Test facility*

The current scientific study was a follow-up investigation based on previous research by the Institute of Agricultural Engineering (University of Kiel) using the inhibitor in respiration chambers. The investigations were carried out in the chambers of the Leibniz Institute for Farm Animal Biology (FBN), Institute of Nutritional Physiology “Oskar Kellner”. Respiration chambers offer continuous monitoring of the single animal and its behaviour. Up to four respiration chambers could be used simultaneously, each containing one animal at a time. An acrylic glass provided visual contact between two animals (Derno, Elsner, Paetow, Scholze, & Schweigel, 2009). The chambers had a total size of 4 m x 2 m x 2 m and the cow had a space of 2.5 m x 1.5 m inside an implemented stanchion (Derno et al., 2009) (Figure 7). In order to observe the animals from every angle, the workers could walk around the stanchion. The chamber construction can be related to tie stall housings (Derno et al., 2009). The chamber’s surface was covered with a rubber mattress and three foils were attached to the sides of the stanchion (Figure 7 and Figure 8). The size and length of the foils were measured prior to the start of the experiment. The foils were required to prevent the distribution of faeces and urine inside the chamber and on the small path around the stanchion. The faeces and urine of the cows stained the foils and were seen as a potential source of emissions (Figure 9 and Figure 10). The rubber mattress inside the chambers was cleaned twice a day by removing the urine and the faeces into tanks in

a cellar underneath the chamber. The airtight tanks were emptied every morning (Metges, Kuhla, & Derno, 2014). The milking of the cows was carried out at 7 a.m. and between 5 p.m. and 6 p.m. Milking was performed normally with all doors closed to prevent air leakage. Mobile milking devices were located in front of the chambers and connected to two vacuum and one milk tube from inside the chamber (Metges, Kuhla, & Derno, 2014). Further details of the costs, calorimetric system and equipment of the respiration chambers can be found in Derno et al. (2009) and Metges, Kuhla, and Derno (2014).



**Figure 7 (left)** View inside a cleaned respiration chamber. Front: water and feed bin. Metal frame: stanchion inside the chamber. End: cellar with funnel for the urine and faeces. Surface: rubber mattress (not pictured: three plastic foils attached to the metal frame). Red line: the inhibitor was only applied to the end of the rubber mattress. **Figure 8 (right)** Sketch of the respiration chamber. Numbers 1-3: plastic foils attached to the metal frame

A consistent airflow of  $30 \text{ m}^3 \text{ h}^{-1}$  through the chamber was provided by rotary vane vacuum pumps (VT 4.40, Fuergut, Aichstetten, Germany). A flowmeter (McCrometer, Hemet, CA, accuracy 0.5 %) was used to measure the airflow of the exhaust air. The air temperature inside the chambers was set to a constant  $15 \text{ }^\circ\text{C}$ . The relative humidity inside the chamber was at average 77 % and was measured in the exhaust air. Air samples for the analysis were collected by membrane pumps (KNF Neuburger Laboport, Freiburg, Germany) and an infrared absorption-based analyser (GMS800, SICK, Reute, Germany, accuracy 1 %) was used to analyse the ammonia content of the exhaust air. Gas concentration and airflow were measured every six minutes. The following additional side parameters were also measured on each test day: feed intake, water consumption, milk yield and animal weight (Derno et al., 2009; Metges, Kuhla, & Derno, 2014). Data logging began as soon as the cow entered the chamber and the length of stay inside the respiration chamber was a maximum of three days.





**Figure 9 (left) and Figure 10 (right)** Faeces distributed at the end of the rubber mattress and on the inside of the foils.

The test animals were Holstein Friesian cows and were adapted to the experimental circumstances before the investigations began. In order to prevent abnormal behaviour and possible data outliers, the cows were adapted to the chambers at least three times for four hours each. Successful adaptation was expressed by regular behaviour patterns, such as eating and lying down (Metges, Kuhla, & Derno, 2014). The inhibitor experiments were part of ongoing investigations carried out by the FBN. Therefore, the animals were already divided into different groups (A–C). The groups were based on different feed compositions and the addition of supplements. However, only group A received supplements based on different fatty acid compositions. Based on a random selection, cows received either coconut oil (1), linseed and safflower oil (2), Lutalin (3) and linseed, safflower oil and Lutalin (4). A detailed description of the supplements can be found in the *Appendix* (Text A1). Lactating cows, dry cows and cows with inserted fistulas were used in the experiments (Table 3). Because the previously explained adaptation process is very time consuming, a number of cows were tested twice (one thrice) over the duration of the experiment (Table 4).

**Table 3** Overview of cow status according to group.

Group	Status	Animal (n)
A (all cows had fistulas)	Lactating	12
	Dry cow	9
B	Lactating	11
	Dry cow	1
C	Lactating	3
	Dry cow	2

*Experimental design*

In order to examine the reduction potential of the inhibitor, the maximum experiment duration of three days was divided into two phases. The reference phase had a duration of at least 24 hours. The values included the ammonia emissions measured before the inhibitor was applied. The application phase began after the inhibitor was applied and ended after 24 hours.

*Data processing and statistical analysis*

The process of adapting each cow to the chamber was time-consuming and required effort, therefore, cows were used more than once (Table 4).

**Table 4** Overview of the number of animals per group and repetitions.

Group	Animal (n)	Animals tested twice
A	21	8
B	12	5 (1 thrice)
C	5	-

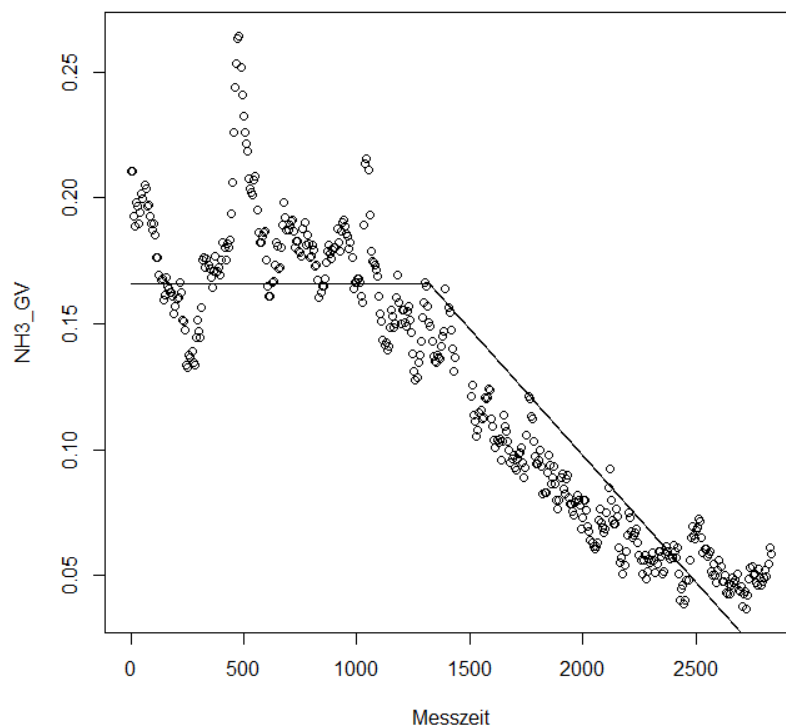
However, the cows that were used more than once were treated as different individuals for data processing purposes. The most challenging part of the data processing was the different inhibitor application times between the cows. The applications took place between 7 a.m. and 4 p.m., hence the applications took place at different times of the day. However, in order to estimate reduction potential, all cows were pooled to have the application at time “0”, disregarding the “real time” (Table 5).

**Table 5** Overview of the different application times.

Time slot (hour)	Applications during the time slot (n)	Animals (n)
07:00 – 07:30	2	
07:30 – 08:00	7	
08:00 – 08:30	10	
08:30 – 09:00	1	
13:30 – 14:00	1	38
14:00 – 14:30	2	
14:30 – 15:00	3	
15:00 – 15:30	8	
15:30 – 16:00	4	

The purpose of the statistical analysis was to investigate the chronological response of the urease inhibitor. Therefore, the reference phase and the application phase were divided into time slots. In order to obtain feasible ammonia emissions, only the four hours (-4-hour time slot)

before the application point were used for the reference phase. The 24 hours of the application phase were apportioned into 4-hour time slots (4 hours, 8 hours, 12 hours, 16 hours, 20 hours and 24 hours). Ammonia concentration was converted into  $\text{g h}^{-1}$  per 500 kg livestock unit (LU). The newly built data set included animal number, group (A–C), time slots (-4 hours to 24 hours) and the mean values of the ammonia emissions ( $\text{g h}^{-1} \text{LU}^{-1}$ ) for the scaled time slots. A prior statistical analysis (broken line regression, Figure 11) with the software R (R Core Team, 2020) (not published) was used in an attempt to estimate a breaking point. The breaking point is the time point at which the inhibitor significantly reduces the ammonia emissions and an increase in reductions is visible.



**Figure 11** Pretest example of a breaking point graph for one dairy cow inside the chamber.

The dataset was based on the 4-hour values of the reference phase and on the 24 hours of the application phase. However, no time slot division took place. Only a manually selected time frame of two hours after the urease inhibitor application point was fixed. It was assumed that the breaking point would occur within two hours after application. Therefore, a range of between zero minutes and 120 minutes was defined. However, no reliable results were detected. Most of the breaking points occurred at time zero minutes or at 120 minutes. Only very few feasible time points were found, but these few results might also be coincidental. No fixed time frame resulted in a breaking point before the application occurred. The huge variations within the data might be a possible reason why no clear evidence was found, even though the data was

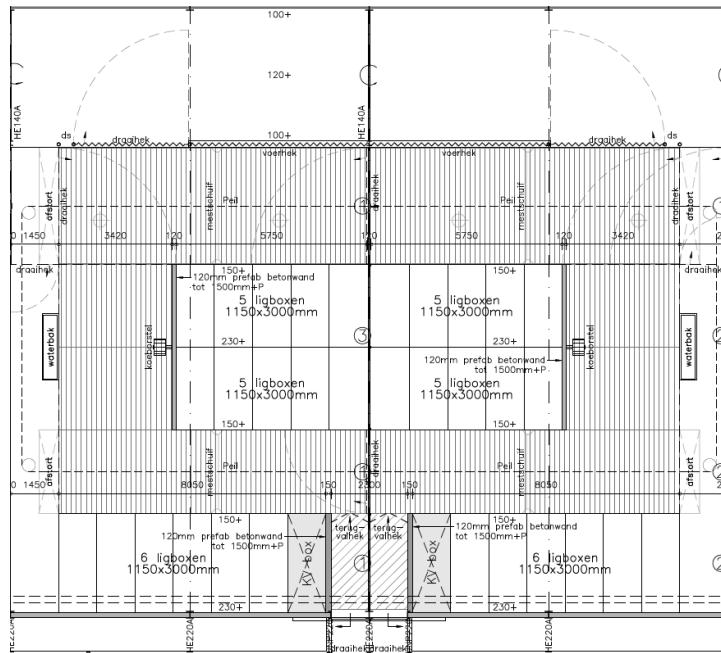
previously smoothed to prevent this. Upcoming investigations concerning the breaking point should therefore extend the manually selected time frame for a greater chance to find a resilient result. In order to continue investigating the chronological development of the urease inhibitor, a time slot analysis was used based on the separate time slot data. Data evaluation using the software R (R Core Team, 2020) first defined an appropriate statistical model based on generalized least squares (Box, Jenkins, & Reinsel, 1994; Carroll & Ruppert, 1988). The model included the factors group (A–C) and the different time slots (-4, 4, 8, 12, 16, 20, 24 hours), as well as their interactions. Correlation of the measurement values due to the several time slot levels were taken into account. The residuals were assumed to be normally distributed and to be heteroscedastic with respect to the different levels of the fixed factors. These assumptions are based on a graphical residual analysis. Based on this model, a Pseudo  $R^2$  was calculated (Nakagawa & Schielzeth, 2013) and an analysis of variances (ANOVA) was conducted, followed by multiple contrast tests (Bretz, Hothorn, & Westfall, 2011; Schaarschmidt & Vaas, 2009) in order to compare the different levels of time, respectively. After this, the percentage reduction per group and time slots were calculated based on the mean differences and model estimates.

#### Chapter 4: Reduction of ammonia emissions by using a urease inhibitor in a mechanically ventilated dairy housing system

- Test facility
- Experimental design
- Urease inhibitor (see General material and methods)
- Application technique (see General material and methods, *Field investigations*)
- Measurement setup
- Data processing and statistical analysis

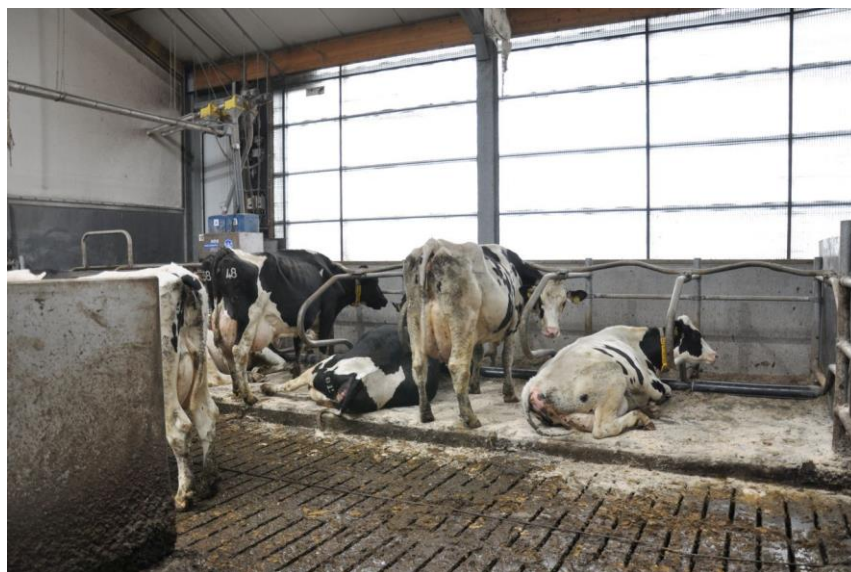
##### *Test facility*

The emission measurements were carried out at the Dairy Campus, a dairy research farm located near Leeuwarden in the province of Friesland in the Netherlands. The farm provided a barn that was established to focus on emissions investigations. The emissions barn consisted of six separate units, four of which were equipped with cubicles, all of them mechanically ventilated. For this study, two identical cubicle units (referred to as Unit A and Unit B) were used (Figure 12). A detailed description of the facility can be found in Dairy Campus (2019).



**Figure 12** Sketch of two (of the four) identical units of the Dairy Campus.

Inside the unit, each cow was provided with 7.6 m<sup>2</sup> space including 4.5 m<sup>2</sup> of concrete slatted floor (Figure 13). The bedding material in the cubicles consisted of mattresses covered with a thin layer of sawdust that was renewed manually twice a day. The liquid manure was stored in a pit underneath the slatted floor and cubicles. A capacity of 185 m<sup>3</sup> was available. The cows were milked twice a day at around 5 a.m. and 4 p.m. During milking, the doors between the units were opened (in general for two hours), the cubicles were cleaned, the feed was pushed up or a fresh TMR ration (consisting mainly of grass and maize silage) was provided. The total ration mass was 50 kg per cow per day and consisted of 32 kg silage, ± 13 kg corn and minerals, 6 kg TGC (wheat yeast concentration) and 1.5 kg soya. An automatic feeding station in each unit provided concentrates based on individual cow needs and a drinking trough provided unlimited fresh water. The indoor climate was regulated by two mechanical ventilators (Fancom) mounted on a shaft (Ø800) and combined with a measuring and control unit (Fancom ATM80). The ventilation rate inside Unit A and B was set at a fixed capacity of around 11,000 m<sup>3</sup> h<sup>-1</sup> (40 % of maximum capacity) during spring and around 17,000 m<sup>3</sup> h<sup>-1</sup> (50 % of maximum capacity) during summer and winter. The indoor temperatures in both units were measured using two temperature and relative humidity sensors per unit (Rotronic Instrument Corp., Huntington, USA) with a precision of ± 1.0 °C and ± 2 % relative humidity. For the outside temperature and humidity, the weather station number 270 of the Royal Netherlands Meteorological Institute (KNMI) at the air force base in Leeuwarden was used.



**Figure 13** Inside view of one of the units at the Dairy Campus.

Each unit housed 16 lactating Holstein Friesian cows with comparable daily milk yields, average milk urea concentration, age and lactation number (Table 6). The cows had no access to grazing or to an exercise yard.

**Table 6** Similar side parameters of the cows inside both units

Season	Unit	Average urea concentration (mg L <sup>-1</sup> )	Average milk yield (kg d <sup>-1</sup> )	Average lactation number (n)	Average age (a)
Spring	Unit A	260	28	2	3.2
	Unit B	280	29	2	3.7
Summer	Unit A	240	29	2	3.2
	Unit B	250	29	2	3.9
Winter	Unit A	180	33	2	3.9
	Unit B	180	31	3	4.0

### *Experimental design*

The emission measurements were carried out in two identical units of a dairy research barn using the two different experimental setups (in time and case-control approach) of the VERA test protocol (International VERA Secretariat, 2018) (see Figure 5 and Figure 6). The basic idea of both approaches was to test the inhibitor by comparing the ammonia emissions measured before the application with the emissions after the inhibitor application. The in time approach involved the application of inhibitor K (application phase) and no application (reference phase) in the same unit (Table 7), while a direct case-control approach (reference phase-application

phase) measured emissions between the units (Table 8). The measurements were conducted over the course of one year (winter, summer and spring) to include the potential effects of seasonal interaction. The measurement of each unit was repeated twice for each season. A break of nine days was implemented between the first and the second measurement periods in order to begin the second measurement without any remaining inhibitor effects. After the first measurement period, case and control treatments were switched between the units.

**Table 7** Measurement schedule for the case-control in time approach. TP = transition period (spring for both Unit A and B).

Unit	Date	Season	Measurement period	Days per phase (n)	Phase
A	17–18.11.2017	Winter	1	2	Reference
A	19–21.11.2017	Winter	1	3	Application
A	22–30.11.2017	Winter	-	9	Decay
A	01–02.12.2017	Winter	2	2	Reference
A	03–05.12.2017	Winter	2	3	Application
A	10–11.04.2017	TP	1	2	Reference
A	12–14.04.2017	TP	1	3	Application
A	15–23.04.2017	TP	-	9	Decay
A	24–25.04.2017	TP	2	2	Reference
A	26–28.04.2017	TP	2	3	Application
A	30.06.–01.07.2017	Summer	1	2	Reference
A	02–04.07.2017	Summer	1	3	Application
A	05–13.07.2017	Summer	-	9	Decay
A	14–15.07.2017	Summer	2	2	Reference
A	16–18.07.2017	Summer	2	3	Application
B	28–29.11.2017	Winter	1	2	Reference
B	30–02.12.2017	Winter	1	3	Application
B	03–11.12.2017	Winter	-	9	Decay
B	12–13.12.2017	Winter	2	2	Reference
B	14–16.12.2017	Winter	2	3	Application
B	21–22.04.2017	TP	1	2	Reference
B	23–25.04.2017	TP	1	3	Application
B	26.04.–04.05.2017	TP	-	9	Decay
B	05–06.05.2017	TP	2	2	Reference
B	07–09.05.2017	TP	2	3	Application
B	11–12.07.2017	Summer	1	2	Reference
B	13–15.07.2017	Summer	1	3	Application
B	16–24.07.2017	Summer	-	9	Decay
B	25–26.07.2017	Summer	2	2	Reference
B	27–29.07.2017	Summer	2	3	Application

**Table 8** Measurement schedule for the case-control approach. TP = transition period (spring for both Unit A and B).

Date	Season	Block	Days per phase (n)	Reference	Application
19–21.11.2017	Winter	1	3	Unit B	Unit A
30.11.– 02.12.2017	Winter	2	3	Unit A	Unit B
14–16.12.2017	Winter	3	3	Unit A	Unit B
12–14.04.2017	TP	1	3	Unit B	Unit A
23–25.04.2017	TP	2	3	Unit A	Unit B
07–09.05.2017	TP	3	3	Unit A	Unit B
02–04.07.2017	Summer	1	3	Unit B	Unit A
13–15.07.2017	Summer	2	3	Unit A	Unit B
27–29.07.2017	Summer	3	3	Unit A	Unit B

*Measurement setup*

Two ventilators were mounted to the ceiling of each unit (Figure 14). Exhaust air from each ventilator (two sampling lines each) and background air from two sampling points outside the units was constantly sampled through polyethylene sampling lines (0.6 cm inner diameter). The sampling lines were connected to a multiplexer together with those from the other units. The multiplexer switched every 10 minutes to a different sampling point. The sampled air passed through a thermal ammonia converter with known efficiency before being analysed in a chemiluminescence NO<sub>x</sub> analyser (Teledyne API, T200, precision: 0.5 % of reading). Due to the retarded response of the thermal ammonia converters to concentration differences, only the last minute of the 10-minute measurement period was used in further calculations. The calibration was made using a bottle of certified gas of around 40 ppm NO and a maximum interval of one month. Further details of this setup are described in Phillips et al. (1998) and Phillips, Lee, Scholtens, Garland, and Sneath (2001). The pulse signal of each Fancor ATM 80 ventilator was logged at an interval of one minute and stored in a data logger (Campbell Scientific, C1000X) together with the gas concentrations.





**Figure 14** View of the two ventilators inside the units.

Ventilation rates were calculated using a calibration line separately determined in a wind tunnel. The calibration was carried out according to the Deutsche Landwirtschafts Gesellschaft (Prüfungs-Nr.: 12-00892):

$$Q \text{ (m}^3 \text{ h}^{-1}\text{)} = 141.23 \text{ (intercept)} + 218.33 * f \text{ (Hz)}, \quad (1)$$

where  $Q$  ( $\text{m}^3 \text{ h}^{-1}$ ) is the ventilation rate and  $f$  is the recorded pulse signal of the free running propeller in Hz. Average concentrations and ventilation rates per hour were used to calculate the ammonia emissions for each unit.

#### *Data processing and statistical analysis*

All statistical tests and graphs were performed using the open access software R (R Core Team, 2020). In order to estimate the reduction potential of the in time approach, the data used was based on the hourly data of the two reference days before inhibitor application and the hourly data of the two last application days of the original three application days. The first application day had to be excluded since the night-time hours between midnight and before the application would cause an underestimation of the reduction effect. For the analysis of the case-control approach, the hourly values of the three reference days and all three application days were taken into account. However, due to an overlapping and remainder effect, only three of the four measurement periods per season could be used. The aim of the case-control analysis was to investigate the chronological reduction response. Therefore, the selected days of the reference and application phase were divided into four-hour time slots (similar to the analysis in Chapter 3).

The final dataset contained the reference and application days divided into six time slots (4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 24 hours). The dataset was based on hourly ammonia emission values of the three reference days of one unit and on the hourly values of the three application days of the other unit, since the emissions were measured in parallel for the case-control approach.

### *In time approach*

The basic approach was to estimate the reduction potential by using the in time approach to compare ammonia emissions before and after the application in both units and over all seasons. Data evaluation began by defining an appropriate statistical mixed model (Laird & Ware, 1982; Verbeke & Molenberghs, 2000) as follows:

$$Y_{ijklmo} \sim a_i * b_j * c_k * d_l + e_m + (ef)_{mn} + r_{ijklmno}, \quad (2)$$

where

$Y$ : ammonia emissions ( $\text{g h}^{-1} \text{LU}^{-1}$ ),

and the fixed factors

$a_i$ : season (spring, summer, winter),

$b_j$ : measurement period (one, two),

$c_k$ : phase (reference phase, application phase),

$d_l$ : time (0, 1 ... 23 hour).

The “\*” means that the main effects, including all corresponding interaction terms, are covered.

The random factors were:

$e_m$ : barn (Unit A, Unit B),

$(ef)_{mn}$ : measurement unit (combination of phase, measurement period and season),

$r_{ijklmno}$ : residual error, correlated due to the time.

In addition, the index  $o$  represents the application day (two days: day 1, day 2). Based on a graphical residual analysis, the residuals  $r_{ijklmno}$  were assumed to be normally distributed and to be heteroscedastic due to the different levels of season, measurement period and phase. Based on this model, a pseudo  $R^2$  was calculated (Nakagawa & Schielzeth, 2013) and an analysis of

variances (ANOVA) was conducted, followed by a multiple contrast test (Bretz et al., 2011; Schaarschmidt & Vaas, 2009) in order to compare the levels of phase. The subsequent reduction estimation percentage was based on the mean values of both the reference and the application phases.

### *Case-control approach*

In order to investigate the chronological reduction response, the days of the reference and application phase were divided into four-hour time slots. The hours were not accumulated and each time slot always consisted of four values, based on the four hours. A mean value was calculated for each time slot. The evaluation of the time slot dataset began by defining an appropriate statistical mixed model (Laird & Ware, 1982; Verbeke & Molenberghs, 2000) based on the following equation:

$$Y \sim a_i * b_j * c_k * d_l + e_m + (ef)_{mn} + (efa)_{mni} + r_{ijklmn}, \quad (3)$$

where

$Y$ : ammonia emissions ( $\text{g h}^{-1} \text{LU}^{-1}$ ),

and the fixed factors

$a_i$ : season (spring, summer, winter),

$b_j$ : phase (reference, application),

$c_k$ : day (day 1, day 2, day 3),

$d_l$ : time slot (4, 8, 12, 16, 20, 24 hours).

The “\*” means that main effects, including all corresponding interaction terms, are covered.

The random factors were:

$e_m$ : unit (Unit A, Unit B),

$f_n$ : measurement period per season (period 1, period 2, period 3),

$r_{ijklmn}$ : residual error, correlated due to time slot.

The residuals were assumed to be normally distributed and to be heteroscedastic with respect to the different levels of season. These assumptions were based on a graphical residual analysis. A pseudo  $R^2$  was calculated from this model (Nakagawa & Schielzeth, 2013) and an analysis of variances (ANOVA) was conducted, followed by multiple contrast tests (e.g., see Bretz et

al., 2011, Schaarschmidt & Vaas, 2009) in order to compare the several levels of phase. Subsequently, percentage reductions per day and time slot were calculated based on the mean differences of the application and reference phases and the model estimates.

#### Chapter 5: Reduction of ammonia emissions by applying a urease inhibitor in naturally ventilated dairy barns

- Test facilities
- Experimental design
- Urease inhibitor (see General material and methods)
- Application technique (see General material and methods, *Field investigations*)
- Measurement setup
- Data processing and statistical analysis

##### *Test facilities*

The measurements were carried out on two dairy farms in Germany. Farm A was located in the federal state of Mecklenburg-Vorpommern and Farm B in the federal state of Brandenburg. The herd size of Farm A was divided into four cow groups based on their lactating status and their feeding regime. Each group went to the milking parlour separately. The cows were milked three times a day (6 a.m.–12 p.m., 2 p.m.–8 p.m. and 10 a.m.–4 p.m.). Feeding took place in the morning (twice) and the push-up of feed was carried out 6–8 times a day. The cubicles were provided with straw and chalk twice a week. The barn was equipped with four ventilators (three Big Ass Fans and one ARNTJEN® Fresh Air Fan) to supply fresh air by allowing it to circulate down onto the cows. Air exits the building through the opened side walls. The ventilators were turned on at a threshold of 13 °C. During winter, the curtains were half closed in order to protect the cows from cold temperatures and wind. The barn has four outside liquid manure channels from where the liquid manure was mixed and pumped daily to a nearby biogas system.

On Farm B, the feed was provided at 6 a.m. and 10 a.m. Three push-up times were distributed over the day (12 a.m., 4 p.m. and 8 p.m.). The cubicles were raked twice a day and new bedding material was provided every 14 days. Three ventilators were mounted to the ceiling, but not used during the experiments. The barn is equipped with two inside pits where the liquid manure was mixed and pumped to a storage tank from where it was delivered to a nearby biogas system. Further housing and animal information is provided in Table 9 and by Hempel et al. (2018), who provides a good overview of the structure of the two farms.

**Table 9** Overview of the two farms.

Parameter	Farm A	Farm B
Building length (m)	96.15	35.43
Building width (m)	43.2	17.30
Housing type	Naturally ventilated (windbreaker net)	Naturally ventilated (windbreaker net)
Sidewall	curtains	curtains
Treated area (m <sup>2</sup> )	1618.6	280.3
Area per cow (m <sup>2</sup> )	8.9	8.6
Grazing	None (only one group, occasionally)	None
Floor surface	Concrete, solid	Concrete, solid, slats in front of the automatic milking system (AMS)
Bedding design	Cubicle + deep bedding	Cubicle + deep bedding
Bedding material	Chopped straw/chalk	Chopped straw/chalk
Manure channels (n)	4 outside pits	2 inside pits
Scraper type	Winch-drawn	Winch-drawn
Scraper (n)	4	2
Scraper frequency	12 times/day in summer, every half hour in winter	Every hour
Milking	Herringbone milking system	AMS (Lely Astronaut A4)
Breed	Holstein Friesian	Holstein Friesian
Weight (kg)	650	650
Herd size (n)	Ø 374	Ø 52
Cow groups (n)	4	1
Milking times (n)	3	-
Milk yield (kg d <sup>-1</sup> )	39.23 (group 1–4)	ca. 28
Milk yield (kg a <sup>-1</sup> )	11.557	10.300

### *Experimental design*

The test design was based on the case-control in time approach of the VERA test protocol (*see General material and methods, 4. Verification of Environmental Technologies for Agricultural Production test protocol*, Figure 6). The measurements were carried out over all seasons (winter, summer and transition period: spring/autumn), in order to exclude any seasonal influences. The aim of the experimental setup was to compare the measured ammonia emissions before (reference phase) and after (application phase) the urease inhibitor was applied. The reference phase consisted of two days and the inhibitor was applied once a day (over a duration of three days) in the application phase (Table 10). A decay phase of nine days was implemented between

the first and second measurement period to exclude any remaining effects of the inhibitor. Each season consisted of two identical measurement periods.

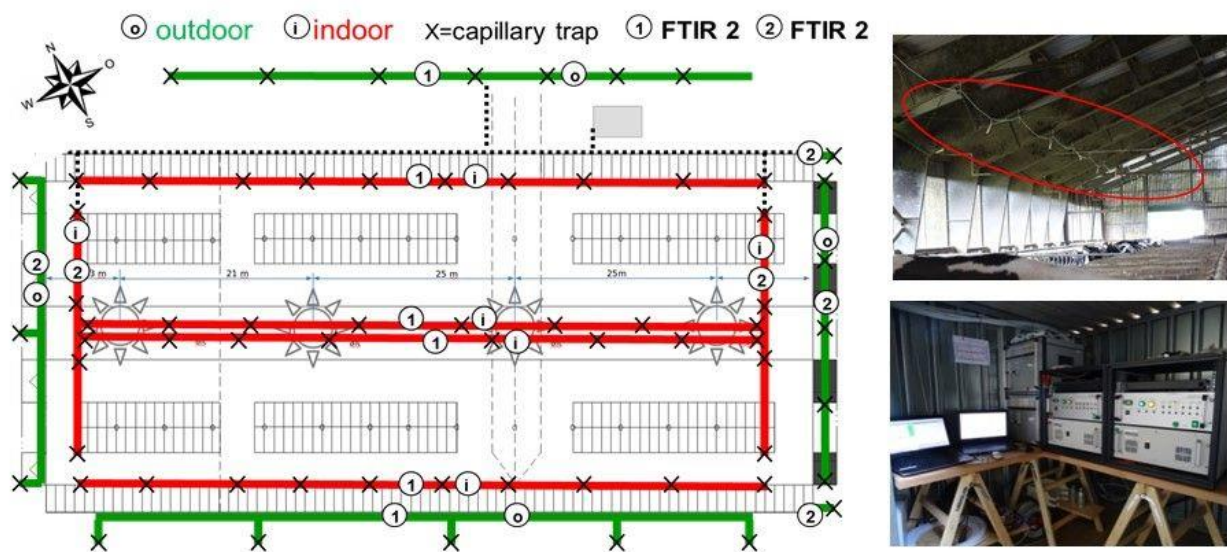
**Table 10** Experimental schedule of the two measurement periods for all seasons of Farm A and Farm B; TP = transition period (Farm A = spring, Farm B = fall).

Farm	Date	Season	Measurement period	Days per phase (n)	Phase
A	19–20.02.2017	Winter	1	2	Reference
A	21–23.02.2017	Winter	1	3	Application
A	24.02.–03.03.2017	Winter	-	9	Decay
A	04–05.03.2017	Winter	2	2	Reference
A	06–08.03.2017	Winter	2	3	Application
A	21–22.05.2017	TP	1	2	Reference
A	23–25.05.2017	TP	1	3	Application
A	26.05.–03.06.2017	TP	-	9	Decay
A	04–05.06.2017	TP	2	2	Reference
A	06–08.06.2017	TP	2	3	Application
A	06–07.08.2017	Summer	1	2	Reference
A	08–10.08.2017	Summer	1	3	Application
A	11–19.08.2017	Summer	-	9	Decay
A	20–21.08.2017	Summer	2	2	Reference
A	22–24.08.2017	Summer	2	3	Application
B	28–29.01.2018	Winter	1	2	Reference
B	30.01.–01.02.2018	Winter	1	3	Application
B	02–10.02.2018	Winter	-	9	Decay
B	11–12.02.2018	Winter	2	2	Reference
B	13–15.02.2018	Winter	2	3	Application
B	15–16.10.2017	TP	1	2	Reference
B	17–19.10.2017	TP	1	3	Application
B	20–28.10.2017	TP	-	9	Decay
B	29–30.10.2017	TP	2	2	Reference
B	31.10.–02.11.2017	TP	2	3	Application
B	10–11.09.2017	Summer	1	2	Reference
B	12–14.09.2017	Summer	1	3	Application
B	15–23.09.2017	Summer	-	9	Decay
B	24–25.09.2017	Summer	2	2	Reference
B	26–28.09.2017	Summer	2	3	Application

### *Measurement setup*

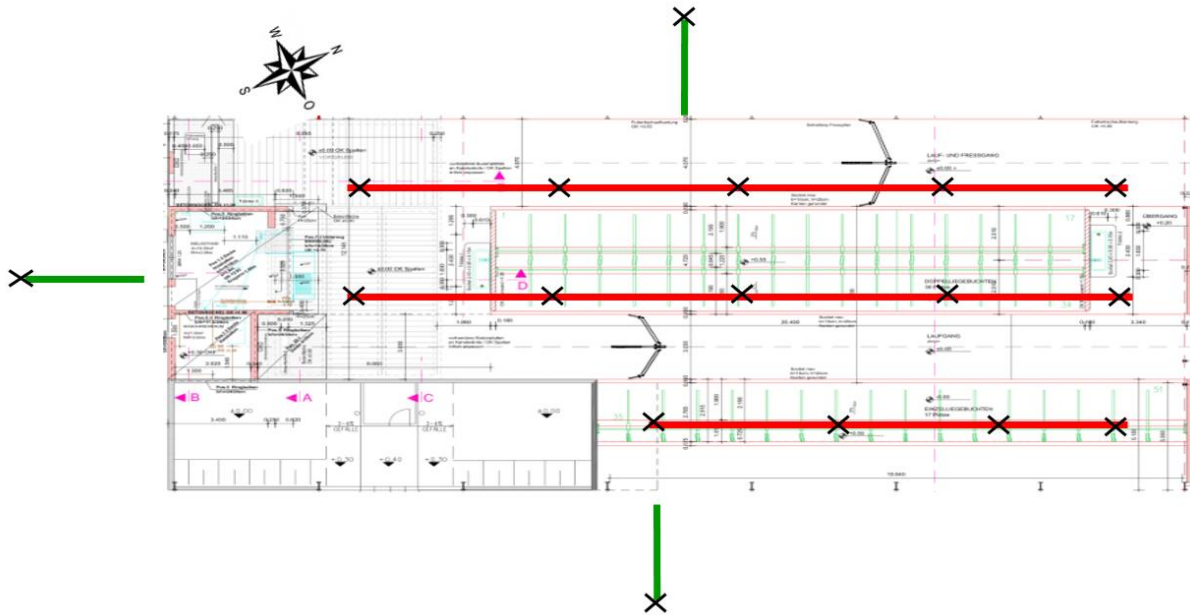
The values of the emissions, ammonia concentration, carbon dioxide concentration, ventilation rate, indoor temperature, wind direction and speed, humidity, operational function and stability of the farms were provided by the Department of Engineering for Livestock Management at the

Leibniz Institute for Agricultural Engineering and Bioeconomy in Potsdam. On both farms, the gas concentrations of ammonia and carbon dioxide were measured using two high-resolution Fourier-transform infrared spectrometer (FTIR) measurement devices (Gasetm CX4000; detection limit: 0.1 ppm; limit of quantitation: 0.3 ppm). Samples of air were drawn through polytetrafluoroethylene (PTFE) tubes with an inner diameter of 6 mm. The tubes had an orifice with a capillary trap every 10 m to ensure uniform volumetric flow at every orifice. An ultrasonic anemometer (USA, Windmaster Pro ultrasonic anemometer, Gill Instruments Limited, Lymington, Hampshire, UK) was installed on the roof of the barn of Farm A to measure wind velocity and direction. Six sample lines were installed inside the barn so that each of the four openings was equipped with a line, with two lines placed in the middle. All lines were positioned at a height of approximately 3 m, except the second middle line, which had a height of 6 m. Figure 15 shows a sketch of the installations on Farm A. Six sampling lines were placed outside the barn, one on each side or opening of the barn and two for additional measurements of potential hot spots. Other buildings are located only on the northern side and have a distance of around 20 m. The main wind direction was south-west.



**Figure 15** Sketch of the measurement setup on Farm A (left and top right) and the two FTIR measurement devices (bottom right).

The duration of the measurements for each line was 10 minutes and each FTIR was connected to six lines so that one cycle was measured per hour, providing data for the hourly estimation of air exchange rate and ammonia emissions. In the barn of Farm B, three sampling lines were installed inside the barn and three outside the barn to measure the gas concentrations (Figure 16).



**Figure 16** Sketch of the sampling lines at Farm B.

The next building was located in a north-west direction and had a distance of 5 m. The main wind direction was south-west. The tubes and capillary traps were the same as for Farm A. One difference was the distance between the traps, which was set to 6 m in this barn. One FTIR measurement device (same model as on Farm A) was used to analyse gas concentrations with a measurement duration of 10 minutes per sample line; 7 minutes were used for flushing and the other 3 minutes were used for the measuring. One cycle with 6 lines was completed in one hour and hourly emissions and air exchange rates (AERs) could be provided. To calculate the emissions, the following formula was used:

$$E = (c_{in} - c_{out}) * Q, \quad (4)$$

where  $E$  is the emission of ammonia in ( $\text{kg s}^{-1}$ ),  $c_{in}$  the inside and  $c_{out}$  the outside measured concentrations of ammonia in ( $\text{kg m}^{-3}$ ) and  $Q$  is the volume flow through the barn in ( $\text{m}^3 \text{s}^{-1}$ ). To estimate the volume flow  $Q$ , the  $\text{CO}_2$  mass balance CIGR method according to Pedersen and Sällvik, (2002) was used. To ensure the results were comparable, the emissions were converted to the emission factor EF, which refers to one livestock unit (LU) of 500 kg for dairy cows:

$$EF = \frac{E}{LU}, \quad (5)$$



where  $EF$  has a unit of  $\text{g (h}^{-1} \text{LU}^{-1})$ . More details about this method can be found in König, Hempel, Janke, Amon, and Amon (2018). Outliers emerged with high air exchange rates that occurred at very small inlet outlet differences of  $\text{CO}_2$ . Hourly values lower than 30 ppm  $\text{CO}_2$  and negative values were regarded as outliers and were therefore excluded from further calculations.

#### *Data processing and statistical analysis*

For the analysis database, the first application day (of the three) was excluded over all seasons and for both farms. The application began in the early morning with the hours from midnight included in the first day of the application phase, although no inhibitor was applied during these hours. In order to avoid underestimating the effect of the inhibitor, a new database was created using hourly emissions of the two reference days before the application and on the second and third application days.

##### *a) Reduction of the ammonia emissions*

The statistical analysis and graphs were created with the statistical software R (R Core Team, 2020). Data evaluation began by defining the following statistical mixed model:

$$Y_{ijklmo} \sim a_i * b_j * c_k * d_l + e_m + (ef)_{mn} + r_{ijklmno}, \quad (6)$$

where

$Y$ : ammonia emissions ( $\text{g h}^{-1} \text{LU}^{-1}$ )

and the fixed factors are

$a_i$ : season (summer, winter, transitional period),

$b_j$ : measurement period (1, 2),

$c_k$ : phase (reference and application),

$d_l$ : time (0, 1 ... 23 hours).

The “\*” refers to the main effects covered, including all corresponding interaction terms. The random factors were:

$e_m$ : barn (Farm A, Farm B),

$(ef)_{mn}$ : measurement unit (combination of phase, measurement phase and season),

$r_{ijklmno}$ : residual error, correlated due to the time.

Based on a graphical residual analysis, the residuals  $r_{ijklmno}$  were assumed to be normally distributed and to be heteroscedastic due to the different levels of season, measurement period and phase. Based on the model, a pseudo  $R^2$  was calculated (Nakagawa & Schielzeth, 2013) and an analysis of variances (ANOVA) was conducted, followed by a multiple contrast test (Bretz et al., 2011; Schaarschmidt & Vaas, 2009) in order to compare the levels of phase. In order to estimate the direct reduction potential, the following formula, in imitation of Hagenkamp-Korth et al. (2015), was used:

$$\text{Seasonal reduction} = 100 - \left( \frac{\text{mean ammonia emissions}_{\text{treated}} * 100}{\text{mean ammonia emissions}_{\text{untreated}}} \right), \quad (7)$$

*Seasonal reduction* (%) was calculated for both farms and over all seasons. The *mean ammonia emissions*<sub>treated</sub> (g h<sup>-1</sup> LU<sup>-1</sup>) was based on the mean values from the application phase, whereas the *mean ammonia emissions*<sub>untreated</sub> (g h<sup>-1</sup> LU<sup>-1</sup>) was based on the mean values of the days in the reference phase.

#### b) Annual reduction scenarios

In order to estimate the annual reduction for the two farms, the seasonal reductions were summed and divided by the number of seasons. Two additional theoretical scenarios were created to investigate the inhibitor effect under different circumstances. The direct exposure of naturally ventilated barns to the different seasons might impact the application technique used. Therefore, it was important to test the mitigation potential of different weather conditions that could impact inhibitor function or the application equipment.

In the first scenario, no inhibitor was applied in winter. This could be the case in sub-zero temperatures with one reason being the potential malfunctioning of the nozzles due to small ice crystals in the inside filters. The annual reduction was estimated based on reductions in both the transitional periods and summer (a value of zero reduction was used for winter to calculate the annual reduction).

In the second scenario, no inhibitor was applied in either of the transition periods. This scenario represents the most drastic case and could arise from the early onset of winter towards the end of autumn or an extended winter period going into spring. The second scenario provides information for a “worst-case” scenario. The annual reduction was estimated based on the reduction values of both summer and winter and zero reduction in the two transition periods.

## References

- Box, G. E. P., Jenkins, G. M., & Reinsel, G. C. (1994). *Time Series Analysis: Forecasting and Control* (3rd Edition). Englewood Cliff, New Jersey: Prentice Hall.
- Bretz, F., Hothorn, T., & Westfall, P. (2011). *Multiple Comparisons Using R*. London: Chapman and Hall/CRC.
- Carroll, R. J., & Ruppert, D. (1988). *Transformation and Weighting in Regression*: Chapman & Hall. <https://doi.org/10.1201/9780203735268>
- Dairy Campus (2020). Environment and measuring house. Retrieved August 12, 2020, from <https://www.dairycampus.nl/en/Home/Research/Research-facilities/8.-Environment-and-measuring-house.htm>
- Derno, M., Elsner, H.-G., Paetow, E.-A., Scholze, H., & Schweigel, M. (2009). Technical note: A new facility for continuous respiration measurements in lactating cows. *Journal of Dairy Science*, 92(6), 2804–2808. <https://doi.org/10.3168/jds.2008-1839>
- DIN EN 12880 (2001). *Characterization of sludges - Determination of dry residue and water content; German version EN 12880:2000*: Beuth Verlag. <https://dx.doi.org/10.31030/9066387>
- DIN EN 13342 (2001). *Characterization of sludges - Determination of Kjeldahl nitrogen; German version EN 13342:2000*: Beuth Verlag. <https://dx.doi.org/10.31030/9066386>
- DIN EN 38406-5-2 (1983). *German standard methods for the examination of water, waste water and sludge; cations (group E); determination of ammonia-nitrogen (E 5)*: Beuth Verlag. <https://dx.doi.org/10.31030/1209472>
- EC [European Commission]. (2016). Directive (EU) 2016/2284 of the European Parliament and of the Council of 14 December 2016 on the reduction of national emissions of certain atmospheric pollutants, amending Directive 2003/35/EC and repealing Directive 2001/81/EC. *OJL*, 344, 1-31. Retrieved August 28, 2018 from <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L:2016:344:FULL&from=EN>
- Hagenkamp-Korth, F., Haeussermann, A., & Hartung, E. (2015). Effect of urease inhibitor application on urease activity in three different cubicle housing systems under practical conditions. *Agriculture, Ecosystems & Environment*, 202, 168–177. <https://doi.org/10.1016/j.agee.2015.01.010>
- Hempel, S., König, M., Menz, C., Janke, D., Amon, B., Banhazi, T. M., . . . Amon, T. (2018). Uncertainty in the measurement of indoor temperature and humidity in naturally ventilated

- dairy buildings as influenced by measurement technique and data variability. *Biosystems Engineering*, 166, 58–75. <https://doi.org/10.1016/j.biosystemseng.2017.11.004>
- Hristov, A. N., Hanigan, M., Cole, A., Todd, R., McAllister, T. A., Ndegwa, P. M., & Rotz, A. (2011). Review: Ammonia emissions from dairy farms and beef feedlots. *Canadian Journal of Animal Science*, 91(1), 1–35. <https://doi.org/10.4141/CJAS10034>
- International VERA Secretariat (2018). VERA test protocol for Livestock Housing and Management Systems: Version 3:2018-09, 1–60. Retrieved January 21, 2019 from [https://www.vera-verification.eu/app/uploads/sites/9/2019/05/VERA\\_Testprotocol\\_Housing\\_v3\\_2018.pdf](https://www.vera-verification.eu/app/uploads/sites/9/2019/05/VERA_Testprotocol_Housing_v3_2018.pdf)
- König, M., Hempel, S., Janke, D., Amon, B., & Amon, T. (2018). Variabilities in determining air exchange rates in naturally ventilated dairy buildings using the CO<sub>2</sub> production model. *Biosystems Engineering*, 174, 249–259. <https://doi.org/10.1016/j.biosystemseng.2018.07.001>
- Laird, N. M., & Ware, J. H. (1982). Random-Effects Models for Longitudinal Data. *Biometrics*, 38(4), 963–974.
- Leinker, M. (2007). *Entwicklung einer Prinziplösung zur Senkung von Ammoniakemissionen aus Nutztierställen mit Hilfe von Ureaseinhibitoren*. Ph.D. Thesis, Martin-Luther-University, Germany: Forschungsbericht Agrartechnik VDI-MEG Nr. 462.
- Menke, K. H., & Steingass, H. (Eds.) (1987). *Schätzung des energetischen Futterwerts aus der in vitro mit Pansensaft bestimmten Gasbildung und der chemischen Analyse. II. Regressionsgleichungen. Übersichten zur Tierernährung* (15:59-94.).
- Metges, C. C., Kuhla, B., & Derno, M. (2014). Chapter 7: Large and Laboratory Animal Respiration Facilities, Leibniz Institute for Animal Biology, Dummerstorf, Germany. In C. Pinares & G. Waghorn (Eds.), *Technical Manual on Respiration Chamber Designs* (pp. 107–119). <https://doi.org/10.13140/RG.2.1.4511.6244>
- Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R<sup>2</sup> from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4(2), 133–142. <https://doi.org/10.1111/j.2041-210x.2012.00261.x>
- Pedersen, S., & Sällvik, K. (2002). Climatization of animal houses heat and moisture production at animal and house levels. *Research Centre Bygholm, Danish Institute of Agricultural*, 1–46.
- Phillips, V. R., Holden, M. R., Sneath, R. W., Short, J. L., White, R. P., Hartung, J., . . . Wathes, C. M. (1998). The Development of Robust Methods for Measuring Concentrations and

- Emission Rates of Gaseous and Particulate Air Pollutants in Livestock Buildings. *Journal of Agricultural Engineering Research*, 70(1), 11–24. <https://doi.org/10.1006/jaer.1997.0283>
- Phillips, V. R., Lee, D. S., Scholtens, R., Garland, J. A., & Sneath, R. W. (2001). A Review of Methods for measuring Emission Rates of Ammonia from Livestock Buildings and Slurry or Manure Stores, Part 2: monitoring Flux Rates, Concentrations and Airflow Rates. *Journal of Agricultural Engineering Research*, 78(1), 1–14. <https://doi.org/10.1006/jaer.2000.0618>
- R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from <https://www.R-project.org/>
- Schaarschmidt, F., & Vaas, L. (2009). Analysis of Trials with Complex Treatment Structure Using Multiple Contrast Tests. *Hortscience*, 44(1), 188–195. <https://doi.org/10.21273/HORTSCI.44.1.188>
- Stickstoffwerke Piesteritz GmbH (2020). Retrieved June 14, 2020, from <https://www.skwp.de/en/company/>
- VDLUFA [Verband Deutscher landwirtschaftlicher Untersuchungs- und Forschungsanstalten] (Ed.) (2007). *Verband Deutscher landwirtschaftlicher Untersuchungs- und Forschungsanstalten. Methodenbuch. Bd. III. Die chemische Untersuchung von Futtermitteln*. Darmstadt, Deutschland.
- Verbeke, G., & Molenberghs, G. (2000). *Linear Mixed Models for Longitudinal Data*. New York: Springer. [https://doi.org/10.1007/978-0-387-22775-7\\_3](https://doi.org/10.1007/978-0-387-22775-7_3)

## Chapter Three

### **Investigating the chronological reduction potential of a urease inhibitor in respiration chambers**

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## Abstract

Ammonia emissions have wide-ranging, negative consequences for human, animal and environmental health. Regulations including the Directive 2016/2284 require European Union member states to reduce emissions. Dairy farming has one of the highest levels of ammonia emissions. Most dairy barns in Germany are naturally ventilated for improved animal welfare standards. However, these housing systems allow an uncontrolled, direct release of emissions into the atmosphere. A novel mitigation method could be the use of a urease inhibitor. Although previous studies have noted the reduction potential of the inhibitor type K, the chronological course of the reduction is still unknown. This study investigates the inhibitory reaction by dividing the experiment into time slots to examine the chronological reduction in emissions. The inhibitor was found to reduce ammonia emissions by up to 37 % at the 12-hour time slot. This leads to the assumption that the reduction potential of the inhibitor evolves over time.

**Keywords:** ammonia reduction, urease inhibitor, dairy cows, respiration chambers

## Introduction

Ammonia emissions have a negative impact on the environment and on human and animal health (Erisman, Bleeker, Hensen, & Vermeulen, 2008; Sutton, Erisman, Dentener, & Möller, 2008; Loftus et al., 2015). The German livestock sector contributes a large amount of the ammonia emitted into the atmosphere. Dairy cow husbandry, in particular, contributes directly to these ammonia emissions (Haenel et al., 2020). The Directive 2016/2284 (EC, 2016) urges all member states of the European Union to reduce their ammonia emissions output. Since Germany needs to substantially decrease its ammonia emissions, there is an urgent need to find solutions that will reduce emissions to the agreed emissions ceiling. Much research has been conducted over recent years to reduce ammonia emissions in the industry. This research mainly focused on housing improvements (Baldini, Borgonovo, Gardoni, & Guarino, 2016; Mendes et al., 2017; Rotz, 2004; Snoek, Haesen, Groot Koerkamp, & Monteny, 2010) or enhanced feeding (Cole et al., 2005; Erickson & Klopfenstein, 2010; Frank, Persson, & Gustafsson, 2002; McGinn, Koenig, & Coates, 2002; Todd, Cole, & Clark, 2006). These changes in farm equipment and management could be time-consuming and/or a financial liability (Webb et al., 2005). Another reliable method could be the use of a urease inhibitor. Unlike the feeding and housing approaches, no changes in the farm management schedule would be necessary. Moreover, the liquid manure would benefit by becoming a valuable fertiliser and nutrient medium for crops. In the process, the enzyme urease is inhibited, thus preventing hydrolysis and the conversion of urea to ammonia (Varel, 1997). An overview of the different inhibitors and their potential can be found in Andersson (1994), Mobley, Hausinger, and Robert (1989), Monteny and Erisman (1998) and Ndegwa, Hristov, Arogo, and Sheffield (2008). Over the years, several laboratory and field work studies have been carried out to investigate the potential of the different inhibitors (Hagenkamp-Korth, Haeussermann, & Hartung, 2015a; Hagenkamp-Korth, Haeussermann, Hartung, & Reinhardt-Hanisch, 2015b; Hagenkamp-Korth, Ohl, & Hartung, 2015c; Leinker, 2007; Parker et al., 2005; Parker et al., 2016; Parker, Rhoades, Cole, & Sambana, 2012; Reinhardt-Hanisch, 2008; Varel, 1997; Varel, Nienaber, & Freetly, 1999). Even though different approaches and different types of inhibitors were used, in general, a reduction potential was observed. However, no information on the chronological response of the inhibitor was presented. The time frame of the speed and duration of the reaction involving the inhibitor with respect to the reduction in emissions was still unknown. Therefore, this scientific study aims to investigate the chronological response of the inhibitor by analysing the time slots where most of the emissions were reduced. The experiments were carried out in respiration chambers. The chambers provide a predefined, stable environment where the focus was on the decrease in

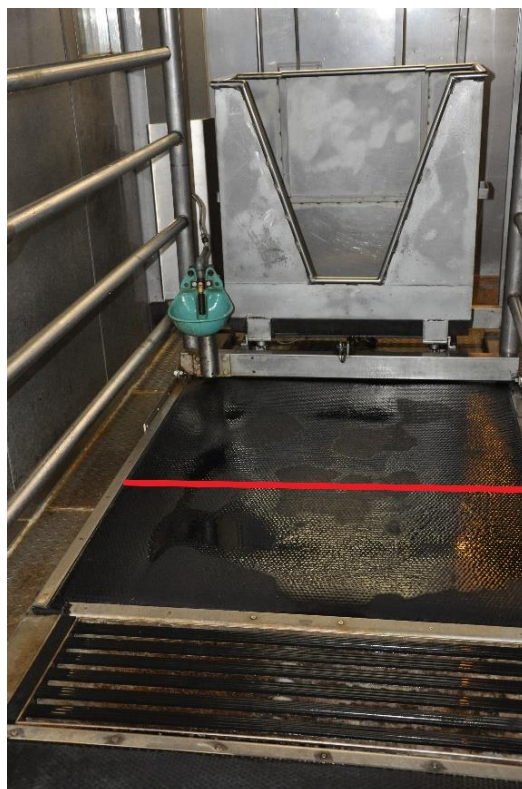


ammonia emissions whenever the inhibitor was applied. In order to focus directly on the aims of this study, a common inhibitor type K with a known reduction potential (Hagenkamp-Korth et al., 2015a) was used.

## Material and Methods

### *Test facility*

The investigations were carried out in the respiration chambers of the Institute of Nutritional Physiology “Oskar Kellner” at the Leibniz Institute for Farm Animal Biology (FBN). Up to four respiration chambers were used simultaneously, each containing one animal at a time. The chambers had a total size of 4 x 2 x 2 m and each cow had a space of 2.5 x 1.5 m inside the stanchion (Derno, Elsner, Paetow, Scholze, & Schweigel, 2009). The chamber floor was covered with a rubber mattress with three foils attached to the sides of the stanchion (Figure 1). The foils were used to prevent the excessive distribution of faeces and urine inside the chamber. However, the foil was also seen as a potential source of emissions due to faeces and urine splashing against it. The rubber mattresses inside the chambers were cleaned twice a day by removing the urine and the faeces to tanks in a cellar underneath the chamber. Milking was carried out at 7 a.m. and between 5 p.m. and 6 p.m. Not all cows were milked, since dry cows as well as cows with inserted fistulas were used over the experimental period. Milking was normally performed with all doors closed to prevent air leakage. Air leakage is considered unlikely since the chamber can only be entered through an air lock filled with chamber air (Derno et al. 2009).



**Figure 1** View inside one of the four cleaned respiration chambers. Front: water and feed trough; metal frame: stanchion inside the chamber; end: cellar for the urine and faeces; floor: rubber mattress (not pictured: three plastic foils attached to the metal frame); red line: the inhibitor was only applied to the end of the rubber mattress.

The inside air temperature was set at a constant 15 °C with an even airflow of 30 m<sup>3</sup> h<sup>-1</sup> through the chamber provided by rotary vane vacuum pumps (VT 4.40, Fuergut, Aichstetten, Germany). A flowmeter (McCrometer, Hemet, CA, accuracy 0.5 %) was used to measure the airflow of the exhaust air. Air samples for the analysis were collected by membrane pumps (KNF Neuberger Laboport, Freiburg, Germany) and an infrared absorption-based analyser (GMS800, SICK, Reute, Germany, accuracy 1 %) was used to analyse the ammonia content of the exhaust air. Data logging began as soon as the cow entered the chamber, and the length of the stay inside the respiration chamber was a maximum of three days. Gas concentration and airflow were measured every six minutes. The following additional side parameters were also measured: feed intake, measured via a decrease in feed using a scale connected to an electronic registration device (Paari, Erfurt, Germany); water consumption, measured by water meters equipped with electromechanical registration (Elster Messtechnik, Lampertheim, Germany); milk yield, registered after every milking and animal weight, measured before and after the experiment (Derno et al., 2009; Metges, Kuhla, & Derno, 2014). The barometric pressure and relative humidity of each chamber and of the exhaust lines were measured three times per second and averaged over

the six-minute interval (Derno et al. 2009). The test animals were Holstein Friesian cows and were adapted to the experimental circumstances before the start of the investigation.

### *Experimental design*

In order to examine the reduction potential of the inhibitor, the general experimental design was based on both a reference and application phase. The reference phase consisted of the ammonia emissions before the inhibitor was applied and the application phase of the ammonia emissions after the inhibitor was applied (Table 1). Therefore, the general experimental setup was divided into two phases. The experiments were implemented in ongoing research at the FBN. In order to prevent bias caused by the different FBN experimental schemes, the inhibitor investigations were conducted on different dairy cow groups. The classification of the groups was done by the FBN (groups A–C) and adapted for this study. The differences between the animal groups was the result of different feed compositions and the addition of supplements (see *Side parameters*).

**Table 1** General experimental design.

<b>Time (h)</b>	<b>Phase</b>
24 (at least)	Reference
Application of the inhibitor	
24	Application

### *Urease inhibitor type K*

The company Stickstoffwerke Piesteritz GmbH (2020) (SKWP) supplied the inhibitor K, a phosphorodiamidate, liquid formulated. In this form, the inhibitor K was easily dispensable and mixable with water. The optimised synthesis process and urease inhibitor formulations, together with the necessary application and stability properties, were developed by SKWP and are protected via patents (two registrations). The inhibitor K concentration used was  $2.5 \text{ mg m}^{-2}$ . This concentration was adapted from a previous study (Hagenkamp-Korth et al., 2015a). The inhibitor K formulation was mixed together with an amount of  $100 \text{ ml m}^{-2}$  water and applied on the floor surface.

### *Application technique*

The application of the inhibitor was carried out with a commercial hand sprayer (GLORIA Haus- und Gartengeräte GmbH) at a maximum pressure of three bar inside the respiration chamber. This application technique proved to be suitable due to the limited space inside the chambers. The hand sprayer was filled with the water-inhibitor solution outside the chamber. To

prevent any air leakages, the application took place with all doors closed. The inhibitor was applied to half of the lower end of the rubber mattress surface and to the three foils attached to the stanchion (Figure 1, red line). The end of the rubber mat was regarded as the part of the chamber with the highest emissions potential. The cow was unable to turn around and urine and faeces could only be secreted in this particular area. The duration of the application process was recorded following a protocol (see *Appendix*, Table A1).

#### *Side parameters*

The side parameters were defined based on the previously categorised groups (A–C) of the FBN. For the investigations, 13 cows were used twice and one cow was used three times, since the adaption process required some effort. Compared to groups A and C, the highest water and feed consumption was recorded in group B, which might correlate with its higher milk yield (Table 2).

**Table 2** Overview of the side parameters of the groups per day.

Group	Animals [n]	Milk yield (kg d <sup>-1</sup> )	Water intake (L d <sup>-1</sup> )	Fresh feed intake (kg d <sup>-1</sup> )
A	21	23.5	65.0	29.5
B	12	35.5	87.6	43.2
C	5	28.6	62.1	35.6

The most important dietary factors affecting the ammonia emissions of the groups (A–C) are shown in Table 3. Group A received supplements (1–4) with different fatty acid compositions. Based on a random selection, cows received either coconut oil (1), linseed and safflower oil (2), Lutalin (3) and linseed, safflower oil and Lutalin (4). A detailed description of the supplements can be found in the *Appendix* (Text A1). The nutrient requirement of the cows (Table 3) mostly conforms with the guidelines in Kirchgeßner (2014) and DLG (2020). Even though, the dry cows had slightly higher nXP and NEL values according to the DLG (2020).

**Table 3** Overview of the feed analysis of the most important feeding parameters per group.

Ingredient	Group A		Group B		Group C	
	Dry	Lactating	Dry	Lactating	Dry	Lactating
Dry matter intake (kg)	15	22	15	24	24	23
nXP (g kg <sup>-1</sup> )	141.0	142.8	146.0	153.8	155.0	154.7
NEL (MJ kg <sup>-1</sup> )	6.5	7.1	6.4	6.8	6.8	6.8

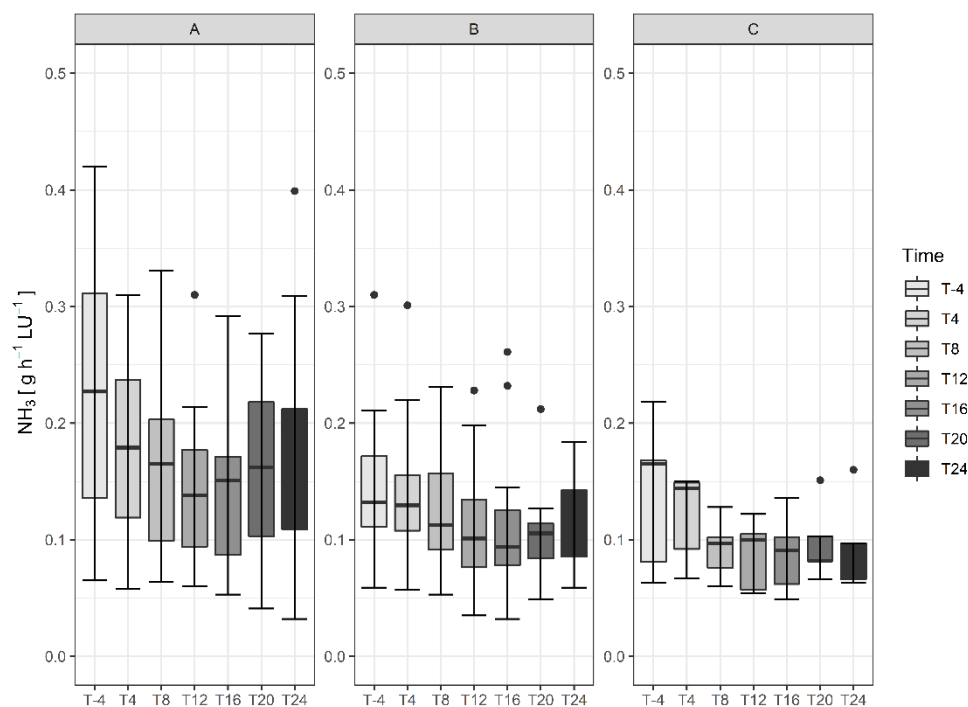
#### *Data processing and statistical analysis*

The focus of this study was the chronological response to the urease inhibitor. Therefore, the reference and application phases were divided into time slots. The inhibitor was applied after the animal had been in the chamber for at least 24 hours. It was noted that ammonia emissions inside the chamber increased over time (also recognised by Brose 2000). Pretests (not published) investigated possible time slot frames. Based on the pretest observations, the time slots were set at durations of four hours. For the reference phase, the last four-hour time slot (-4 hours) before the application point was used, while for the application phase, 24 hours were divided into four-hour time slots (4, 8, 12, 16, 20 and 24 hours). The ammonia concentration sampled was converted into g per h per livestock unit (LU). The final data set included the animal number, group (A–C), time slots (-4 hours to 24 hours) and mean values of the ammonia emissions (g h<sup>-1</sup> LU<sup>-1</sup>) for the respective time slots. The statistical software R (R Core Team, 2020) was used to evaluate the data. Data evaluation began with the definition of an appropriate statistical model based on generalised least squares (Box, Jenkins, & Reinsel, 1994; Carroll & Ruppert, 1988). The model included the factors group (A–C) and time slot (-4, 4, 8, 12, 16, 20, 24), as well as the interaction term. Correlations of the measurement values from the several time slot levels were taken into account. The residuals were assumed to be normally distributed and to be heteroscedastic with respect to the different levels of the fixed factors. These assumptions were based on a graphical residual analysis. Based on this model, a pseudo R<sup>2</sup> was calculated (Nakagawa & Schielzeth, 2013) and an analysis of variances (ANOVA) was conducted and followed by multiple contrast tests (Bretz, Hothorn, & Westfall, 2011; Schaarschmidt & Vaas, 2009) in order to compare the time slot levels. After this, the percentage reductions per group and time slot were calculated based on the mean differences and model estimates.

## Results

### *Course of the ammonia emissions*

In general, a similar ammonia emissions course was observed for all groups (A–C). The highest ammonia emissions were registered in the reference phase (-4 hours, T -4) and the lowest values occurred after the inhibitor was applied. However, the specific courses differed between the groups. A main reason for this was the different feeding regimes defining the different groups. Additionally, different starting times, different animal numbers per group and different cow status (lactating, fistula, dry) were considered reasons for the different courses between the groups. Group A had the highest emissions at  $0.42 \text{ g h}^{-1} \text{ LU}^{-1}$ , group B recorded emissions of  $0.31 \text{ g h}^{-1} \text{ LU}^{-1}$ , while group C's highest ammonia emissions were  $0.22 \text{ g h}^{-1} \text{ LU}^{-1}$  (Figure 2). The inhibitor application decreased the ammonia emissions over all groups and the lowest values were observed at different time slots. Group A's lowest values of  $0.03 \text{ g h}^{-1} \text{ LU}^{-1}$  were recorded at the 24-hour time slot, group B's lowest value of  $0.03 \text{ g h}^{-1} \text{ LU}^{-1}$  was measured at the 16-hour time slot and group C's lowest emissions value of  $0.05 \text{ g h}^{-1} \text{ LU}^{-1}$  was recorded at the 16-hour time slot (Figure 2). Table 4 and Figure 2 highlight these results.



**Figure 2** Ammonia emissions of groups A–C over the different time slots. Reference phase: T-4 (-4-hour time slot); application phase: T4 (4-hour time slot); T8 (8-hour time slot), T12 (12-hour time slot), T16 (16-hour time slot), T20 (20-hour time slot), T24 (24-hour time slot).

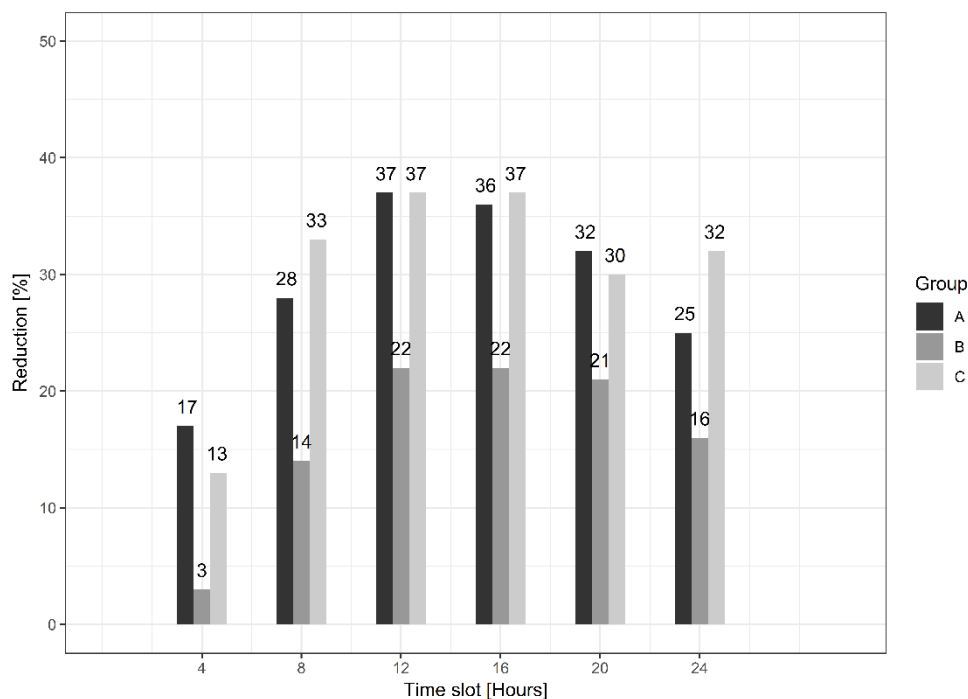
Significant differences between the time slots were detected for groups A and B, but not for group C (Table 4). The highest time slot difference for group A ( $-0.084 \text{ g h}^{-1} \text{ LU}^{-1}$ ) and B ( $-0.033 \text{ g h}^{-1} \text{ LU}^{-1}$ ) was observed at the 12-hour time slot. Although the corresponding difference for group C ( $-0.051 \text{ g h}^{-1} \text{ LU}^{-1}$ ) was higher than for B, it was not significant. This is clearly due to the fact that the data was heteroscedastic and had unbalanced sample sizes (see Table 2).

**Table 4** Differences between the time slots of the application (4, ... 24) and reference (-4) phases for all groups.

Comparison	Group	Estimate (g h <sup>-1</sup> LU <sup>-1</sup> )	Std. Error	t value	Pr(> t )
4 - -4	A >= 0	-0.038	0.010	-3.748	0.00117 **
8 - -4	A >= 0	-0.062	0.013	-4.690	< 0.001 **
12 - -4	A >= 0	-0.084	0.017	-4.708	< 0.001 ***
16 - -4	A >= 0	-0.081	0.018	-4.351	< 0.001 ***
20 - -4	A >= 0	-0.072	0.020	-3.572	0.00227 **
24 - -4	A >= 0	-0.057	0.020	-2.782	0.02707 *
4 - -4	B >= 0	-0.004	0.005	-0.875	0.75816
8 - -4	B >= 0	-0.020	0.007	-2.939	0.01726 *
12 - -4	B >= 0	-0.033	0.010	-3.075	0.01169 *
16 - -4	B >= 0	-0.032	0.013	-2.398	0.07353 .
20 - -4	B >= 0	-0.031	0.013	-2.251	0.10355
24 - -4	B >= 0	-0.023	0.013	-1.748	0.27769
4 - -4	C >= 0	-0.018	0.012	-1.499	0.40597
8 - -4	C >= 0	-0.046	0.018	-2.498	0.05776 .
12 - -4	C >= 0	-0.051	0.020	-2.527	0.05362 .
16 - -4	C >= 0	-0.050	0.020	-2.432	0.06767 .
20 - -4	C >= 0	-0.042	0.022	-1.898	0.21345
24 - -4	C >= 0	-0.044	0.021	-2.030	0.16542

Based on the observed decrease in ammonia emissions, a reduction potential (in %) could be estimated for every time slot (Figure 3). In general, a similar reduction course was observed for all groups. The reduction potential increased over time, reached a plateau and subsequently decreased. The lowest reduction potential was found at the time slot closest to the application point. In combination with the increasing reduction, it could be assumed that the inhibitor potential developed over time. However, different reduction potentials were registered between the three groups. Groups A and C showed an almost equal course for the reduction potential over the time slots. For both groups, the value 37 % at the 12-hour time slot was observed. Group B, however, had a similar course, but the value at the 12-hour time slot was lower at 22 %. This corresponds with the data in Table 4 where the absolute time slot difference for group B was also the lowest compared to groups A and C.





**Figure 3** Estimated reduction potential (%) of each time slot for groups A–C.

## Discussion

### *Test facility*

Most of the previous urease inhibitor experiments were conducted under laboratory conditions, (Hagenkamp-Korth et al., 2015b; Reinhardt-Hanisich, 2008; Varel, 1997), therefore respiration chambers were considered to be a valuable interface between laboratory and more practical field investigations. The animal inside the chamber can be regarded as being closer to a field scenario, while the chamber with its airflow rate, temperature, feeding etc. regulations (Derno et al., 2009) is similar to a laboratory setting. The chamber's structure is similar to that of tie stall housing where the cow can change only its lying or standing position. However, nowadays, due to animal welfare reasons, most common dairy housings in Germany are based on loose housing systems (Tergast, Schickramm, Lindena, Ellßel, & Hansen, 2019). Besides the housing aspect, the constant airflow inside the chambers of 30 m<sup>3</sup> per hour could influence the emissions. According to DIN 18910 (2017), an airflow of at least 73 m<sup>3</sup> per hour is recommended for a dairy cow of 400 kg, whereas 115 m<sup>3</sup> per hour is recommended for a dairy cow of 700 kg. Therefore, any comparison to the ammonia emissions of free or tie stalls is unrealistic considering the laboratory conditions and the low and untypical airflow. However, the course of the reduction potential could be investigated independently of the test facility used.

*Application technique*

In comparison to a prior study under practical conditions, where a commercial backpack sprayer was used to apply the inhibitor K (Hagenkamp-Korth et al., 2015a), a small manual hand sprayer was considered most suitable for this experimental setup. Moreover, only a limited space inside the chamber and stanchion was available for the application. To prevent injuries caused by the cow and to avoid any unnecessary feed contact, the application could only be carried out from and at the rear of the chamber. This is where the urine and faeces mix and removed to tanks in the cellar underneath the chamber (Figure 1, red line). The cow was unable to turn around inside the chamber, therefore the application area was assumed to be the place where most of the ammonia emissions occurred. The chamber's funnel could be a possible emissions area, but no inhibitor could be applied here due to its constructional design. The use of a hand sprayer might be suitable for the respiration chambers, however, field studies in different cubicle housing systems carried out by Hagenkamp-Korth et al., (2015a) used a backpack sprayer. Upcoming investigations should give further information on developments relating to automated processes for the application. The additional use of 100 ml m<sup>2</sup> water was considered to have no impact on the reductions in this study. Kroodsmma, Huis in 't Veld, and Scholtens (1993) investigated the reduction effect of flushing water, where 50–110 L water per day per cow were used over different intervals, pressures and times, and a reduction potential from 25 % up to 72 % was observed. In contrast, only 1 L of water per cow per application day was used in this study and therefore no reduction potential was expected due to the added water.

*Reduction course of the time slot analysis*

The time slot analysis aimed to investigate the chronological response of the urease inhibitor on ammonia emissions. Based on their different groups (A–C), the cows went into the chambers at different times, hence the start of the experiment was different for each cow. These circumstances resulted in different application times. Therefore, all cows were pooled with an application time “0” which did not represent the “real time”. This pooling might have had an influence on reduction levels. Wu, Zhang, and Kai (2012) found that ammonia emissions peak one hour after feeding time (around or after noon). In this study, the analyses were conducted using previously selected (from pretests) four-hour time slots. Shorter time slots lead to unfeasible results due to a high individual distribution of the measurement values. Further investigations concerning the extension or contraction of the time slots may have an impact on reduction levels. Brose (2000) investigated ammonia emissions inside respiration chambers and found low ammonia emissions at the beginning of the experiment and an increase in values as soon as the

animal excreted urine and faeces. At the beginning of the investigations in this study, the animals were placed inside extremely clean chambers that had been cleaned under high pressure. Similar to Brose (2000), a slow increase in emission levels began as soon as the animal entered the chamber. Therefore, the urease inhibitor application was carried out after 24 hours in order to take regular emission levels into account. Based on the data, it was observed that ammonia emissions increased over time. Therefore, any influence from the lower values at the start of the experiment was excluded by only using the last four hours before the application of the reference phase.

Reinhardt-Hanisch (2008) investigated the potential of different urease inhibitors under laboratory conditions. Up to 60 hours (2.5 days) in the reference phase ensured constant, stable test conditions before the inhibitor application. However, for animal welfare reasons, the animals' duration in the chamber was limited to a maximum of three days. After the application, it was found that the inhibitor effect increased over time and the lowest reduction potential was found at four hours after the application (Figure 3). The highest reductions were observed at the 12-hour time slot (Figure 3) and consequently it could be assumed that the inhibitor needed this time to develop its potential. Similar progression courses were found in the laboratory investigations of Hagenkamp-Korth et al. (2015b) and Reinhardt-Hanisch (2008), in the pretests of this study (not published). In general, emissions dropped as soon as the inhibitor was applied. The course of the ammonia emissions in Hagenkamp-Korth et al. (2015b) and Reinhardt-Hanisch (2008), decreased more dramatically due to the limited surface area and the "flushing" effect of the inhibitor. However, the manually applied urea solution was only used once over 24 hours. Chambers are considered to be a step closer to field conditions by using animals and providing more realistic conditions. Leinker (2007) investigated two different urease inhibitors under laboratory conditions by measuring the urease activity four hours and 19 hours after the inhibitor application. Leinker (2007) found the lowest urease activity at the 19-hour time slot measurement after the inhibitor application. Similar observations were made in this study: the reduction potential increased over time and a higher reduction was found at the 12-hour time slot compared to the 4-hour time slot.

Reinhardt-Hanisch (2008) tested the long-range potential of two urease inhibitors at three different substrate temperatures. It was found that higher temperatures contributed to an earlier ammonia emissions increase. After 3–11 days, ammonia emissions started to increase again at a temperature of 15 °C, depending on the previously applied inhibitor (type C or D). In this study, however, a small decrease in reduction potential was already noted at the 20-hour time slot. Therefore, a residual potential was assumed. One main reason for this could be due to a

differing urea/urine frequency. Reinhardt-Hanisch (2008) added 12 urea solution doses (100 ml) over a period of 18 days, whereas in this study the cows frequently urinated on the floor surface inside the chambers. A reduction potential from 3 % up to 37 % was observed in the time slot analysis. Hagenkamp-Korth et al. (2015b) investigated the reduction potential of the same inhibitor K under different temperatures and found a general reduction of 64 %. However, these different reduction values might be a consequence of different experimental setups, research object (here, time slot analysis) and measurement techniques.

### Conclusion

This study investigated the urease inhibitor K as an effective mitigation method for ammonia emissions. Investigations from previous studies (Hagenkamp-Korth et al., 2015a; Leinker, 2007; Reinhardt-Hanisch, 2008) concerning the duration of effect could be extended. In general, a reduction potential from 3 % up to 37 % could be calculated. Furthermore, this study showed a continuous increase over the 24 hours of the reduction period after the application, where a peak trend towards the 12-hour time slot was seen. However, optimal test conditions using the same start and end times for all test animals should be provided. Moreover, enough time (at least 24 hours) should be scheduled at the start of the experiment to allow emissions to stabilise and provide realistic reference data. In order to investigate the inhibitor under different climate conditions, the respiration chambers offer the possibility of modifying air temperature and airflow rate. Follow-up studies should therefore focus on the temperature setup. The manual application tool in this investigation was used under the given conditions.

However, the urease inhibitor K can also be used in different housing systems and future aims should be to investigate the benefit of using the inhibitor in practical situations. Therefore, upcoming studies should focus on the implementation of fully-automated application systems that can be adapted to different housing shapes and sizes. Faster application processes and thus less interruption to and influence on the test animals may be expected.

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### Declarations of interest

None.

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## References

- Andersson, M. 1994. *Performance of additives in reducing ammonia emissions from cow slurry*. Swedish Swedish University of Agricultural Sciences, Department of Agricultural Biosystems and Technologies. Report No. 93. JBL Publ., Stockholm, Sweden.
- Baldini, C., Borgonovo, F., Gardoni, D., & Guarino, M. (2016). Comparison among NH<sub>3</sub> and GHGs emissive patterns from different housing solutions of dairy farms. *Atmospheric Environment*, 141, 60–66. <https://doi.org/10.1016/j.atmosenv.2016.06.047>
- Box, G. E. P., Jenkins, G. M., & Reinsel, G. C. (1994). *Time Series Analysis: Forecasting and Control* (3rd Edition): Holden-Day.
- Bretz, F., Hothorn, T., & Westfall, P. (2011). *Multiple Comparisons Using R*. London: Chapman and Hall/CRC. ISBN 9781584885740.
- Brose, G. (2000). *Emission von klimarelevanten Gasen, Ammoniak und Geruch aus einem Milchviehstall mit Schwerkraftlüftung*. Ph.D. Thesis. Forschungsbericht Agrartechnik des Arbeitskreises Forschung und Lehre der Max-Eyth-Gesellschaft Agrartechnik VDI-MEG Nr. 362.
- Carroll, R. J., & Ruppert, D. (1988). *Transformation and Weighting in Regression*: Chapman & Hall. <https://doi.org/10.1201/9780203735268>
- Cole, N. A., Clark, R. N., Todd, R. W., Richardson, C. R., Gueye, A., Greene, L. W., & McBride, K. (2005). Influence of dietary crude protein concentration and source on potential ammonia emissions from beef cattle manure. *Journal of Animal Science*, 83(3), 722–731. <https://doi.org/10.2527/2005.833722x>
- Derno, M., Elsner, H.-G., Paetow, E.-A., Scholze, H., & Schweigel, M. (2009). Technical note: A new facility for continuous respiration measurements in lactating cows. *Journal of Dairy Science*, 92(6), 2804–2808. <https://doi.org/10.3168/jds.2008-1839>
- DIN 18910 (2017). *Thermal insulation for closed livestock buildings - Thermal insulation and ventilation - Principles for planning and design for closed ventilated livestock buildings*: Beuth Verlag. <https://dx.doi.org/10.31030/2680106>

- DLG [Deutsche Landwirtschafts-Gesellschaft] (2020). Berücksichtigung N- und P-reduzierter Fütterungsverfahren bei den Nährstoff - ausscheidungen von Milchkühen. DLG-Merkblatt 444. Retrieved July 03, 2020, from [https://www.dlg.org/fileadmin/downloads/landwirtschaft/themen/publikationen/merkblaetter/dlg-merkblatt\\_444.pdf](https://www.dlg.org/fileadmin/downloads/landwirtschaft/themen/publikationen/merkblaetter/dlg-merkblatt_444.pdf)
- EC [European Commission]. (2016). Directive (EU) 2016/2284 of the European Parliament and of the Council of 14 December 2016 on the reduction of national emissions of certain atmospheric pollutants, amending Directive 2003/35/EC and repealing Directive 2001/81/EC. *OJL*, 344, 1-31. Retrieved August 28, 2018 from <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L:2016:344:FULL&from=EN>
- Erickson, G., & Klopfenstein, T. (2010). Nutritional and management methods to decrease nitrogen losses from beef feedlots. *Journal of Animal Science*, 88(13 Suppl), E172-80. <https://doi.org/10.2527/jas.2009-2358>
- Erismann, J. W., Bleeker, A., Hensen, A., & Vermeulen, A. (2008). Agricultural air quality in Europe and the future perspectives. *Atmospheric Environment*, 42(14), 3209–3217. <https://doi.org/10.1016/j.atmosenv.2007.04.004>
- Frank, B., Persson, M., & Gustafsson, G. (2002). Feeding dairy cows for decreased ammonia emission. *Livestock Production Science*, 76(1-2), 171–179. [https://doi.org/10.1016/S0301-6226\(02\)00021-0](https://doi.org/10.1016/S0301-6226(02)00021-0)
- Haenel, H. D., Rösemann, C., Dämmgen, U., Döring, U., Wulf, S., Eurich-Menden, B., ... & Fuß, R. (2020). *Calculations of gaseous and particulate emissions from German agriculture 1990-2018: Report on methods and data (RMD) Submission 2020* (No. 77). Thünen Report. <https://doi.org/10.3220/REP1584363708000>
- Hagenkamp-Korth, F., Haeussermann, A., & Hartung, E. (2015a). Effect of urease inhibitor application on urease activity in three different cubicle housing systems under practical conditions. *Agriculture, Ecosystems & Environment*, 202, 168–177. <https://doi.org/10.1016/j.agee.2015.01.010>
- Hagenkamp-Korth, F., Haeussermann, A., Hartung, E., & Reinhardt-Hanisich, A. (2015b). Reduction of ammonia emissions from dairy manure using novel urease inhibitor formulations under laboratory conditions. *Biosystems Engineering*, 130, 43–51. <https://doi.org/10.1016/j.biosystemseng.2014.12.002>
- Hagenkamp-Korth, F., Ohl, S., & Hartung, E. (2015c). Effects on the biogas and methane production of cattle manure treated with urease inhibitor. *Biomass and Bioenergy*, 75, 75–82. <https://doi.org/10.1016/j.biombioe.2015.02.014>

- Kirchgeßner, M. (2014). *Tierernährung: Leitfaden für Studium, Beratung und Praxis*, 14. Auflage. DLG Verlag. Frankfurt am Main.
- Kroodsma, W., Huis in 't Veld, J.W.H., & Scholtens, R. (1993). Ammonia emission and its reduction from cubicle houses by flushing. *Livestock Production Science*, 35(3-4), 293–302. [https://doi.org/10.1016/0301-6226\(93\)90099-4](https://doi.org/10.1016/0301-6226(93)90099-4)
- Leinker, M. (2007). *Entwicklung einer Prinziplösung zur Senkung von Ammoniakemissionen aus Nutztierställen mit Hilfe von Ureaseinhibitoren*. Ph.D. Thesis, Martin-Luther-University, Germany: Forschungsbericht Agrartechnik VDI-MEG Nr. 462.
- Loftus, C., Yost, M., Sampson, P., Arias, G., Torres, E., Vasquez, V. B., . . . Karr, C. (2015). Regional PM2.5 and asthma morbidity in an agricultural community: A panel study. *Environmental Research*, 136, 505–512. <https://doi.org/10.1016/j.envres.2014.10.030>
- McGinn, S. M., Koenig, K. M., & Coates, T. (2002). Effect of diet on odorant emissions from cattle manure. *Canadian Journal of Animal Science*, 82(3), 435–444. <https://doi.org/10.4141/A02-015>
- Mendes, L. B., Pieters, J. G., Snoek, D., Ogink, N. W.M., Brusselman, E., & Demeyer, P. (2017). Reduction of ammonia emissions from dairy cattle cubicle houses via improved management- or design-based strategies: A modeling approach. *Science of the Total Environment*, 574, 520–531. <https://doi.org/10.1016/j.scitotenv.2016.09.079>
- Metges, C. C., Kuhla, B., & Derno, M. (2014). Chapter 7: Large and Laboratory Animal Respiration Facilities, Leibniz Institute for Animal Biology, Dummerstorf, Germany. In C. Pinares & G. Waghorn (Eds.), *Technical Manual on Respiration Chamber Designs* (pp. 107–119). <https://doi.org/10.13140/RG.2.1.4511.6244>
- Mobley, H. L., & Hausinger, R. P. (1989). Microbial ureases: significance, regulation, and molecular characterization. *Microbiology and Molecular Biology Reviews*, 53(1), 85-108. <https://doi.org/10.1128/MMBR.53.1.85-108.1989>
- Monteny, G. J., & Erisman, J. W. (1998). Ammonia emission from dairy cow buildings: a review of measurement techniques, influencing factors and possibilities for reduction. *Netherlands Journal of Agricultural Science*. (46), 225–247. <https://doi.org/10.18174/njas.v46i3.481>
- Nakagawa, S. and Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. Edited by Robert B. O'Hara. *Methods in Ecology and Evolution* 4(2), 133-42. <https://doi.org/10.1111/j.2041-210x.2012.00261.x>

- Ndegwa, P. M., Hristov, A. N., Arogo, J., & Sheffield, R. E. (2008). A review of ammonia emission mitigation techniques for concentrated animal feeding operations. *Biosystems Engineering*, 100(4), 453–469. <https://doi.org/10.1016/j.biosystemseng.2008.05.010>
- Parker, D. B., Pandrangi, S., Greene, L. W., Almas, L. K., Cole, N. A., Rhoades, M. B., & Koziel, J. A. (2005). Rate And Frequency Of Urease Inhibitor Application For Minimizing Ammonia Emissions From Beef Cattle Feedyards. *Transactions of the ASAE*, 48(2), 787–793. <https://doi.org/10.13031/2013.18321>
- Parker, D. B., Rhoades, M. B., Baek, B. H., Koziel, J. A., Waldrip H. M., & Todd, R. W. (2016). Urease Inhibitor for Reducing Ammonia Emissions from an Open-Lot Beef Cattle Feedyard in the Texas High Plains. *Applied Engineering in Agriculture*, 32(6), 823–832. <https://doi.org/10.13031/aea.32.11897>
- Parker, D. B., Rhoades, M. B., Cole, N. A., & Sambana, V. P. (2012). Effect of Urease Inhibitor Application Rate and Rainfall on Ammonia Emissions from Beef Manure. *American Society of Agricultural and Biological Engineers*. (55), 211–218. <https://doi.org/10.13031/2013.41248>
- R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from <https://www.R-project.org/>
- Reinhardt-Hanisch, A. (2008). *Grundlagenuntersuchungen zur Wirkung neuartiger Ureaseinhibitoren in der Nutztierhaltung*. Ph. D. Thesis, University of Hohenheim, Germany: Forschungsbericht Agrartechnik VDI-MEG Nr. 471.
- Rotz, C. A. (2004). Management to reduce nitrogen losses in animal production. *Journal of Animal Science*, 82 E-Suppl, E119-137. [https://doi.org/10.2527/2004.8213\\_supplE119x](https://doi.org/10.2527/2004.8213_supplE119x)
- Schaarschmidt, F., & Vaas, L. (2009). Analysis of Trials with Complex Treatment Structure Using Multiple Contrast Tests. *Hortscience*. 44(1), 188–195. <https://doi.org/10.21273/HORTSCI.44.1.188>
- Snoek J. W., Haesen G. P. M. J., Groot Koerkamp P. W. G., & Monteny G. J. (2010). Effect of floor design in a dairy cow house on ammonia emission - Design, test and preliminary results with an experimental set-up for run off experiments. *International Conference on Agricultural Engineering. Cemagref, Clermont-Ferrand*. p. 1-10. <https://doi.org/10.13140/RG.2.1.1957.0168>
- Stickstoffwerke Piesteritz GmbH (2020). Retrieved June 14, 2020, from <https://www.skwp.de/en/company/>



- Sutton, M. A., Erisman, J. W., Dentener, F., & Möller, D. (2008). Ammonia in the environment: From ancient times to the present. *Environmental Pollution (Barking, Essex : 1987)*, *156*(3), 583–604. <https://doi.org/10.1016/j.envpol.2008.03.013>
- Tergast, H., Schickramm, L., Lindena, T., Ellßel, R., & Hansen, H. (2019) Steckbriefe zur Tierhaltung in Deutschland: Milchkühe, 1–17. Retrieved August 24, 2020 from [https://literatur.thuenen.de/digbib\\_extern/dn061460.pdf](https://literatur.thuenen.de/digbib_extern/dn061460.pdf)
- Todd, R. W., Cole, N. A., & Clark, R. N. (2006). Reducing crude protein in beef cattle diet reduces ammonia emissions from artificial feedyard surfaces. *Journal of Environmental Quality*, *35*(2), 404–411. <https://doi.org/10.2134/jeq2005.0045>
- Varel, V. H., Nienaber, J. A., & Freetly, H. C. (1999). Conservation of nitrogen in cattle feedlot waste with urease inhibitors. *Journal of Animal Science*, *77*(5), 1162. <https://doi.org/10.2527/1999.7751162x>
- Varel, V. H. (1997). Use of urease inhibitors to control nitrogen loss from livestock waste. *Bioresource Technology*, *62*(1-2), 11–17. [https://doi.org/10.1016/S0960-8524\(97\)00130-2](https://doi.org/10.1016/S0960-8524(97)00130-2)
- Webb, J., Menzi, H., Pain, B. F., Misselbrook, T. H., Dämmgen, U., Hendriks, H., & Döhler, H. (2005). Managing ammonia emissions from livestock production in Europe. *Environmental Pollution (Barking, Essex : 1987)*, *135*(3), 399–406. <https://doi.org/10.1016/j.envpol.2004.11.013>
- Wu, W., Zhang, G., & Kai, P. (2012). Ammonia and methane emissions from two naturally ventilated dairy cattle buildings and the influence of climatic factors on ammonia emissions. *Atmospheric Environment*, *61*, 232–243. <https://doi.org/10.1016/j.atmosenv.2012.07.050>

## Chapter Four

### **Reduction of ammonia emissions by using a urease inhibitor in a mechanically ventilated dairy housing system**

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### Abstract

Ammonia emissions have a negative influence on both human and animal health as well as on the environment. Livestock farming, in particular the dairy sector, is a major emitter of ammonia emissions. Urease inhibitors may act as a suitable mitigation measure without requiring the high investments connected with, for e.g., new floor surfaces in barn refurbishments. The main aim of this field study was to a) quantify the reduction potential of the urease inhibitor K and b) provide information on the chronological reduction response of the inhibitor. The experimental setup was based on an in time and case-control approach, in compliance with the Verification of Environmental Technologies for Agricultural Production test protocol. The investigations were carried out in two mechanically ventilated units of the Leeuwarden Dairy Campus. Both sets of investigations were performed over all seasons. A new liquid chemical formulation of the urease inhibitor K, using a concentration of  $2.5 \text{ mg m}^{-2}$  was mixed with  $50 \text{ ml m}^{-2}$  water. Urease activity was measured to confirm the reduction potential of the inhibitor. Urease activity could be reduced by 69 %. The in time approach found a seasonal reduction potential, where ammonia emissions were reduced by 17–23 % in summer, 10–31 % in winter and 22 % in spring. The case-control approach found a reduction pattern with the highest reduction potential at the 16-hour time slot. However, the time slot analysis found a decrease in reduction after the inhibition peak.

**Keywords:** Ammonia emissions, dairy cows, urease inhibitor, reduction potential

## Introduction

Ammonia emissions contribute to negative acidification and eutrophication effects on the environment (Draaijers, Ivens, Bos, & Bleuten, 1989; Erisman, Bleeker, Hensen, & Vermeulen, 2008; Guthrie et al., 2018). Moreover, ammonia supports the formation of fine particles which have a negative impact on human health (Erisman et al., 2008; Gauderman et al., 2004). The agricultural sector is responsible for 95 % of the ammonia emissions in Germany (UBA, 2018), with dairy farming being the main contributor (Haenel et al., 2020). In order to reduce air pollution, the Directive 2016/2284, (EC, 2016) requires the establishment of mandatory emissions ceilings for each European member state. Much research has therefore been carried out in recent years to reduce ammonia emissions (Cole et al., 2005; Kroodsma, Huis in 't Veld, & Scholtens, 1993; Mendes et al., 2017; Metz, Ogink, & Smits, 1995; Monteny, Bannink, & Chadwick, 2006; Ndegwa, Hristov, Arogo, & Sheffield, 2008; Ogink & Kroodsma, 1995; Shi, Parker, Cole, Auvermann, & Mehlhorn, 2001; Smits, Valk, Elzing, & Keen, 1995; Snoek, Haesen, Groot Koerkamp, & Monteny, 2010; Swierstra, Braam, & Smits, 2001; Webb et al., 2005). However, most mitigation measures require huge financial or management inputs. A possible mitigation approach with lower input requirements could be the use of urease inhibitors. Ammonia gas originates from the microbial conversion of urea in urine through hydrolysis. The enzyme urease works as a catalyst in this hydrolysis process. This enzyme is present in faeces and is ubiquitous on floor surfaces (Varel, 1997). The hydrolysis process could be reduced by inhibiting the enzyme. Previous studies have tested the mitigation potential of different urease inhibitors (Hagenkamp-Korth, Haeussermann, & Hartung, 2015a; Hagenkamp-Korth, Haeussermann, Hartung, & Reinhardt-Hanisch, 2015b; Hagenkamp-Korth, Ohl, & Hartung, 2015c; Leinker, 2007; Parker et al., 2005; Parker et al., 2016; Parker, Rhoades, Cole, & Sambana, 2012; Reinhardt-Hanisch, 2008; Varel, 1997; Varel, Nienaber, & Freetly, 1999). Out of the above mentioned studies, Hagenkamp-Korth et al., 2015a tested the urease inhibitor K under practical conditions by measuring urease activity and a relative reduction potential of 66 % up to 96 % was detected. However, the measurement of urease activity represents an indirect ammonia detection method and is therefore limited in certain assumptions. Only a direct ammonia emissions measurement can provide a comprehensive picture.

Therefore, this study aims to a) quantify the reduction potential through a direct measurement of emissions and b) examine the chronological reduction response of the urease inhibitor in a mechanically ventilated dairy barn. Many of the previous studies used different measurement equipment and approaches. Therefore, this study was based on the Verification of Environmen-

tal Technologies for Agricultural Production (VERA) test protocol (International VERA Secretariat, 2018) in order to be comparable with other studies. Two different experimental setups (in time and case-control approach) were used and the ammonia emission measurements were carried out using a NO<sub>x</sub> converter. In order to verify the inhibitor effect on the urease, urease activity was measured.

## Material & Methods

### *Housing system*

The emission measurements were carried out at the Dairy Campus, a dairy research farm located near Leeuwarden in the province of Friesland in the Netherlands. The farm has a research barn for the investigation of emissions. A detailed description of the facility can be found in Dairy Campus (2019) and a sketch of the barn is given (see General material and methods, Chapter 4; Figure 12). The research barn consists of six separated units, four of which are equipped with cubicles, all of them mechanically ventilated. For the investigations, two identical cubicle units (referred to as Unit A and Unit B) were used. Each unit housed 16 lactating Holstein-Friesian cows with comparable daily milk yields (30 kg d<sup>-1</sup>), average milk urea concentrations (230 - 240 mg L<sup>-1</sup>), age (3.4 – 3.9 years) and number of lactation (2). Each cow was provided with 7.6 m<sup>2</sup> space, including 4.5 m<sup>2</sup> of concrete slatted floor. The bedding material in the cubicles consisted of mattresses covered with a thin layer of saw dust, which was manually renewed twice a day. The liquid manure was stored in a pit underneath the slatted floor. The cows were milked twice a day, at around 5 a.m. and 4 p.m. During milking, the doors between the units were opened (for approximately two hours), the cubicles were cleaned and the feed was pushed up or a fresh TMR ration (mainly consisting of grass and maize silage) was provided. The cows had no access to grazing or to an exercise yard. An automatic feeding station in each unit provided mineral concentrate at amounts based on individual cow needs and a drinking trough provided ad libitum fresh water. The indoor climate was regulated by two mechanical ventilators (Fancom) mounted in a shaft (Ø800) and combined with a measuring and control unit (Fancom ATM80). The ventilation rate inside the units was set at a fixed capacity of around 11,000 m<sup>3</sup> h<sup>-1</sup> (40 % of maximum capacity) during spring and around 17,000 m<sup>3</sup> h<sup>-1</sup> (50 % of maximum capacity) during summer and winter.

### *Experimental design*

The experimental setup was based on the measurement protocol of the VERA Test Protocol for Livestock Housing and Management Systems; Version 3:2018-09 (International VERA Secretariat, 2018). The emission measurements were carried out in parallel in the two identical units

(Unit A and Unit B) and two different experimental setups were used. One setup was based on an in time approach with a reference phase (no inhibitor application) and an application phase, with application of the inhibitor K taking place in the same unit. The second experimental setup was based on a direct case-control approach (reference phase-application phase) between both units (Table 1). The measurements were conducted over the course of one year (winter, summer and spring), in order to include the potential effects of seasonal interaction. For each season, the measurement of every unit was repeated twice. Each measurement period consisted, in general, of two reference days and three following application days. In between the first and the second measurement period, a break of nine days was implemented in order to start the second measurement without any residual inhibitor effects. After the first measurement period, the case and control treatments were switched between units so that each unit was used twice as both a case and a control. Urease activity was also measured as a back-up check for the direct ammonia emissions measurement. Therefore, a small sample size was selected prior to the measurement start. In order to confirm the blocking effect of the inhibitor, urease activity was measured one day before the inhibitor was applied and on the last (third) application day. A measurement pattern (see General material and methods, 3. *Urease activity measurement*, Figure 2) was created to cover all seasons and both units. The experimental setup and measurements were carried out according to Braam and Swierstra (1999) and Hagenkamp-Korth, et al. (2015a). Further side parameters, such as feed and liquid manure samples, were collected twice per unit and season in order to consider any possible influences on the ammonia emissions. All samples were frozen immediately and shipped to a laboratory for standard analysis (see General Material & methods, 5. *Side parameters*).

**Table 1** Experimental scheme of one experimental period in spring. The in time approach was used in Unit A (10.04.2017 – 14.04.2017) and the case-control approach was used in both Unit A and Unit B (12.04.2017 – 14.04.2017).

Date	Unit A	Unit B
10.04.2017	Reference	
11.04.2017	Reference	
12.04.2017	Application	Reference
13.04.2017	Application	Reference
14.04.2017	Application	Reference

*Setup of the ammonia emissions measurement*

Exhaust air from each ventilator and background air from two sampling points outside the units was sampled constantly using polyethylene sampling lines (0.64 cm inner diameter). The sampling lines were connected to a multiplexer together with those from the other units. The multiplexer switched every 10 minutes to a different sampling point. The sampled air passed through a thermal ammonia converter with known efficiency before being analysed in a chemiluminescence NO<sub>x</sub> analyzer (Teledyne API, T200, precision: 0.5 % of reading). Due to a retarded response of the thermal ammonia converters to concentration differences, only the last minute of the 10-minute measurement period was used in further calculations. The calibration took place using a bottle of certified gas of around 40 ppm NO and a maximum interval of one month. Further details of this setup are described in Phillips et al. (1998) and Phillips, Lee, Scholtens, Garland, and Sneath (2001). The pulse signal of each Fancom ATM 80 was logged at an interval of one minute and, together with the gas concentrations, stored in a datalogger (Campbell Scientific, C1000X). Ventilation rates were calculated using a calibration line separately determined in a wind tunnel. Average concentrations and ventilation rates per hour were used to calculate ammonia emissions for each unit. Indoor temperatures were measured in the unit using two temperature and relative humidity sensors per unit (Rotronic Instrument Corp., Huntington, USA) with a precision of  $\pm 1.0$  °C and  $\pm 2$  % relative humidity.

*Urease inhibitor*

The company Stickstoffwerke Piesteritz GmbH (2020) (SKWP) supplied the chemical formulation with the inhibitor K, a phosphorodiamidate. In this form, the inhibitor K was easily dispensable and mixable with water. For the investigations, the same inhibitor K and the same concentration of 2.5 mg m<sup>-2</sup> that was used in tests of a cubicle housing system by Hagenkamp-Korth et al. (2015a) were used. The formulated inhibitor K was mixed with an amount of 50 ml m<sup>-2</sup> water as a carrier medium to ensure a consistent spraying pattern. The optimized synthesis process and urease inhibitor formulations with the necessary application and stability properties were developed by SKWP and are protected with patents (two registrations).

*Application technique*

In a previously conducted study (Hagenkamp-Korth et al., 2015a), a manually operated backpack sprayer was used to apply the inhibitor to the floor surface. In this study, the manual setup was replaced with an automatic backpack sprayer. The backpack sprayer was placed on a mod-

ified application system for increased flexibility (see Material and method, 2. *Application technique, Field investigations*; Figure 1). Two anti-drift nozzles were mounted onto a spray bar in the front. The manual speed was based on the length of the barn and the flow rate of the sprayer. In the three application days, application of the inhibitor took place during the morning milking after all cows had gone to the milking parlour. After every application, the tanks were checked for remaining liquids.

## Statistics

### *Data processing and statistical analysis*

#### *In time approach*

All statistical tests and graphs were performed using the open access software R (R Core Team, 2020). The basic approach was to estimate the reduction potential by using the in time approach to compare ammonia emissions before and after the application in both units and over all seasons. The application began early in the morning and the first application day still included the night-time hours before the inhibitor was applied. In order to not underestimate the reduction potential, the first day was excluded from the analysis of the in time approach. Therefore, the final dataset consisted of hourly ammonia emission values from the two days before the application and from the second and third application days of both units over all seasons. The data evaluation began with the definition of an appropriate statistical mixed model (Laird & Ware, 1982; Verbeke & Molenberghs, 2000). The model was as follows:

$$Y_{ijklmo} \sim a_i * b_j * c_k * d_l + e_m + (ef)_{mn} + r_{ijklmno}, \quad (1)$$

where

$Y$ : ammonia emissions ( $\text{g h}^{-1} \text{LU}^{-1}$ ),

and the fixed factors

$a_i$ : season (spring, summer, winter),

$b_j$ : measurement period (one, two),

$c_k$ : phase (reference phase, application phase),

$d_l$ : time (0, 1 ... 23 hour).

The “\*” means that the main effects, including all corresponding interaction terms, are covered.

The random factors are:



$e_m$ : barn (Unit A, Unit B),

$(ef)_{mn}$ : measurement unit (combination of phase, measurement period and season),

$r_{ijklmno}$ : residual error, correlated due to the time.

In addition, the index  $o$  represents the application day (two used days; day 1, day 2). Based on a graphical residual analysis, the residuals  $r_{ijklmno}$  were assumed to be normally distributed and to be heteroscedastic due to the different levels of season, measurement period and phase. Based on this model, a pseudo  $R^2$  was calculated (Nakagawa & Schielzeth, 2013) and an analysis of variances (ANOVA) was conducted, followed by a multiple contrast test (Bretz, Hothorn, & Westfall, 2011; Schaarschmidt, & Vaas, 2009) in order to compare the levels of phase. The subsequent reduction estimation percentage was based on the mean values of the reference and the mean values of the application phase.

#### *Case-control approach*

The dataset was based on the hourly ammonia emission values of the three reference days of one unit and on the hourly values of the three application days of the other unit since the emissions were measured in parallel for the case-control approach (see *Experimental design*, Table 1). In order to investigate the chronological reduction response, the days of the reference and application phase were divided into four-hour time slots. Each day consisted of six time slots (4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 24 hours). The hours were not accumulated and each time slot always consisted of four values, based on the four hours. A mean value was calculated for each time slot. The evaluation of the time slot dataset began with the definition of an appropriate statistical mixed model (Laird & Ware, 1982; Verbeke & Molenberghs, 2000). The statistical model was based on the following equation:

$$Y \sim a_i * b_j * c_k * d_l + e_m + (ef)_{mn} + (efa)_{mni} + r_{ijklmn}, \quad (2)$$

where

$Y$ : ammonia emissions ( $\text{g h}^{-1} \text{LU}^{-1}$ ),

and the fixed factors

$a_i$ : season (spring, summer, winter),

$b_j$ : phase (reference, application),

$c_k$ : day (day 1, day 2, day 3),

$d_t$ : time slot (4, 8, 12, 16, 20, 24 hours).

The “\*” means that main effects, including all corresponding interaction terms, are covered.

The random factors are:

$e_m$ : unit (Unit A, Unit B),

$f_n$ : measurement period per season (period 1, period 2, period 3),

$r_{ijklmn}$ : residual error, correlated due to time slot.

The residuals were assumed to be normally distributed and to be heteroscedastic with respect to the different levels of season. These assumptions are based on a graphical residual analysis. A pseudo  $R^2$  was calculated from this model (Nakagawa & Schielzeth, 2013) and an analysis of variances (ANOVA) was conducted, followed by multiple contrast tests (e.g., see Bretz et al., 2011, Schaarschmidt & Vaas, 2009) in order to compare the several levels of phase. Subsequently, percentage reductions per day and time slot were calculated based on the mean differences of the application and reference phases and the model estimates.

## Results

### *Side parameters*

The measured inside temperatures were in line with what was expected according to the annual seasons. The highest air temperatures were measured in summer and the lowest in winter (Table 2). No unusual temperatures were detected for the seasons. The ventilation rate had the highest values in summer and winter and the lowest values during spring (Table 2). The ventilation rate was comparable to naturally ventilated barns. As expected, the relative humidity was highest during the winter period.

**Table 2** Side parameters (air temperature, ventilation rate, relative humidity) of the in time setup in both units and over all seasons.

Season	Unit	Minimum (°C)	Mean (°C)	Maximum (°C)	Ventilation rate (per animal $\text{m}^3 \text{h}^{-1}$ )	Relative humidity (%)
Spring	A	5.2	12.1	20.4	11,352 (710)	83.4
	B	4.7	12.0	19.5	11,060 (691)	85.2
Summer	A	13.6	19.6	26.2	17,355 (1085)	88.4
	B	13.5	19.7	26.1	16,469 (1029)	87.2
Winter	A	0.4	7.9	14.3	16,166 (1010)	97.3
	B	0.5	8.1	14.4	15,439 (965)	98.0

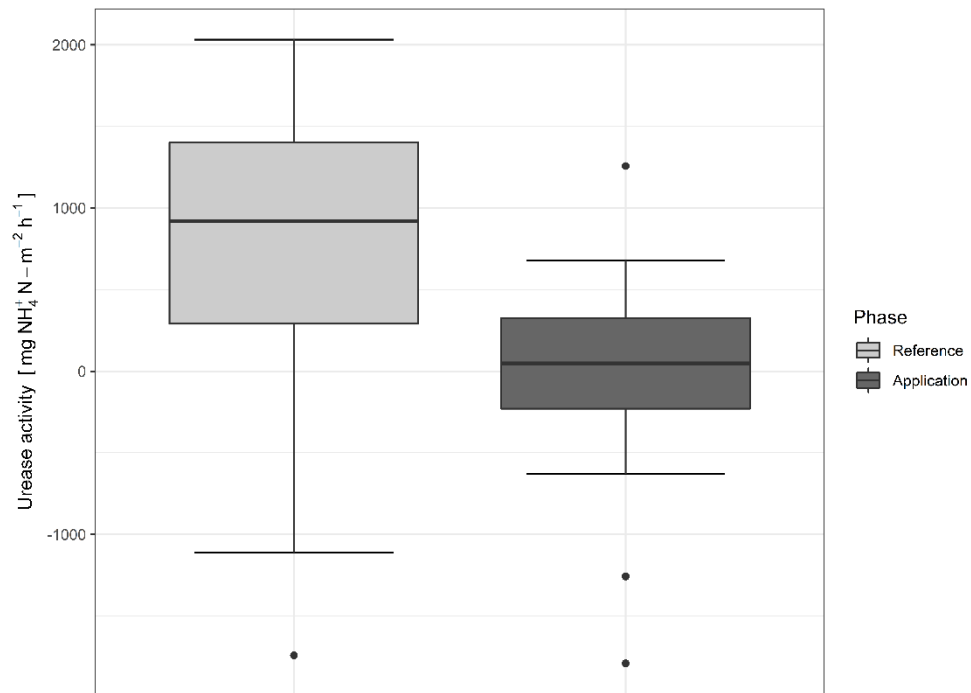
The feed and liquid manure samples were frozen and shipped to a laboratory in order to be analysed for the additional side parameters. Feed composition influences ammonia emissions. However, the feeding of the cows in this study (Table 3) was in line with the guidelines recommended by DLG (2020) and Kirchgeßner (2014). Average dairy cow liquid manure has nitrogen content of 3.7 kg m<sup>-3</sup> and an NH<sub>4</sub>-N content of 1.7 kg m<sup>-3</sup> (Landwirtschaftskammer Niedersachsen, 2018). The nitrogen content in this study was slightly higher, but the NH<sub>4</sub>-N content was comparable to the average content. The slightly higher nitrogen content might be also due to the collection method of the liquid manure. The liquid manure was collected using a small pipe which was inserted between the slats. Some slats were closer together, which might have meant the liquid manure collector didn't function correctly. Multiple samples per unit were collected and mixed before taking the final sample.

**Table 3** Overview of the analysed feed and liquid manure samples.

Parameter	Unit A			Unit B			
	Winter	Spring	Summer	Winter	Spring	Summer	
Feed	Dry matter (g kg <sup>-1</sup> )	326.4	274.1	376.9	391.4	301.3	343.2
	Crude protein (g kg <sup>-1</sup> )	156.7	142.2	156.4	154.9	137	162.4
	Net energy (MJ kg <sup>-1</sup> DM <sup>-1</sup> )	7.06	6.28	6.93	7.01	6.54	6.91
	Dry matter (%)	12.3	10.5	10.8	11.0	10.3	11.5
Manure	Nitrogen (kg m <sup>-3</sup> )	4.8	4.5	4.6	4.3	4.1	4.8
	NH <sub>4</sub> -N (kg m <sup>-3</sup> )	1.9	2.1	1.9	1.9	1.8	1.8

### *Urease activity*

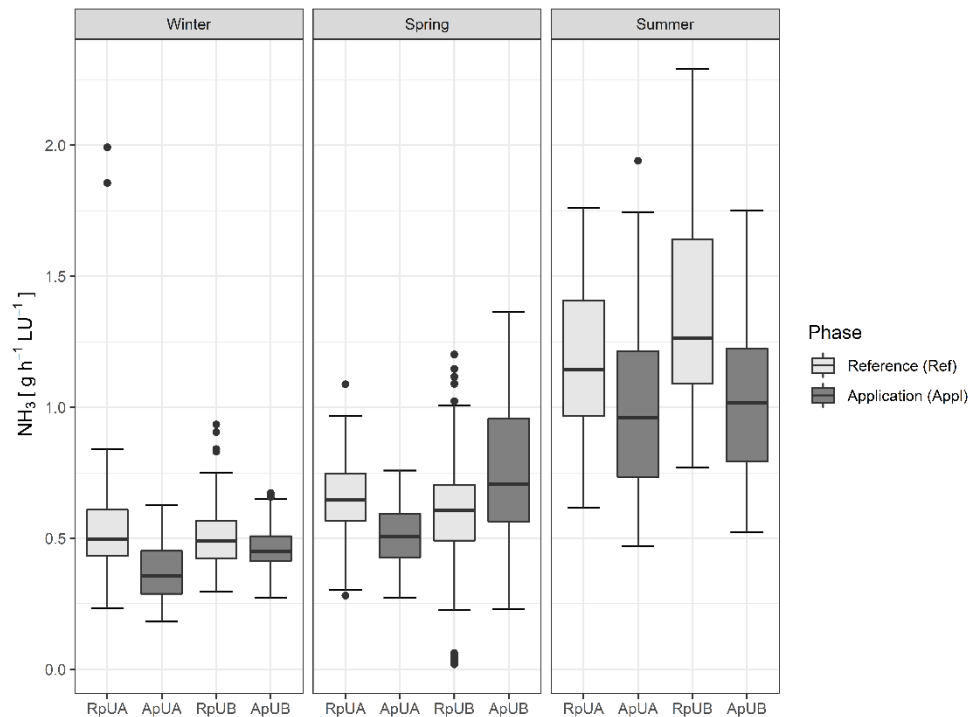
Urease activity was measured as an additional back-up method to verify the general inhibiting effect of the inhibitor. The minimum-maximum values of the reference phase ranged between -1741 mg NH<sub>4</sub><sup>+</sup>-N m<sup>-2</sup> h<sup>-1</sup> and 2032 mg NH<sub>4</sub><sup>+</sup>-N m<sup>-2</sup> h<sup>-1</sup> and the minimum-maximum values of the application phase varied between -1790 mg NH<sub>4</sub><sup>+</sup>-N m<sup>-2</sup> h<sup>-1</sup> and 1258 mg NH<sub>4</sub><sup>+</sup>-N m<sup>-2</sup> h<sup>-1</sup>. A clear decrease in urease activity after inhibitor application could be detected (Figure 1). A urease activity median of 919 mg NH<sub>4</sub><sup>+</sup>-N m<sup>-2</sup> h<sup>-1</sup> was estimated before the inhibitor was applied and a median of 48 mg NH<sub>4</sub><sup>+</sup>-N m<sup>-2</sup> h<sup>-1</sup> was observed after the inhibitor application. A reduction of 69 % in urease activity was calculated for both units and all seasons.



**Figure 1** Urease activity of the reference (n=33) and application (n=34) phases of both units over all seasons.

### *In time approach*

The ammonia emissions were estimated according to the experimental setup (Table 1) over all seasons in both units. A seasonal effect on the ammonia emissions was observed (Figure 2). The lowest ammonia emissions of  $0.02 \text{ g h}^{-1} \text{ LU}^{-1}$  were measured in Unit B during the application phase in winter and the highest ammonia emissions of  $2.29 \text{ g h}^{-1} \text{ LU}^{-1}$  in Unit B during the reference phase in summer. In contrast with the clear difference between the reference and the application phase in summer and winter, the spring measurement of Unit B showed higher ammonia emission values after the inhibitor was applied. A very likely reason for this was an unscheduled and urgent liquid manure removal in the underneath pits. The liquid manure was mixed and then pumped into a tank, and during this time, the curtains and doors were opened to minimize the exposure of the animals to the gases generated from the liquid manure pit.



**Figure 2** Measured ammonia emissions in Unit A and Unit B before (reference phase) and after the application of the inhibitor (application phase) in winter, spring and summer. RpUA = Reference phase, Unit A; ApUA = Application phase, Unit A; RpUB = Reference phase, Unit B; ApUB = Application phase, Unit B; All phases: n=96.

The model (see *Statistics; In time approach*) found a significant difference of  $0.12 \text{ g h}^{-1} \text{ LU}^{-1}$  ( $p$ -value  $< 0.01$ ) between the reference and application phase (pooled over the remaining parameters of season, units and measurement periods). However, the model referred to absolute ammonia emissions, while relative reduction displayed a quite different picture. The relative reductions registered different values in each season. The highest reduction potential of 31 % was observed in winter (Unit A) and the lowest in winter 10 % (Unit B) (Table 4). The negative value of -30 % during spring in Unit B was based on the urgent liquid manure removal and is therefore excluded from any interpretations.

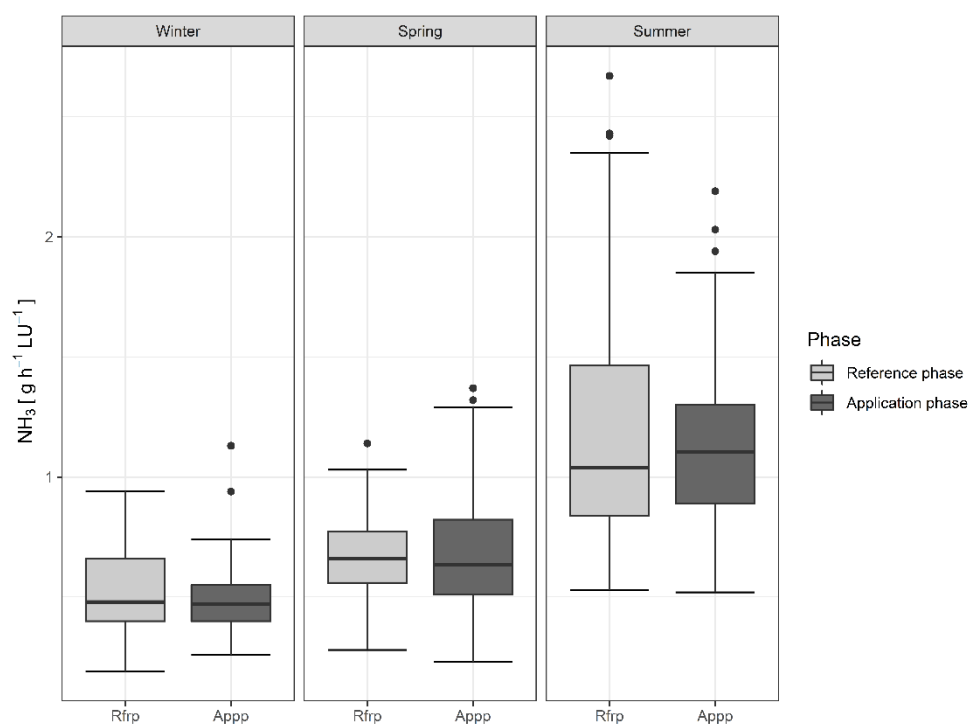
**Table 4** Estimated reduction potential of ammonia emissions in Unit A and Unit B for all seasons

Season	Reduction [%]	
	Unit A	Unit B
Winter	31	10
Spring	22	(-30)*
Summer	17	23

\*unfeasible data due to liquid manure removal

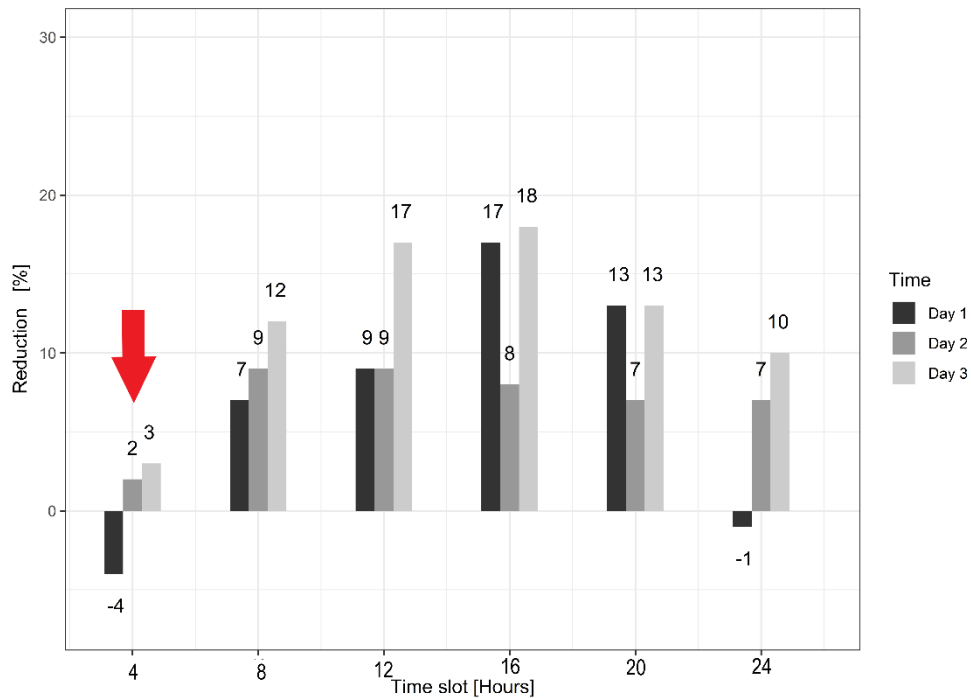
### Case-control approach

The case-control approach was used to investigate the chronological reduction response of the inhibitor. The highest absolute emission values of  $2.67 \text{ g h}^{-1} \text{ LU}^{-1}$  were measured in the reference phase in summer and the lowest values of  $0.19 \text{ g h}^{-1} \text{ LU}^{-1}$  in the reference phase during winter (Figure 3). These values were in agreement with the temperature dependence of ammonia emissions. The boxplots for spring were influenced by the urgent liquid manure removal and should therefore be interpreted with caution.



**Figure 3** Boxplot overview of the ammonia emission values in the reference (n=216; only summer n=205) and application (n=216) phases for each season. Rfrp = Reference phase; App= Application phase.

The ANOVA output found a significant interaction between the timeslot parameter and phase (p-value 0.0076). In addition, the contrast test found no significant differences between the reference and application phase over the three application days. The highest estimate of  $0.58 \text{ g h}^{-1} \text{ LU}^{-1}$  was found on the third day during the 20-hour timeslot.



**Figure 4** Overview of the estimated reduction potential (all values in %) of the different time slots over the three application days for all seasons and both units. The red arrow represents the time slot in which the application was made.

Based on the model used, the reduction potential was estimated using the difference between the reference and application phases in the six time slots (4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 24 hours). The reduction potential of every application day was estimated (Figure 4). The highest difference was found between the first and third application day. In general, the reduction potential increases over all application days until the 16-hour time slot and decreases in the 20-hour time slot. The lowest reduction potential was observed in the 4-hour time slot, and this was the time slot when the inhibitor was applied. The first day registered a negative reduction at the 24-hour time slot. This value is likely to be a function of the hours between midnight and the application time in the early morning. During this time, no inhibitor was applied, meaning this is a pre-application value. Day 2 and day 3 of the application phase had small reductions at the 24-hour time slot, probably caused by the declining inhibitor effect.

## Discussion

### *Housing system*

Although the test facility at the Dairy Campus was specially designed for emissions measurements, no special test circumstances were implemented and the milking and feeding of the cows was similar to conventional dairy barns. Both units were equipped with a mechanical ventilation system. This is in contrast to the current most common freestall housing systems (naturally ventilated) for dairy cows (Tergast, Schickkramm, Lindena, Ellßel, & Hansen, 2019). The ventilation rate of 688–1062 m<sup>3</sup> h<sup>-1</sup> per cow (Table 2), however, lies well between minimum and maximum ventilation rates of naturally ventilated barns measured in practice (Mosquera, Hol, Huis in 't Veld, Ploegaert, & Ogink, 2012). However, a disadvantage of the units used was the fact that during the feeding and milking times, the curtains and doors of both units were opened for approximately four hours (two hours per milking). The air inside the units could become mixed between the units and with fresh air from outside. Air entering the units could result in higher pit ventilation and could have an impact on the ammonia emissions measured. Furthermore, the exchange with the outside air could also result in an underestimation of the ammonia emissions. However, such occurrences happened simultaneously in both units to the same extent and this unit effect was taken into account in the models of the in time approach and the case-control approach.

### *Urease inhibitor and application technique*

Compared to a previous study (Hagenkamp-Korth et al., 2015a), the inhibitor type K in this study was supplied by SKWP in a liquid form. This resulted in a simplified handling. However, the newly added formulation solidified below the threshold temperature of 5 °C because of a low fusion point of the solvent. Although this solid form could be liquified using a water bath, field condition temperatures below this point are common during winter. Therefore, the formulation of the inhibitor needs to be improved. However, this occurrence was well known, but in order to be consistent and therefore, comparable to previous studies, the same inhibitor was used.

The application technique in this study was based on a previous investigation by Hagenkamp-Korth et al. (2015a). The automatic backpack sprayer delivered an equal pressure and an even spraying pattern of the inhibitor-water solution. During the application process, low temperatures meant that small particles blocked the nozzles. However, these occurrences were considered to have no effect on the reduction potential because this inadequate spraying and the blocked nozzles were followed up by an immediate cleaning. Due to technical problems, a small



amount of inhibitor in combination with the solvent couldn't be applied on one application day of the summer measurements. However, no influence on the inhibitor effect was observed, for e.g. with inexplicable values.

#### *Urease activity measurement*

The measurement of urease activity is an indirect method to confirm the inhibitor effect. Previous research using different urease inhibitors also measured the urease activity in order to investigate the mitigation potential (Hagenkamp-Korth et al., 2015a; Leinker, 2007). Leinker (2007) measured urease activity before and after the application of a urease inhibitor (type D). The inhibitor was applied to a concrete surface that was previously fouled with cattle manure. Mean values during the reference phase of  $1052 \text{ mg NH}_4^+\text{-N m}^{-2} \text{ h}^{-1}$  were found with a 90 % reduction in urease activity 19 hours after the application. Hagenkamp-Korth et al. (2015a) used the same inhibitor K under practical conditions in a cubicle housing system. During the reference phase the urease activity ranged between  $704 \text{ mg NH}_4^+\text{-N m}^{-2} \text{ h}^{-1}$  and  $2402 \text{ mg NH}_4^+\text{-N m}^{-2} \text{ h}^{-1}$ . A reduction potential of between 66 % and 96 % was observed. In the current study, a median of  $919 \text{ mg NH}_4^+\text{-N m}^{-2} \text{ h}^{-1}$  was registered and a reduction potential of 69 % was found in the reference phase. However, the lowest values were detected in the application phase. Values of  $48 \text{ mg NH}_4^+\text{-N m}^{-2} \text{ h}^{-1}$  were found in this study compared to  $36 \text{ mg NH}_4^+\text{-N m}^{-2} \text{ h}^{-1}$  in Hagenkamp-Korth et al. (2015a). Leinker (2007) detected values of around  $106 \text{ mg NH}_4^+\text{-N m}^{-2} \text{ h}^{-1}$  19 hours after inhibitor application. Negative values were also observed by Hagenkamp-Korth et al. (2015) and Leinker (2007). The sensitive testing method, the accuracy of the photometer test tubes used and the sampling process might offer an explanation for this occurrence. The different distribution of urease activity and the different reduction values between this study and the investigations by Hagenkamp-Korth et al. (2015a) and Leinker (2007) could be a result of the different experimental setups, the sensitive measuring method and the different sample size number.

#### *Measured ammonia emissions from the in time and case-control approaches*

The two experimental designs were supplied by the VERA test protocol (International VERA Secretariat, 2018). No comparison was made between the two approaches, since two different reduction estimations were performed. The mean values of the reference phase of the in time approach between the units varied between  $1.18\text{--}1.34 \text{ g h}^{-1} \text{ LU}^{-1}$  in summer,  $0.59\text{--}0.65 \text{ g h}^{-1} \text{ LU}^{-1}$  in spring and  $0.51\text{--}0.54 \text{ g h}^{-1} \text{ LU}^{-1}$  in winter. The mean values of the reference phase in the case-control approach varied between  $1.19 \text{ g h}^{-1} \text{ LU}^{-1}$  in summer,  $0.67 \text{ g h}^{-1} \text{ LU}^{-1}$  in spring and  $0.52 \text{ g h}^{-1} \text{ LU}^{-1}$  in winter. The ammonia emissions in both the in time and case-control

approaches were measured in the same units using the same measurement equipment. Therefore, equal ammonia emission values were expected (Figure 2 and Figure 3). However, in order to verify the estimated reduction potential, the measured ammonia emissions were compared to values of two naturally ventilated farms. A study conducted by Bobrowski et al. (under review**b**) measured ammonia emissions in two naturally ventilated dairy farms with solid floors. The mean values of the two farms, ranged between 0.96–2.12 g h<sup>-1</sup> LU<sup>-1</sup> in summer, 0.68–2.63 g h<sup>-1</sup> LU<sup>-1</sup> in the transition period (spring/autumn) and between 0.57–0.71 g h<sup>-1</sup> LU<sup>-1</sup> in winter. A seasonal influence on emissions was observed in both the mechanically ventilated units and the two naturally ventilated farms, as shown by emission levels being lowest in winter. The emission values were, in general, higher in the two naturally ventilated barns. The different levels of the ammonia emissions could be explained by Zhang et al. (2005), who investigated the emissions on different floor types and manure systems. Higher emissions were found in a building with a solid floor and scraper compared to a building with a slatted floor and a robotic scraper. The two naturally ventilated barns in Germany consisted mainly of solid floor surfaces. This observation could be one reason for the lower emission values of the units with slatted floors compared to the farms with mainly solid floors.

#### *Reductions in the in time approach*

The dataset was selected based on pretests (not published). Of the three application days, only the second and third days were used. The application took place early in the morning, meaning that the few hours before the first application point were considered part of the first application day. The reduction potential, however, would be underestimated if those hours before the application time were taken into account in the calculation. Another option relating to data selection was to exclude the feeding and milking times, which comprised approximately four hours per day. During this time, the doors and curtains were opened, resulting in the inside and outside air mixing. However, it was observed that this would have a negligible impact on the reduction. In addition, the feeding and milking times also differed and the closing of the doors/curtains after two hours couldn't be always confirmed. Another main reason for the final data selection was so that the results could be compared to a previous in time investigation by Bobrowski et al. (under review**b**). The reduction of the in time approach by Bobrowski et al. (under review**b**) was estimated using the mean ammonia emission values before and after the inhibitor application. However, in this study a reduction potential for each unit could be calculated. Unit A had a reduction potential of 17 % in summer, 22 % in spring and 31 % in winter. For Unit B, a reduction of 23 % in summer, -32 % in spring and 10 % in winter was observed. The liquid

manure removal during the spring measurement resulted in higher values during the application phase (Figure 3) and caused a negative reduction in Unit B. Bobrowski et al. (under review**b**) estimated the reduction in two naturally ventilated dairy farms. Here, ammonia emissions were reduced by 65 % and 68 % in winter, 64 % and 53 % in the transition period and 40 % and 54 % in summer on Farm A and Farm B, respectively. The generally lower reductions in this study can be explained by the floor surface design. In contrast to the solid floor on the two farms, the units were equipped with slatted floors with liquid manure pits underneath. A possible explanation might be a lack of inhibitor on the slatted floor. Also, the slatted floor provides more surface compared to a solid floor surface. The source of ammonia emissions extends to the sides and bottom of the slats as well as to the whole liquid manure pit. However, despite the different reduction levels in the two studies, the same observation was made that the highest reduction potential was found in the winter measurement and the lowest in the summer measurement. During winter, emissions are already at a lower level and during the summer, multiple sources contribute to higher emission levels (*see* Figure 2 and Figure 3).

#### *Emissions reduction in the case-control approach*

The case-control approach was used to investigate the chronological reduction response to the inhibitor at different time slots after the application. The three application days were divided into four-hour time slots (4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 24 hours). Four measurements per season were originally scheduled in the experimental setup (two measurement periods/unit). Due to the overlapping of the residual inhibitor effects, only three measurement periods per season could be used in the calculations. In general, reduction potential increased and peaked (18 %) at the 16-hour time slot, followed by a decrease (Figure 4). The application took place early in the morning, and the negative values of the 24-hour time slot (day 1) result from the time before the application took place. The inhibitor was applied in the 4-hour time slot, thus the negative values from the 4-hour time slot on day 1 are due to the open doors/curtains and the fact that the inhibitor had not yet been applied. The reduction values from the 4-hour and 24-hour time slots of day 2 and day 3 were probably caused by residual inhibitor effects (Figure 4). A residual effect after the peak was also observed in Bobrowski et al. (under review**a**). However, the reduction potential increased over time on all application days (days 1–3). A pattern of the chronological reduction response of the inhibitor was observed. The highest reductions were found between the 12-hour and 16-hour time slots. Bobrowski et al. (under review**a**) investigated the chronological reduction response of the inhibitor K in respiration

chambers using the same time slot analysis. The inhibitor was applied once in during the experiment and an increase in reduction of up to 37 % until the 12-hour time slot was observed. At the 20-hour and 24-hour time slots, a clear decrease in reductions was observed. These observations confirm the assumption that the inhibitor releases its full potential over time. However, in the respiration chambers, the reduction potential was based on a fixed application time (point “0”) and the reduction was calculated using previously measured ammonia values with no inhibitor effect (Bobrowski et al., under review<sup>a</sup>). In contrast, this study used a case-control approach and included the application point in the different time slots since the application took place within a certain time frame. This time frame was dependent on the management structure of the location, since the application had to take place in an empty unit.

### Conclusion

This study used two different approaches (in time and case-control) in order to investigate the reduction potential and the chronological reduction response of a urease inhibitor on the ammonia emissions in a mechanically ventilated dairy housing system. The advantage of the in time approach could be the calculation of the reduction potential for each season, even though only two application days could be used. Future studies could focus on a selection based on hourly values, in order to provide additional data. The advantage of the case-control approach is a direct comparison between the two units and its ability to use all application days. However, even though the units had the same structure, the emission levels differed slightly. Also, due to an overlapping experimental schedule, only three measurement periods instead of four could be used per season.

However, since the experiments were conducted in accordance with the VERA test protocol, it will be possible to compare the results with upcoming studies and extend the repeatability. The VERA protocol, however, is only used in a limited number of countries. The mitigation effect of the inhibitor K could be confirmed by a 69 % reduction in urease activity. In order to classify the ammonia emissions measured in both setups, they were compared with the ammonia values measured in a naturally ventilated barn. Reductions for the in time approach ranged between 10 % (Unit B, winter) and 31 % (Unit A, winter). The case-control approach found a similar reduction pattern when compared with a previous study. The highest reduction was recorded on the second application day and it was observed that the inhibitor reached a reduction peak of 18 % at 16 hours before decreasing again. However, a residual effect of the inhibitor was still observed at the 24-hour time slot. These observations will be valuable for further investigations relating to the frequency of inhibitor application. Furthermore, a connection between the daily

patterns of the animals' behaviour and the time slot reduction should be examined in order to investigate a connection between the timely response and feeding, night times or milking times. Moreover, the dataset used could be improved by using hourly values to include the first application day in the in time approach. The case-control dataset could be improved by fixing the application time at time "0" and creating the different time slots after this. In doing so, the investigation of the chronological response of the inhibitor could be refined. Upcoming studies should also focus on the application procedure which should preferably rely on a fully automatic system. Unnecessary extra investments could be avoided by using already existing farm equipment in the future application technique. A scraping robot with nozzles could carry a tank with the inhibitor-water solution.

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#### Declarations of interest

None.

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#### References

- Bobrowski, A. B., Derno, M., Kuhla, B., Hagenkamp-Korth, F., Hasler, M., & Hartung, E. (under review<sup>a</sup>). Investigating the chronological reduction potential of a urease inhibitor in respiration chambers. *Biosystems Engineering*.
- Bobrowski, B. A., Willink, D., Janke, D., Amon, T., Hagenkamp-Korth, F., Hasler, M., & Hartung, E. (under review<sup>b</sup>). Reduction of ammonia emissions by applying a urease inhibitor in naturally ventilated dairy barns. *Biosystems Engineering*.
- Braam, C. R., & Swierstra, D. (1999). Volatilization of Ammonia from Dairy Housing Floors with Different Surface Characteristics. *Journal of Agricultural Engineering Research*. (72), 59–69. <https://doi.org/10.1006/jaer.1998.0345>

- Bretz, F., Hothorn, T., & Westfall, P. (2011). *Multiple Comparisons Using R*. London: Chapman and Hall/CRC. ISBN 9781584885740.
- Cole, N. A., Clark, R. N., Todd, R. W., Richardson, C. R., Gueye, A., Greene, L. W., & McBride, K. (2005). Influence of dietary crude protein concentration and source on potential ammonia emissions from beef cattle manure. *Journal of Animal Science*, 83(3), 722–731. <https://doi.org/10.2527/2005.833722x>
- Dairy Campus (2020). Environment and measuring house. Retrieved August 12, 2020, from <https://www.dairycampus.nl/en/Home/Research/Research-facilities/8.-Environment-and-measuring-house.htm>
- DLG [Deutsche Landwirtschafts-Gesellschaft] (2020). Berücksichtigung N- und P-reduzierter Fütterungsverfahren bei den Nährstoff - ausscheidungen von Milchkühen. DLG-Merkblatt 444. Retrieved July 03, 2020, from [https://www.dlg.org/fileadmin/downloads/landwirtschaft/themen/publikationen/merkblaetter/dlg-merkblatt\\_444.pdf](https://www.dlg.org/fileadmin/downloads/landwirtschaft/themen/publikationen/merkblaetter/dlg-merkblatt_444.pdf)
- Draaijers, G.P.J., Ivens, W.P.M.F., Bos, M. M., & Bleuten, W. (1989). The contribution of ammonia emissions from agriculture to the deposition of acidifying and eutrophying compounds onto forests. *Environmental Pollution*, 60(1-2), 55–66. [https://doi.org/10.1016/0269-7491\(89\)90220-0](https://doi.org/10.1016/0269-7491(89)90220-0)
- EC [European Commission]. (2016). Directive (EU) 2016/2284 of the European Parliament and of the Council of 14 December 2016 on the reduction of national emissions of certain atmospheric pollutants, amending Directive 2003/35/EC and repealing Directive 2001/81/EC. *OJL*, 344, 1-31. Retrieved August 28, 2018 from <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L:2016:344:FULL&from=EN>
- Erisman, J. W., Bleeker, A., Hensen, A., & Vermeulen, A. (2008). Agricultural air quality in Europe and the future perspectives. *Atmospheric Environment*, 42(14), 3209–3217. <https://doi.org/10.1016/j.atmosenv.2007.04.004>
- Gauderman, W. J., Avol, E., Gilliland, F., Vora, H., Thomas, D., Berhane, K., . . . Peters, J. (2004). The Effect of Air Pollution on Lung Development from 10 to 18 Years of Age. *The New England Journal of Medicine*. (Vol. 351; No. 11), 1057–1067. <https://doi.org/10.1056/NEJMoa040610>
- Guthrie, S., Giles, S., Dunkerley, F., Tabaqchali, H., Harshfield, A., Ioppolo, B., & Manville, C. (2018). The impact of ammonia emissions from agriculture on biodiversity: An evidence synthesis. *RAND Corporation and The Royal Society, Cambridge, UK*. Retrieved April 04,

- 2019, from <https://royalsociety.org/-/media/policy/projects/evidence-synthesis/Ammonia/Ammonia-report.pdf>
- Haenel, H. D., Rösemann, C., Dämmgen, U., Döring, U., Wulf, S., Eurich-Menden, B., ... & Fuß, R. (2020). *Calculations of gaseous and particulate emissions from German agriculture 1990-2018: Report on methods and data (RMD) Submission 2020* (No. 77). Thünen Report. <https://doi.org/10.3220/REP1584363708000>
- Hagenkamp-Korth, F., Haeussermann, A., & Hartung, E. (2015a). Effect of urease inhibitor application on urease activity in three different cubicle housing systems under practical conditions. *Agriculture, Ecosystems & Environment*, *202*, 168–177. <https://doi.org/10.1016/j.agee.2015.01.010>
- Hagenkamp-Korth, F., Haeussermann, A., Hartung, E., & Reinhardt-Hanisch, A. (2015b). Reduction of ammonia emissions from dairy manure using novel urease inhibitor formulations under laboratory conditions. *Biosystems Engineering*, *130*, 43–51. <https://doi.org/10.1016/j.biosystemseng.2014.12.002>
- Hagenkamp-Korth, F., Ohl, S., & Hartung, E. (2015c). Effects on the biogas and methane production of cattle manure treated with urease inhibitor. *Biomass and Bioenergy*, *75*, 75–82. <https://doi.org/10.1016/j.biombioe.2015.02.014>
- International VERA Secretariat (2018). VERA test protocol for Livestock Housing and Management Systems: Version 3:2018-09, 1–60. Retrieved January 21, 2019 from [https://www.vera-verification.eu/app/uploads/sites/9/2019/05/VERA\\_Testprotocol\\_Housing\\_v3\\_2018.pdf](https://www.vera-verification.eu/app/uploads/sites/9/2019/05/VERA_Testprotocol_Housing_v3_2018.pdf)
- Kirchgeßner, M. (2014). *Tierernährung: Leitfaden für Studium, Beratung und Praxis*, 14. Auflage. DLG Verlag. Frankfurt am Main.
- Kroodsma, W., Huis in 't Veld, J.W.H., & Scholtens, R. (1993). Ammonia emission and its reduction from cubicle houses by flushing. *Livestock Production Science*, *35*(3-4), 293–302. [https://doi.org/10.1016/0301-6226\(93\)90099-4](https://doi.org/10.1016/0301-6226(93)90099-4)
- Laird, N. M., & Ware, J. H. (1982). Random-Effects Models for Longitudinal Data. *Biometrics*, *38*(4), 963–974.
- Landwirtschaftskammer Niedersachsen (2018). Nährstoffgehalte in organischen Düngern. Retrieved May 20, 2020, from <https://www.lwk-niedersachsen.de/index.cfm/portal/96/nav/2280/article/32460.html>

- Leinker, M. (2007). *Entwicklung einer Prinziplösung zur Senkung von Ammoniakemissionen aus Nutztierställen mit Hilfe von Ureaseinhibitoren*. Ph.D. Thesis, Martin-Luther-University, Germany: Forschungsbericht Agrartechnik VDI-MEG Nr. 462.
- Mendes, L. B., Pieters, J. G., Snoek, D., Ogink, N. W. M., Brusselman, E., & Demeyer, P. (2017). Reduction of ammonia emissions from dairy cattle cubicle houses via improved management- or design-based strategies: A modeling approach. *The Science of the Total Environment*, 574, 520–531. <https://doi.org/10.1016/j.scitotenv.2016.09.079>
- Metz, J. H. M., Ogink, N. W. M., & Smits, M. C. J. (1995). Research on housing systems and manure treatment to reduce ammonia emission in dairy husbandry. *Applied Research for sustainable dairy farming, Research Station for Cattle, Sheep and Horse husbandry (PR), Lelystad*, 36-39.
- Monteny, G.-J., Bannink, A., & Chadwick, D. (2006). Greenhouse gas abatement strategies for animal husbandry. *Agriculture, Ecosystems & Environment*, 112(2-3), 163–170. <https://doi.org/10.1016/j.agee.2005.08.015>
- Mosquera, J., Hol, J.M.G., Huis in 't Veld, J.W.H., Ploegaert, J.P.M., & Ogink, N.W.M. (2012). Emissies uit een ligboxenstal voor melkvee met het "vrije keuze" systeem. Meetprogramma Integraal Duurzame Stallen: Wageningen UR Livestock Research, 13.
- Nakagawa, S. and Schielzeth, H. (2013). A General and Simple Method for Obtaining R2 from Generalized Linear Mixed-Effects Models. Edited by Robert B. O'Hara. *Methods in Ecology and Evolution* 4(2), 133-42. <https://doi.org/10.1111/j.2041-210x.2012.00261.x>
- Ndegwa, P. M., Hristov, A. N., Arogo, J., & Sheffield, R. E. (2008). A review of ammonia emission mitigation techniques for concentrated animal feeding operations. *Biosystems Engineering*, 100(4), 453–469. <https://doi.org/10.1016/j.biosystemseng.2008.05.010>
- Ogink, N. W. M., & Kroodsma, W. (1996). Reduction of Ammonia Emission from a Cow Cubicle House by Flushing with Water or a Formalin Solution. *Journal of Agricultural Engineering Research*. (63), 197–204. <https://doi.org/10.1006/jaer.1996.0021>
- Parker, D. B., Pandrangi, S., Greene, L. W., Almas, L. K., Cole, N. A., Rhoades, M. B., & Koziel, J. A. (2005). Rate And Frequency Of Urease Inhibitor Application For Minimizing Ammonia Emissions From Beef Cattle Feedyards. *Transactions of the ASAE*, 48(2), 787–793. <https://doi.org/10.13031/2013.18321>
- Parker, D. B., Rhoades, M. B., Baek, B. H., Koziel, J. A., Waldrip H. M., & Todd, R. W. (2016). Urease Inhibitor for Reducing Ammonia Emissions from an Open-Lot Beef Cattle Feedyard



- in the Texas High Plains. *Applied Engineering in Agriculture*, 32(6), 823–832. <https://doi.org/10.13031/aea.32.11897>
- Parker, D. B., Rhoades, M. B., Cole, N. A., & Sambana, V. P. (2012). Effect of Urease Inhibitor Application Rate and Rainfall on Ammonia Emissions from Beef Manure. *American Society of Agricultural and Biological Engineers*. (55), 211–218. <https://doi.org/10.13031/2013.41248>)
- Phillips, V. R., Holden, M., R., Sneath, R., W., Short, J., L., White, R., P., Hartung, J., . . . Wathes, C., M. (1998). The Development of Robust Methods for Measuring Concentrations and Emission Rates of Gaseous and Particulate Air Pollutants in Livestock Buildings. *Journal of Agricultural Engineering Research*, (70,), 11–24. <https://doi.org/10.1006/jaer.1997.0283>
- Phillips, V. R., Lee, D. S., Scholtens, R., Garland, J. A., & Sneath, R. W. (2001). A Review of Methods for measuring Emission Rates of Ammonia from Livestock Buildings and Slurry or Manure Stores, Part 2: monitoring Flux Rates, Concentrations and Airflow Rates. *Journal of Agricultural Engineering Research*, 78(1), 1–14. <https://doi.org/10.1006/jaer.2000.0618>
- R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from <https://www.R-project.org/>
- Reinhardt-Hanisch, A. (2008). *Grundlagenuntersuchungen zur Wirkung neuartiger Ureaseinhibitoren in der Nutztierhaltung*. Ph.D. Thesis, University of Hohenheim, Germany: Forschungsbericht Agrartechnik VDI-MEG Nr. 471.
- Schaarschmidt, F., & Vaas, L. (2009). Analysis of Trials with Complex Treatment Structure Using Multiple Contrast Tests. *Hortscience*. 44(1), 188–195. <https://doi.org/10.21273/HORTSCI.44.1.188>
- Shi, Y., Parker, D. B., Cole, N. A., Auvermann, B. W., & Mehlhorn, J. E. (2001). Surface amendments to minimize ammonia emissions from beef cattle feedlots. *American Society of Agricultural Engineers*. (Vol. 44 (3)), 677–682. <https://doi.org/10.13031/2013.6105>
- Smits, M.C.J., Valk, H., Elzing, A., & Keen, A. (1995). Effect of protein nutrition on ammonia emission from a cubicle house for dairy cattle. *Livestock Production Science*, 44(2), 147–156. [https://doi.org/10.1016/0301-6226\(95\)00068-6](https://doi.org/10.1016/0301-6226(95)00068-6)
- Snoek J. W., Haesen G. P. M. J., Groot Koerkamp P. W. G., & Monteny G. J. (2010). Effect of floor design in a dairy cow house on ammonia emission - Design, test and preliminary results

- with an experimental set-up for run off experiments. *International Conference on Agricultural Engineering, Cemagref, Clermont-Ferrand*. p. 1-10.  
<https://doi.org/10.13140/RG.2.1.1957.0168>
- Stickstoffwerke Piesteritz GmbH (2020). Retrieved June 14, 2020, from <https://www.skwp.de/en/company/>
- Swierstra, D., Braam, C. R., & Smits, M. C. (2001). Grooved Floor System For Cattle Housing: Ammonia Emission Reduction And Good Slip Resistance. *Applied Engineering in Agriculture*. (17), 85–90. <https://doi.org/10.13031/2013.1929>
- Tergast, H., Schickramm, L., Lindena, T., Ellßel, R., & Hansen, H. (2019) Steckbriefe zur Tierhaltung in Deutschland: Milchkühe, 1–17. Retrieved August 24, 2020 from [https://literatur.thuenen.de/digbib\\_extern/dn061460.pdf](https://literatur.thuenen.de/digbib_extern/dn061460.pdf)
- UBA [Umweltbundesamt] (2018). Ammoniak-Emissionen. Umweltbundesamt. Retrieved January 22, 2019, from <https://www.umweltbundesamt.de/daten/luft/luftschadstoff-emissionen-in-deutschland/ammoniak-emissionen#textpart-1>
- Varel, V. H., Nienaber, J. A., & Freetly, H. C. (1999). Conservation of nitrogen in cattle feedlot waste with urease inhibitors. *Journal of Animal Science*, 77(5), 1162. <https://doi.org/10.2527/1999.7751162x>
- Varel, V. H. (1997). Use of urease inhibitors to control nitrogen loss from livestock waste. *Bioresource Technology*, 62(1-2), 11–17. [https://doi.org/10.1016/S0960-8524\(97\)00130-2](https://doi.org/10.1016/S0960-8524(97)00130-2)
- Verbeke, G., & Molenberghs, G. (2000). *Linear Mixed Models for Longitudinal Data*. New York: Springer. [https://doi.org/10.1007/978-0-387-22775-7\\_3](https://doi.org/10.1007/978-0-387-22775-7_3)
- Webb, J., Menzi, H., Pain, B. F., Misselbrook, T. H., Dämmgen, U., Hendriks, H., & Döhler, H. (2005). Managing ammonia emissions from livestock production in Europe. *Environmental Pollution (Barking, Essex : 1987)*, 135(3), 399–406. <https://doi.org/10.1016/j.envpol.2004.11.013>
- Zhang, G., Strøm, J. S., Li, B., Rom, H. B., Morsing, S., Dahl, P., & Wang, C. (2005). Emission of Ammonia and Other Contaminant Gases from Naturally Ventilated Dairy Cattle Buildings. *Biosystems Engineering*, 92(3), 355–364. <https://doi.org/10.1016/j.biosystemseng.2005.08.002>

## Chapter Five

### **Reduction of ammonia emissions by applying a urease inhibitor in naturally ventilated dairy barns**

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## Abstract

Ammonia emissions damage the environment and have negative consequences for human and animal health. In the German livestock sector, most ammonia emissions derive from naturally ventilated dairy barns. To reduce emissions, Germany signed the EU Directive 2016/2284 to achieve a stepwise decrease in the ammonia emissions ceiling. The aim of this study was to investigate the seasonal mitigation effect of a urease inhibitor under practical conditions and provide information relating to two theoretical application scenarios in order to estimate an annual application scenario. The experimental design was conducted according to the requirements of the Verification of Environmental Technologies for Agricultural Production test protocol using the CO<sub>2</sub> balance method to obtain the emissions. In a manual process, the inhibitor was applied to the floor surfaces of two dairy farms. The application took place once a day over three days during summer, winter and the transition period (spring/autumn). The ready to use liquid formulation 1 % inhibitor K dissolved in pyrrolidone was mixed with water resulting in 2.5 mg m<sup>-2</sup> inhibitor and 50 ml m<sup>-2</sup> water. The ammonia emissions on Farm A and Farm B were reduced by 40 % and 53 % in summer, 65 % and 68 % in winter and 64 % and 54 % in transition period, respectively. Thus, an annual reduction of 58 % on Farm A and 57 % on Farm B was observed. In the theoretical scenario where no inhibitor was applied during winter, up to 41 % of the annual reduction was observed.

**Keywords:** urease inhibitor, ammonia reduction, dairy cows, natural ventilation

## Introduction

Ammonia gas impacts the environment by both acidification of the soil (Draaijers, Ivens, Bos, & Bleuten, 1989) and eutrophication (Hartung & Phillips, 1994; UBA, 2018a). In addition, air pollution impacts the health of both humans and animals (Loftus et al., 2015). Agriculture contributes around 95 % of the entire amount of ammonia emissions in Germany (UBA, 2018b). Of this, dairy farming emits the most ammonia into the atmosphere in relation to other livestock housings (Haenel et al., 2020). However, the demand for milk is set to increase globally in the future (FAO, 2003). Consequently, dairy husbandry is predicted to increase in the long term. To prevent further harm to the environment and to ensure the health of humans and animals, Germany signed the Directive 2016/2284 (EC, 2016) to decrease ammonia emissions by 5 % between 2020 and 2029 in relation to the year 2005. For every year after 2030, a reduction of 29 % is required. In order to reduce ammonia emissions, numerous investigations have been carried out over the past years (Amon et al., 2007; Braam, Ketelaars, & Smits, 1997a; Braam, Smits, Gunnink, & Swierstra, 1997b; Braam & Swierstra, 1999; Cole et al., 2005; Erickson & Klopfenstein, 2010). However, measures such as installing new floor surfaces or changing nutrition management could require large financial and management inputs.

The use of urease inhibitors could, therefore, be an alternative solution. In general, these inhibitors prevent the hydrolysis process over a certain time (Trenkel, 2010) by blocking the enzyme urease (Mobley & Hausinger, 1989; Varel, 1997), thus preventing urea breakdown. Moreover, a side effect of urease inhibition could be an enrichment of the liquid manure with nitrogen, providing valuable crop nutrition. Much research has been carried out to investigate the potential of different urease inhibitors (Hagenkamp-Korth, Haeussermann, & Hartung, 2015; Leinker, 2007; Parker, Rhoades, Cole, & Sambana, 2012; Parker et al., 2005; Parker et al., 2016; Reinhardt-Hanisch, 2008; Varel, 1997; Varel, Nienaber, & Freetly, 1999). Despite multiple investigations concerning the inhibitor, a direct comparison between these studies is difficult due to the different measurement systems and experimental conditions. Furthermore, the reduction of the urease inhibitor K was tested by measuring urease activity (Hagenkamp-Korth et al., 2015). Even though a connection between decreasing urease activity and the impact on ammonia emissions could be assumed, (Braam & Swierstra, 1999) a direct ammonia emission measurement in combination with the urease inhibitor needs to be investigated. Therefore, the aim of this study was a) to quantify the seasonal mitigation potential of a urease inhibitor under practical conditions in two dairy barns, and b) estimate the annual reduction potential under two different theoretical application scenarios. The investigations were carried out based on the Verification of Environmental Technologies for Agricultural Production (VERA) test protocol

(International VERA Secretariat, 2018). The international VERA protocol contains levels of requirements (e.g. measurement method, sampling and the analysis of liquid manure samples) and a pre-defined experimental setup in order to provide similar measurements.

## Material and Methods

### *Experimental housing*

The measurements were carried out on two dairy farms in Germany. Farm A was located in the federal state of Mecklenburg-Vorpommern and Farm B in the federal state of Brandenburg (Table 1). A detailed description of the two housing sites can be found in Hempel et al. (2018). The herd size of Farm A was divided into four cow groups which were milked separately. The cows were milked three times a day (6–12 a.m., 2–8 p.m. and 10–4 p.m.). The barn was equipped with four ventilators (three Big Ass Fans and one ARNTJEN® Fresh Air Fan) to supply fresh air by downward circulation over the cows. The air exits the building through the opened sidewalls. The ventilators were activated at a threshold of 13°C. During winter, the curtains were half-closed. The barn had four outside liquid manure pits where the liquid manure was mixed and pumped daily to a nearby biogas system. At Farm B, three ventilators were mounted to the ceiling but not used during the experiments. The barn was equipped with two inside pits where the liquid manure was mixed and pumped to a storage tank and afterwards delivered to a nearby biogas system.

**Table 1** Overview of the two farms.

<b>Parameter</b>	<b>Farm A</b>	<b>Farm B</b>
Building length (m)	96.15	35.43
Building width (m)	43.2	17.30
Housing type	Naturally ventilated (wind-breaker net)	Naturally ventilated (windbreaker net)
Side wall	Curtains	Curtains
Treated Area (m <sup>2</sup> )	1618.6	280.3
Area per cow (m <sup>2</sup> )	8.9	8.6
Grazing	None (only one group)	None
Floor surface	Solid	Solid/slats in front of the automatic milking system (AMS)
Bedding design	Cubicle + deep bedding	Cubicle + deep bedding
Bedding material	Chopped straw/chalk	Chopped straw
Manure channels (n)	4 outside pits	2 inside pits
Scraper type	Winch-drawn	Winch-drawn
Scraper (n)	4	2
Scraper frequency	12 times/day in summer, every half hour in winter	Every hour
Milking	Herringbone milking system	AMS (Lely Astronaut T4)
Breed	Holstein Friesian	Holstein Friesian
Weight (kg)	650	650
Herd size (n)	Ø 374	Ø 52
Cow groups (n)	4	1
Milking times (n)	3	-
Milk yield (kg d <sup>-1</sup> )	39 (group 1–4)	ca. 28
Milk yield (kg a <sup>-1</sup> )	11.557	10.300

### *Experimental design*

The experimental design was carried out as a “case-control in time” approach based on the VERA test protocol “for Livestock Housing and Management Systems”, Version 3:2018-09 (International VERA Secretariat, 2018). The measurements were carried out over all seasons (winter, summer and the transition periods of spring/autumn) in order to exclude any seasonal influences. The aim of the experimental setup was to compare the measured ammonia emissions before (reference phase) and after (application phase) the urease inhibitor was applied. The reference phase was two days, while the application phase was three days with the inhibitor being applied once a day (Table 2). In between the first and second measurement periods, a decay phase of nine days was implemented to exclude any residual effects of the inhibitor. Two identical measurement periods were carried out in each season. In addition, in order to confirm

the effect of the inhibitor, the urease activity was measured both before and after the inhibitor was applied.

**Table 2** Exemplary time schedule of the two measurement periods during the winter season of Farm A.

Date	Measurement period	Number of days per phase (n)	Phase
19–20 Feb 2017	1	2	Reference
21–23 Feb 2017	1	3	Application
24–03 March 2017	1	9	Decay
04–05 March 2017	2	2	Reference
06–08 March 2017	2	3	Application
09–16 March 2017	2	9	Decay

### *Side parameters*

For the side parameters, feed samples were taken once every season and liquid manure samples were collected at the beginning and end of each season. The samples were immediately frozen and shipped to a laboratory for further analysis (see General Material & methods, 5. *Side parameters*).

### *Measurement of ammonia emissions*

The values of the emissions, ammonia concentration, carbon dioxide concentration, ventilation rate, temperature, wind direction and speed, indoor humidity, operational function and stability of the farms were provided by the Department of Engineering for Livestock Management at the Leibniz Institute for Agricultural Engineering and Bioeconomy in Potsdam. An overview of the installed sampling lines on both farms can be found in *General material and methods, Chapter 5, Figure 15 and Figure 16*. On both farms, the gaseous concentrations of ammonia and carbon dioxide were measured using two high-resolution Fourier-transform infrared spectrometer (FTIR) measurement devices (Gaset CX4000; Detection limit: 0.1 ppm; Limit of quantitation: 0.3 ppm). Samples of air were sucked through PTFE tubes with an inner diameter of 6 mm. The tubes had an orifice with a capillary trap every 10 m, which ensured uniform volumetric flow at every orifice. An ultrasonic anemometer (USA, WindMaster Pro ultrasonic anemometer, Gill Instruments Limited, Lymington, Hampshire, UK) was installed on the roof of the barn of Farm A to measure wind velocity and direction. Inside the barn, six sample lines were installed so that each of the four openings was equipped with a line, with two lines placed in the middle. All lines were positioned at a height of approximately 3 m, except for the second middle line which had a height of 6 m. Six sampling lines were placed outside the barn, one on each side



or opening of the barn and two for additional measurements at potential hot spots. The duration of measurements per line was 10 minutes and each FTIR was connected to six lines so that one cycle per hour was measured, providing data for the hourly estimation of air exchange rates and ammonia emissions. In the barn of Farm B, three sampling lines were installed inside the barn and three outside the barn to measure gas concentrations. The tubes and capillary traps were the same as for Farm A. There was a difference in the distances between the traps, which, in this case was set to 6 m. One FTIR (the same model as for Farm A) was used to analyse gas concentrations with a measurement duration of 10 minutes per sample line so that one cycle with six lines was completed in one hour and hourly emissions and air exchange rates (AERs) could be provided. To calculate the emissions, the following formula was used:

$$E = (c_{in} - c_{out}) * Q, \quad (1)$$

where  $E$  is the emission of ammonia (in  $\text{kg s}^{-1}$ ),  $c_{in}$  the inside and  $c_{out}$  the outside measured concentrations of ammonia (in  $\text{kg m}^{-3}$ ) and  $Q$  is the volume flow through the barn (in  $\text{m}^3 \text{s}^{-1}$ ). To estimate the volume flow  $Q$ , the  $\text{CO}_2$  mass balance CIGR method according to Pedersen and Sällvik (2002) was used. To ensure comparable results, the emissions were transferred to the emission factor EF, which is referenced to one livestock unit (LU) of 500 kg for dairy cows:

$$EF = \frac{E}{LU}, \quad (2)$$

where  $EF$  has the unit  $\text{g h}^{-1} \text{LU}^{-1}$ . More details of this method can be found in König, Hempel, Janke, Amon, and Amon (2018). Very high air exchanges which occurred due to very small inside-outside differences in  $\text{CO}_2$  resulted in hourly values with differences lower than 30 ppm. These values were regarded as outliers. Moreover, negative emission values were also excluded from further estimations.

#### *Measurement of urease activity*

Urease activity was measured to confirm the inhibitor effect. The experimental setup was performed according to Hagenkamp-Korth et al. (2015). A measurement pattern was established (see General material and methods, *Chapter 5*, Figure 3 and Figure 4) for both farms and the urease activity was measured one day before the application and directly on the application day.

*Urease inhibitor*

The inhibitor K, a phosphorodiamidate ready-made by a liquid chemical formulation based on pyrrolidone were supplied by the company Stickstoffwerke Piesteritz GmbH (2020) (SKW). The inhibitor K concentration of 2.5 mg m<sup>-2</sup> was based on a previous study (Hagenkamp-Korth et al., 2015; Leinker, 2007). The liquid formulated inhibitor K was dissolved and applied in 50 ml m<sup>-2</sup> water. The optimised synthesis process and urease inhibitor formulations, together with the necessary application and stability properties, were developed by the SKW and are protected via patents (two registrations).

*Application system*

On both farms, the application of the inhibitor took place in the early morning, between 5 a.m. and 8 a.m. The application process was conducted using a modified automatic backpack sprayer (SOLO® Kleinmotoren GmbH) and an additional 20 L tank. The automatic backpack sprayer and the tank were placed inside a metal cage fixed to a modified applicator (converted rollator). Two anti-drift fan nozzles were attached to a spray bar in the front. The speed of application was determined by the pressure of the system and size of the barn. After every application, the system was checked any for remaining liquid.

*Data processing and statistics**a) Reduction of the ammonia emissions*

The statistical analysis and graphs were created with the statistical software R (R Core Team, 2020). The first day of the application phase was excluded over all seasons and for both farms due to an underestimation of the reduction potential. Since the application started early in the morning, the hours after midnight were still considered part of the first day of the application phase, although no inhibitor was applied during these hours. In order to focus directly on the effect of the inhibitor, the database for the statistical analysis was created from hourly emissions over the two reference days and the second and third application days. The data evaluation started with the definition of the following statistical mixed model:

$$Y_{ijklmo} \sim a_i * b_j * c_k * d_l + e_m + (ef)_{mn} + r_{ijklmno}, \quad (3)$$

where

$Y$ : ammonia emissions (g h<sup>-1</sup> LU<sup>-1</sup>)

and the fixed factors are

$a_i$ : season (summer, winter, transitional period),

$b_j$ : measurement period (1, 2),

$c_k$ : phase (reference and application),

$d_l$ : time (0, 1 ... 23 hours).

The “\*” refers to the main effects, including all corresponding interaction terms, being covered.

The random factors are:

$e_m$ : barn (Farm A, Farm B)

$(ef)_{mn}$ : measurement unit (combination of phase, measurement phase and season)

$r_{ijklmno}$ : residual error, correlated due to the time.

Based on a graphical residual analysis, the residuals  $r_{ijklmno}$  were assumed to be normally distributed and to be heteroscedastic due to the different levels of season, measurement period and phase. Based on the model, a pseudo  $R^2$  was calculated (Nakagawa & Schielzeth, 2013) and an analysis of variances (ANOVA) was conducted and followed by a multiple contrast test (Bretz, Hothorn, & Westfall, 2011; Schaarschmidt & Vaas, 2009) in order to compare the levels of phase. In order to estimate the direct reduction potential, the following formula, in imitation of Hagenkamp-Korth et al. (2015), was used:

$$\text{Seasonal reduction} = 100 - \left( \frac{\text{mean ammonia emissions}_{\text{treated}} * 100}{\text{mean ammonia emissions}_{\text{untreated}}} \right), \quad (4)$$

The *seasonal reduction* (%) was calculated for both farms and over all seasons. The *mean ammonia emissions*<sub>treated</sub> (g h<sup>-1</sup> LU<sup>-1</sup>) was based on the mean values from the application phase, whereas the *mean ammonia emissions*<sub>untreated</sub> (g h<sup>-1</sup> LU<sup>-1</sup>) were based on the mean values of the days in the reference phase.

#### b) Annual reduction scenarios

In order to estimate the annual reduction for the two farms, the seasonal reductions were summed and divided by the number of seasons. Two additional theoretical scenarios were created to investigate the inhibitor effect under different circumstances. The direct exposure of naturally ventilated barns to the different seasons might impact the application technique used. Therefore, it was important to test the mitigation potential of different weather conditions that may impact the function of the inhibitor or the application equipment. In the first scenario, no

inhibitor was applied in winter. This could be the case during sub-zero temperatures. One reason might be a malfunction of the nozzles due to small ice crystals in the inside filters. The annual reduction was estimated based on reductions in both the transitional periods and summer (a value of zero reduction was used for winter). In the second scenario, there was no inhibitor application in either of the transition periods. This scenario represents the most drastic case and might arise from the early onset of winter towards the end of autumn or an extended winter period going into spring. The second scenario provides information for a “worst-case” scenario. The annual reduction was estimated based on the reduction values of both summer and winter.

## Results

### *Side parameters*

The analysis of feed and liquid manure samples provided information on the management system, where variations could influence emission levels. During each season and measurement period, one feed sample and two slurry samples were collected from both farms. The general herd size of the cows inside Farm A was divided into four groups based on their individual lactating status. The provided rations for each cow group were adapted to the individual milk performance and the actual feeding value. However, collected feed samples of each group were blend together into one sample per season. The values of the feed samples (Table 3) appear to be comparable to typical values for dairy cows in Germany (DLG, 2020; Kirchgeßner, 2014; Landwirtschaftskammer Niedersachsen, 2018a; LUFA Nord-West, 2018). The average liquid manure of a dairy cow should have dry matter of 8 %, total nitrogen of  $3.7 \text{ kg m}^{-3}$  and  $\text{NH}_4\text{-N}$  of  $1.7 \text{ kg m}^{-3}$  (Landwirtschaftskammer Niedersachsen 2018b). The dry matter concentration has a higher fluctuation in Farm A in contrast to Farm B, a reason might be the sampling. Furthermore, the lower nitrogen and  $\text{NH}_4\text{-N}$  values in Farm B might point out a well-adapted feeding regime (Table 3). However, the taken samples (feed/manure) should only represent a broad overview.

**Table 3** Overview of the feed and liquid manure parameters of the two farms.

Parameter	Farm A			Farm B			
	Winter	Spring	Summer	Winter	Autumn	Summer	
Feed	Dry matter (g kg <sup>-1</sup> )	435.5	461.9	405.5	356.8	307.9	370.1
	Crude protein (g kg <sup>-1</sup> )	170.0	170.6	175.7	142.1	128.3	132.1
	Net energy (MJ kg <sup>-1</sup> DM)	7.34	6.62	7.12	7.27	7.3	7.37
Liquid manure	Dry matter (%)	9.6	12.6	10.3	7.5	7.7	9.9
	Nitrogen (kg m <sup>-3</sup> )	3.4	5.0	3.5	2.1	2.0	2.8
	NH <sub>4</sub> -N (kg m <sup>-3</sup> )	1.7	2.0	1.2	0.8	0.6	0.8

Ammonia emissions contribute to higher temperatures. Therefore, it is important to collect air temperature information for each season and each farm (Table 4). This data is based on the hourly values of the reference phase and the application phase in both measurement periods. Due to technical problems, only one measurement period was available in the summer period for Farm B. For both farms, only slight differences between the inside and outside temperatures were registered. However, the highest temperatures were observed during the summer season (Farm A, 18.6 °C, outside) and the lowest air temperatures were measured during the winter season (Farm B, 3.6 °C, outside).

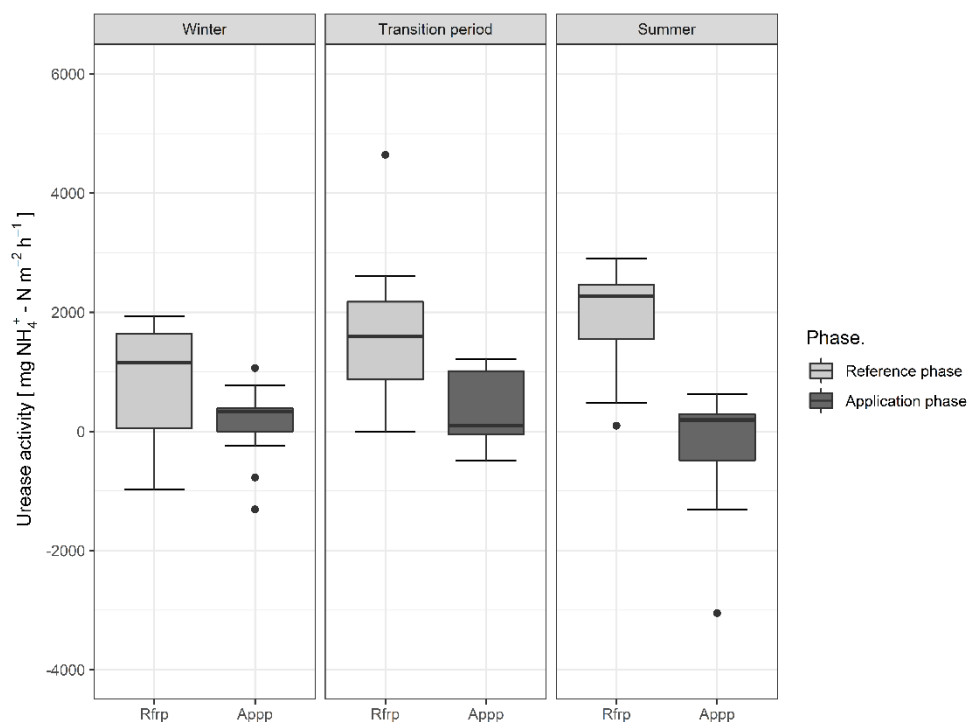
**Table 4** Overview of the mean air temperatures (inside/outside) in °C for the two farms during the selected measurement days.

Season	Farm A		Farm B	
	inside	outside	inside	outside
Summer	18.0	18.6	15.9	15.0
Transitional period	16.0	16.1	10.9	11.3
Winter	6.4	6.6	4.0	3.6

#### *Urease activity*

The winter measurement for urease activity had a mean of 770 mg NH<sub>4</sub><sup>+</sup>-N m<sup>-2</sup> h<sup>-1</sup> in the reference phase and a mean of 134 mg NH<sub>4</sub><sup>+</sup>-N m<sup>-2</sup> h<sup>-1</sup> in the application phase, resulting in a reduction of 83 %. The urease activity during spring had a mean of 1622 mg NH<sub>4</sub><sup>+</sup>-N m<sup>-2</sup> h<sup>-1</sup> in the reference phase and a mean of 339 mg NH<sub>4</sub><sup>+</sup>-N m<sup>-2</sup> h<sup>-1</sup> in the application phase, resulting in a reduction of 79 %. The summer urease activity had a mean of 1914 mg NH<sub>4</sub><sup>+</sup>-N m<sup>-2</sup> h<sup>-1</sup> in the

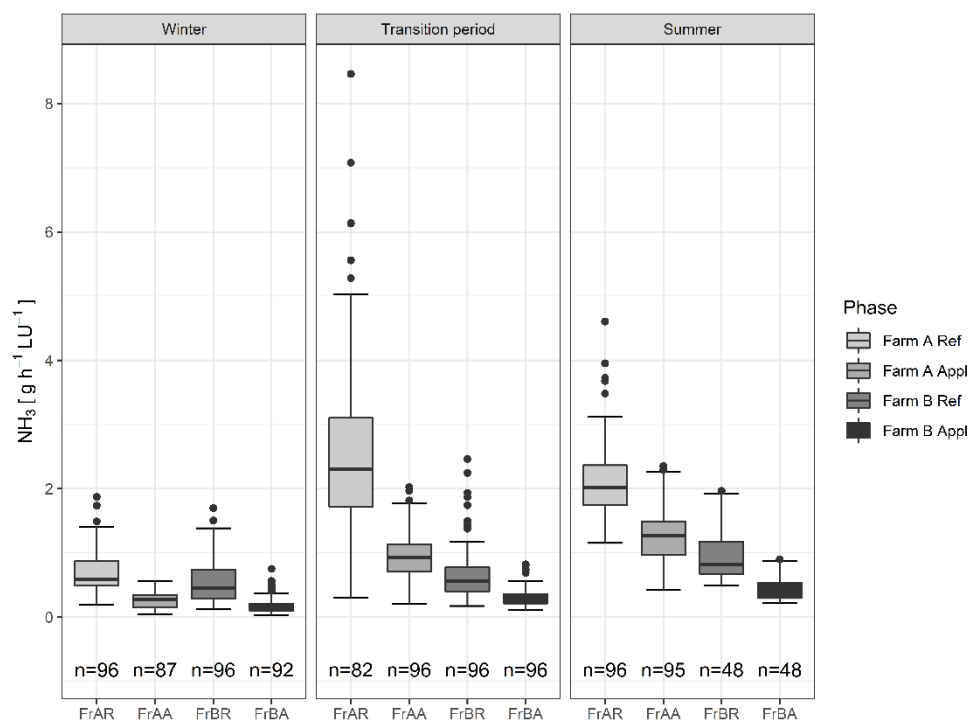
reference phase and a mean of  $-249 \text{ mg NH}_4^+\text{-N m}^{-2} \text{ h}^{-1}$  in the application phase, resulting in a reduction of 113 %. The unusual large reduction in summer could be explained by the great differences between the high reference values and the negative application values. After the inhibitor application, a clear decrease in urease activity was observed for each season (Figure 1).



**Figure 1** Urease activity of both farms for each season. Rfrp = reference phase, n=13 only summer n=14; App = application phase, n=13.

### *Course of the ammonia emissions*

A clear influence of temperature was observed in every season (Figure 2). A decrease in ammonia emissions during the application phase compared to the reference phase was observed for both farms in all seasons (Table 5).



**Figure 2** Overview of ammonia emissions measured during the seasons at both farms; FrAR = Farm A reference phase; FrAA = Farm A application phase; FrBR = Farm B reference phase; FrBA = Farm B application phase.

The highest ammonia emissions were registered in the transition period and the lowest in winter (Figure 2), due to the influence of temperature on ammonia emissions. The temperature effect was also supported by the measured air temperatures in Table 4. In general, higher ammonia emissions were detected on Farm A compared to Farm B (Table 5).

**Table 5** Overview of the average ammonia emissions ( $\text{g h}^{-1} \text{LU}^{-1}$ ) of both farms.

Season	Farm	Reference phase	Application phase
Winter	A	0.71	0.27
Transitional period	A	2.63	0.94
Summer	A	2.12	1.23
Winter	B	0.57	0.19
Transitional period	B	0.68	0.32
Summer	B	0.96	0.44

### *Reduction in ammonia emissions*

The results of the ANOVA showed no significant interaction between the factors of season, measurement period, phase and time. Therefore, a multiple contrast test was performed to compare the reference phase and application phase, pooling all the remaining factors. A significant difference of  $0.72 \text{ g h}^{-1} \text{LU}^{-1}$  ( $p < 0.001$ ) between the reference phase and the application phase

was found for both farms. The estimated reduction potential showed a clear reduction for both farms and all seasons. In general, the highest reductions were measured in winter and the lowest in summer. Farm A had a reduction of 65 % in winter, 64 % in the transition period and 40 % in summer. Farm B had a reduction of 68 % in winter, 54 % in the transition period and 53 % in summer.

#### *Annual reduction scenarios*

Based on the estimated seasonal direct reductions, an annual reduction potential was calculated (Table 6). The highest annual reductions of 57 % to 58 % were observed for both farms. The first theoretical scenario gave lower annual reduction values. For both farms, a reduction potential of between 40 % and 42 % could be estimated. The second theoretical scenario showed the lowest reduction potential. For both farms, an annual reduction of between 26 % and 31 % was observed. This decrease is considered reasonable since the general annual reduction represented the application process over all seasons, whereas the first theoretical scenario excluded the application in the winter season and in the second theoretical scenario, both transitional periods were left out. A stepwise decrease in reduction potential was registered.

**Table 6** Annual reduction (%) for all seasons and two scenarios, calculated separately for each farm and for both farms together.

<b>Object</b>	<b>Over all seasons</b>	<b>First scenario</b>	<b>Second scenario</b>
Farm A	58	42	26
Farm B	57	40	31
Both A and B	58	41	29

#### Discussion

##### *Experimental housing*

Most of the dairy cows (72 %) were kept in free stalls in Germany (Tergast, Schickramm, Lindena, Ellßel, & Hansen, 2019), hereby higher animal welfare standards could be assured in contradiction to tie stalls. In general, both of the selected farms represent a conventional dairy housing system. Snell, Seipelt, and Weghe (2003) investigated the ammonia emissions at four different, naturally ventilated dairy barns during winter. Ammonia emissions of  $1.62 \text{ g h}^{-1} \text{ LU}^{-1}$  up to  $3.56 \text{ g h}^{-1} \text{ LU}^{-1}$  were found. The winter values of this study were, in comparison, lower. Farm A had an average of  $0.71 \text{ g h}^{-1} \text{ LU}^{-1}$  and Farm B an average of  $0.57 \text{ g h}^{-1} \text{ LU}^{-1}$  during the reference phase. One reason for this might be the different measurement methods used. Moreover, Hristov et al. (2011) listed multiple ammonia measurement studies and found a large range



of values between the different investigations. Ammonia emissions between 0.03 up to 10.42 g h<sup>-1</sup> per cow (0.82 up to 250 g d<sup>-1</sup> per cow) were observed. Such distinctions are assumed to be justified by the different measurement methods and mainly different farm working procedures. Hristov et al. (2011) also mentioned that temperature, manure storage, ventilation and frequency of manure removal has a large impact on ammonia emission levels. Differences between the two farms in the present study included building size, herd size and milking management practices. Farm A stored the liquid manure in four outside pits, whereas Farm B collected the liquid manure in two inside pits. Even though both farms were equipped with ventilators, they were only in use at Farm A. The floor surface of both farms consisted mainly of solid concrete. Only a small area around the automatic milking system of Farm B consisted of a slatted floor. Zhang et al. (2005) found a dependency of floor surface and manure on the level of ammonia emissions.

Another possible reason for the different ammonia emission levels might be the calculation method used. Animal activity was extremely high during the three milking times at Farm A and each group went separately to the milking parlour three times a day. The hourly emissions were calculated on the number of cows inside the barn (Pedersen & Sällvik, 2002, CIGR). However, after the milking, the cows returned to the barn, one by one. Thus, each hourly ammonia emission value included the full herd size, despite the milking process as explained above. A consequence of this might be a small overestimation in the emission values. The hourly emission values of Farm B could always be calculated using the daily herd size, since the automatic milking system ensured the presence of all cows.

### *Experimental measurements*

The ammonia emission measurements of a naturally ventilated barn are a rather complex task due to high emission dynamics, instability in wind direction and the high diversity of the ammonia emission sources located in the barn (Fiedler & Müller, 2011; König et al., 2018; Snell, Seipelt, & Weghe, 2003). The CO<sub>2</sub> balance method (CIGR) according to Pedersen and Sällvik (2002) that was used in the study is economically and environmentally friendly compared to the previously used SF<sub>6</sub> tracer gas method. The CO<sub>2</sub> method benefits from the almost equal distribution of CO<sub>2</sub> produced by the animals (Ogink, Mosquera, Calvet, & Zhang, 2013). Tracer gas methods are rather expensive, require much effort and the gas used, for e.g. the radioactive gas krypton (Kr-85), may be considered a health risk (Fiedler & Müller, 2011). In order to identify emissions from neighbouring buildings, outside concentrations were measured on each

of the sides of the barn at a distance of 5 m. The CIGR calculation requires a “days of pregnancy” parameter for the emissions calculation. However, no data was available for Farm B. Therefore, a defined dummy value (150 days) was chosen for all measurement periods. Pretests showed that the use of a dummy value instead of “real” pregnancy days had a negligible impact on ammonia emissions. Wu, Zhang, and Kai (2012) also excluded this variable from their calculations in cases where no data was available.

#### *Measurement of urease activity*

Urease activity was measured in order to confirm the reduction potential and therefore represented a valuable back-up measurement. Similar to the investigations of Hagenkamp-Korth et al. (2015) and Leinker (2007), a clear decrease in urease activity was found after the inhibitor application. The average measured urease activity during the reference phase in this study (770–1914 mg NH<sub>4</sub><sup>+</sup>-N m<sup>-2</sup>h<sup>-1</sup>) are comparable to the values (704–2402 mg NH<sub>4</sub><sup>+</sup>-N m<sup>-2</sup>h<sup>-1</sup>) measured by Hagenkamp-Korth et al. (2015) under practical conditions. However, the values of Hagenkamp-Korth et al. (2015) of the application phase (36–797 mg NH<sub>4</sub><sup>+</sup>-N m<sup>-2</sup>h<sup>-1</sup>) were higher than in this study (134–339 mg NH<sub>4</sub><sup>+</sup>-N m<sup>-2</sup>h<sup>-1</sup>). One possible reason might be the different sample size number and the sensitive measurement method. The negative values (-249 mg NH<sub>4</sub><sup>+</sup>-N m<sup>-2</sup>h<sup>-1</sup>) in this study were found during the summer. Negative values were already observed by Hagenkamp-Korth et al. (2015) and Leinker (2007) and were explained by the sensitive analysing methods (photometer, sampling etc.).

#### *Application technique*

The inhibitor application technique used could be effortlessly implemented into the daily farm schedule. The basic idea of a manually handled backpack sprayer from a previous study Hagenkamp-Korth et al. (2015) was further developed. This study used an automatic commercial backpack sprayer that proved to be a reliable and efficient technique. However, the manually handled system requires extensive human resources and should be replaced by an automatic system.

#### *Reduction in ammonia emissions*

Over the years, much research has been carried out in order to reduce ammonia emissions in agricultural livestock farming (Döhler et al., 2002; Döhler, Eurich-Menden, Rössler, Vandré, & Wulf, 2011; Ndegwa, Hristov, Arogo, & Sheffield, 2008). Measures, such as customised feeding regimes or the handling of manure outside the barn, are mostly nowadays well integrated into farming schedules. The emission reductions discussed in this study were achieved

through a process (involving a urease inhibitor) that was used inside the dairy barns. A reduction potential of 40 % up to 68 % was observed for the two farms. In order to classify the reduction potential of the measures that were implemented inside the barn, the urease inhibitor mitigation was compared to different floor surface structures. Zähler and Schrade (2020) used a urine collection channel and a 3 % cross slope. The aim was to separate the urine and thus prevent further emissions. A reduction of 20 % was found. Swierstra, Braam, and Smits (2001) investigated the possible reduction in emissions through the use of a grooved floor system. A reduction in ammonia emissions of 35 % to 46 % was found. However, retrofitting floor surfaces often results in higher costs and lengthy construction periods. The urease inhibitor, however, in combination with the application technique described here, can be used in different housing systems. Ogink & Kroodsma (1995) demonstrated a possible reduction of 50 % using a water flushing system and formalin solutions. This study followed up previous investigations by Kroodsma, Huis in 't Veld, and Scholtens (1993) which attempted a more sustainable use of water. However, the use of formalin solutions requires high safety standards and, in addition, the application technique has to be permanently implemented.

#### *Annual reduction scenarios*

The two scenarios in this study were used to collect information on the reduction potential where the inhibitor couldn't be applied in certain seasons. An annual reduction potential of 41 % was estimated for the first scenario. This scenario represents the most likely case. The used formulation with urease inhibitor based on pyrrolidone was not easy to handle at temperatures under 5 °C conditioned by a low solidification point of pyrrolidone. During the investigations under practical conditions, the urease inhibitor formulation transformed to the solid state multiple times. Even though the formulation could again be transformed to its liquid phase using a water bath, the process was time consuming. However, when considering fully-automated application processes in the future, an improved formulation and an appropriate storage system should be used. Freezing temperatures could also lead to dysfunctional water pipes, damaged storage tanks and might also effect the application technique. These issues suggest a realistic exclusion of the winter season in the first scenario.

The second scenario, on the other hand, assumed no inhibitor application during the spring and autumn periods. The estimated annual reduction was 29 %. It is, however, unlikely that the application of the inhibitor would be unviable during these times. Disadvantageous weather conditions, including an early winter during autumn or a late winter at the beginning of spring,

may have an impact on the features of the inhibitor formulation. Moreover, management procedures, technical problems or application equipment challenges could result in an unwanted application stop. Of the two scenarios, the first is most likely to occur. Unfavourable usage properties of the used inhibitor formulation at low temperatures in winter are realistic and were experienced during the experimental phases. A second problem arise from frozen water necessary for unique distribution of the inhibitor on the floor surface. However, the reduction potential of the second scenario should be considered mainly for its theoretical value. It provides information on the remaining reduction potential. When considering the implementation of different fully-automated application techniques, such information could be helpful in the decision-making process.

### Conclusion

In this study, the CO<sub>2</sub> balance method was used to measure ammonia emissions when a urease inhibitor was tested in two naturally ventilated dairy barns. The mitigation effect of the inhibitor was tested over different seasons in order to exclude any seasonal effects. A reduction potential of 40 % (summer; Farm A) up to 68 % (winter; Farm B) was observed. If no inhibitor is applied over the winter season, an annual reduction of between 40 % and 42 % was still possible. In order to use the inhibitor at temperatures lower than 5 °C, the formulation of the inhibitor should be improved to avoid the liquid phase transforming into its unsuitable solid phase. The modified application system used proved to be appropriate for the two different existing dairy housing types. However, future studies should focus on an automated, autonomous application system that will function despite changing weather conditions. An existing scraping robot might be a possible solution. The emissions reduction result from the second scenario still provided values between 29 % and 31 %. This potential should serve as additional information and should be taken into account for future studies.

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### Declarations of interest

None.

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## References

- Amon, B., Kryvoruchko, V., Fröhlich, M., Amon, T., Pöllinger, A., Mösenbacher, I., & Hausleitner, A. (2007). Ammonia and greenhouse gas emissions from a straw flow system for fattening pigs: Housing and manure storage. *Livestock Science*, *112*(3), 199–207. <https://doi.org/10.1016/j.livsci.2007.09.003>
- Braam, C. R., Ketelaars, J. J. M. H., & Smits, M. C. J. (1997a). Effects of floor design and floor cleaning on ammonia emission from cubicle houses for dairy cows. *Netherlands Journal of Agricultural Science*. (45), 49–64. <https://doi.org/10.18174/njas.v45i1.525>
- Braam, C. R., Smits M. C. J., Gunnink, H., & Swierstra, D. (1997b). Ammonia Emission from a Double-Sloped Solid Floor in a Cubicle House for Dairy Cows. *Journal of Agricultural Engineering Research*. (68), 375–386. <https://doi.org/10.1006/jaer.1997.0215>
- Braam, C. R., & Swierstra, D. (1999). Volatilization of Ammonia from Dairy Housing Floors with Different Surface Characteristics. *Journal of Agricultural Engineering Research*. (72), 59–69. <https://doi.org/10.1006/jaer.1998.0345>
- Bretz, F., Hothorn, T., & Westfall, P. (2011). *Multiple Comparisons Using R*. London: Chapman and Hall/CRC. ISBN 9781584885740.
- Cole, N. A., Clark, R. N., Todd, R. W., Richardson, C. R., Gueye, A., Greene, L. W., & McBride, K. (2005). Influence of dietary crude protein concentration and source on potential ammonia emissions from beef cattle manure. *Journal of Animal Science*, *83*(3), 722–731. <https://doi.org/10.2527/2005.833722x>
- DLG [Deutsche Landwirtschafts-Gesellschaft] (2020). Berücksichtigung N- und P-reduzierter Fütterungsverfahren bei den Nährstoff - ausscheidungen von Milchkühen. DLG-Merkblatt 444. Retrieved July 03, 2020, from [https://www.dlg.org/fileadmin/downloads/landwirtschaft/themen/publikationen/merkblaetter/dlg-merkblatt\\_444.pdf](https://www.dlg.org/fileadmin/downloads/landwirtschaft/themen/publikationen/merkblaetter/dlg-merkblatt_444.pdf)

- Döhler, H., Dämmgen, U., Eurich-Menden, B., Osterburg, B., Lüttich, M., Berg, W., ... & Brunsch, R. (2002). Anpassung der deutschen Methodik zur rechnerischen Emissionsermittlung an internationale Richtlinien sowie Erfassung und Prognose der Ammoniak-Emissionen der deutschen Landwirtschaft und Szenarien zu deren Minderung bis zum Jahre 2010. *Abschlussbericht im Auftrag von BMVEL und UBA. UBA-Texte*, 5(02). (No. UBA-FB 000249). Berlin, Germany.
- Döhler, H., Eurich-Menden, B., Rössler, R., Vandre, R., & Wulf, S. (2011). UN ECE–Luftreinhaltekonvention–Task Force on Reactive Nitrogen, Systematische Kosten-Nutzen-Analyse von Minderungsmaßnahmen für Ammoniakemissionen in der Landwirtschaft für nationale Kostenabschätzungen. *Kuratorium für Technik und Bauwesen in der Landwirtschaft (KTBL) eV, UBA–Vorhaben FKZ*, 312(01), 287.
- Draaijers, G.P.J., Ivens, W.P.M.F., Bos, M. M., & Bleuten, W. (1989). The contribution of ammonia emissions from agriculture to the deposition of acidifying and eutrophying compounds onto forests. *Environmental Pollution*, 60(1-2), 55–66. [https://doi.org/10.1016/0269-7491\(89\)90220-0](https://doi.org/10.1016/0269-7491(89)90220-0)
- EC [European Commission]. (2016). Directive (EU) 2016/2284 of the European Parliament and of the Council of 14 December 2016 on the reduction of national emissions of certain atmospheric pollutants, amending Directive 2003/35/EC and repealing Directive 2001/81/EC. *OJL*, 344, 1-31. Retrieved August 28, 2018 from <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L:2016:344:FULL&from=EN>
- Erickson, G., & Klopfenstein, T. (2010). Nutritional and management methods to decrease nitrogen losses from beef feedlots. *Journal of Animal Science*, 88(13 Suppl), E172-80. <https://doi.org/10.2527/jas.2009-2358>
- FAO [Food and Agriculture Organisation] (2003). World Agriculture: Towards 2015/2030 An FAO Perspective. Edited by Jelle Bruinsma. Retrieved August 28, 2018, from <http://www.fao.org/3/a-y4252e.pdf>
- Fiedler, A. M., & Müller, H.-J. (2011). Emissions of ammonia and methane from a livestock building natural cross ventilation. *Meteorologische Zeitschrift*, 20(1), 59–65. <https://doi.org/10.1127/0941-2948/2011/0490>
- Haenel, H. D., Rösemann, C., Dämmgen, U., Döring, U., Wulf, S., Eurich-Menden, B., ... & Fuß, R. (2020). *Calculations of gaseous and particulate emissions from German agriculture 1990-2018: Report on methods and data (RMD) Submission 2020* (No. 77). Thünen Report. <https://doi.org/10.3220/REP1584363708000>

- Hagenkamp-Korth, F., Haeussermann, A., & Hartung, E. (2015). Effect of urease inhibitor application on urease activity in three different cubicle housing systems under practical conditions. *Agriculture, Ecosystems & Environment*, 202, 168–177. <https://doi.org/10.1016/j.agee.2015.01.010>
- Hartung, J., & Phillips, V. R. (1994). Control of Gaseous Emissions from Livestock Buildings and Manure Stores. *Journal of Agricultural Engineering Research*. (57), 173–189. <https://doi.org/10.1006/jaer.1994.1017>
- Hempel, S., König, M., Menz, C., Janke, D., Amon, B., Banhazi, T. M., . . . Amon, T. (2018). Uncertainty in the measurement of indoor temperature and humidity in naturally ventilated dairy buildings as influenced by measurement technique and data variability. *Biosystems Engineering*, 166, 58–75. <https://doi.org/10.1016/j.biosystemseng.2017.11.004>
- Hristov, A. N., Hanigan, M., Cole, A., Todd, R., McAllister, T. A., Ndegwa, P. M., & Rotz, A. (2011). Review: Ammonia emissions from dairy farms and beef feedlots. *Canadian Journal of Animal Science*, 91(1), 1–35. <https://doi.org/10.4141/CJAS10034>
- International VERA Secretariat (2018). VERA test protocol for Livestock Housing and Management Systems: Version 3:2018-09, 1–60. Retrieved January 21, 2019 from [https://www.vera-verification.eu/app/uploads/sites/9/2019/05/VERA\\_Testprotocol\\_Housing\\_v3\\_2018.pdf](https://www.vera-verification.eu/app/uploads/sites/9/2019/05/VERA_Testprotocol_Housing_v3_2018.pdf)
- Kirchgeßner, M., Stangl, Gabriele, I., Schwarz, F. J., Roth, F. X., Südekum, K.-H., & Eder, K. (2014). *Tierernährung: Leitfaden für Studium, Beratung und Praxis* (14., aktualisierte Auflage). Frankfurt am Main: DLG Verlag.
- König, M., Hempel, S., Janke, D., Amon, B., & Amon, T. (2018). Variabilities in determining air exchange rates in naturally ventilated dairy buildings using the CO<sub>2</sub> production model. *Biosystems Engineering*, 174, 249–259. <https://doi.org/10.1016/j.biosystemseng.2018.07.001>
- Kroodsma, W., Huis in 't Veld, J.W.H., & Scholtens, R. (1993). Ammonia emission and its reduction from cubicle houses by flushing. *Livestock Production Science*, 35(3-4), 293–302. [https://doi.org/10.1016/0301-6226\(93\)90099-4](https://doi.org/10.1016/0301-6226(93)90099-4)
- Landwirtschaftskammer Niedersachsen (2018a). Weniger Protein für Milchkühe?, Fütterung. Retrieved February 22, 2019, from <https://www.lwk-niedersachsen.de/index.cfm/portal/tier/nav/752/article/24979.html>

- Landwirtschaftskammer Niedersachsen (2018b). Nährstoffgehalte in organischen Düngern. Retrieved May 20, 2020, from <https://www.lwk-niedersachsen.de/index.cfm/portal/96/nav/2280/article/32460.html>
- Leinker, M. (2007). *Entwicklung einer Prinziplösung zur Senkung von Ammoniakemissionen aus Nutztierställen mit Hilfe von Ureaseinhibitoren*. Ph.D. Thesis, Martin-Luther-University, Germany: Forschungsbericht Agrartechnik VDI-MEG Nr. 462.
- Loftus, C., Yost, M., Sampson, P., Arias, G., Torres, E., Vasquez, V. B., . . . Karr, C. (2015). Regional PM<sub>2.5</sub> and asthma morbidity in an agricultural community: A panel study. *Environmental Research*, *136*, 505–512. <https://doi.org/10.1016/j.envres.2014.10.030>
- LUFA Nord-West (2018). *Maissilage - Inhaltsstoffe und Energiegehalt 2012 - 2018*. Retrieved February 22, 2019 from <https://www.lufa-nord-west.de/index.cfm/action/downloadcenter.html>
- Mobley, H. L., & Hausinger, R. P. (1989). Microbial ureases: significance, regulation, and molecular characterization. *Microbiology and Molecular Biology Reviews*, *53*(1), 85-108. <https://doi.org/10.1128/MMBR.53.1.85-108.1989>
- Nakagawa, S. and Schielzeth, H. (2013). A General and Simple Method for Obtaining R<sup>2</sup> from Generalized Linear Mixed-Effects Models. Edited by Robert B. O'Hara. *Methods in Ecology and Evolution* *4*(2), 133-42. <https://doi.org/10.1111/j.2041-210x.2012.00261.x>
- Ndegwa, P. M., Hristov, A. N., Arogo, J., & Sheffield, R. E. (2008). A review of ammonia emission mitigation techniques for concentrated animal feeding operations. *Biosystems Engineering*, *100*(4), 453–469. <https://doi.org/10.1016/j.biosystemseng.2008.05.010>
- Ogink, N. W. M., & Kroodsma, W. (1995). Reduction of Ammonia Emission from a Cow Cubicle House by Flushing with Water or a Formalin Solution. *Journal of Agricultural Engineering Research*. (63), 197–204. <https://doi.org/10.1006/jaer.1996.0021>
- Ogink, N.W.M., Mosquera, J., Calvet, S., & Zhang, G. (2013). Methods for measuring gas emissions from naturally ventilated livestock buildings: Developments over the last decade and perspectives for improvement. *Biosystems Engineering*, *116*(3), 297–308. <https://doi.org/10.1016/j.biosystemseng.2012.10.005>
- Parker, D. B., Pandrangi, S., Greene, L. W., Almas, L. K., Cole, N. A., Rhoades, M. B., & Koziel, J. A. (2005). Rate And Frequency Of Urease Inhibitor Application For Minimizing Ammonia Emissions From Beef Cattle Feedyards. *Transactions of the ASAE*, *48*(2), 787–793. <https://doi.org/10.13031/2013.18321>



- Parker, D. B., Rhoades, M. B., Baek, B. H., Koziel, J. A., Waldrip H. M., & Todd, R. W. (2016). Urease Inhibitor for Reducing Ammonia Emissions from an Open-Lot Beef Cattle Feedyard in the Texas High Plains. *Applied Engineering in Agriculture*, 32(6), 823–832. <https://doi.org/10.13031/aea.32.11897>
- Parker, D. B., Rhoades, M. B., Cole, N. A., & Sambana, V. P. (2012). Effect of Urease Inhibitor Application Rate and Rainfall on Ammonia Emissions from Beef Manure. *American Society of Agricultural and Biological Engineers*. (55), 211–218. <https://doi.org/10.13031/2013.41248>
- Pedersen, S., & Sällvik, K. (2002). Climatization of animal houses heat and moisture production at animal and house levels. *Research Centre Bygholm, Danish Institute of Agricultural*, 1–46.
- R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from <https://www.R-project.org/>
- Reinhardt-Hanisch, A. (2008). *Grundlagenuntersuchungen zur Wirkung neuartiger Ureaseinhibitoren in der Nutztierhaltung*. Ph.D. Thesis, University of Hohenheim, Germany: Forschungsbericht Agrartechnik VDI-MEG Nr. 471.
- Schaarschmidt, F., & Vaas, L. (2009). Analysis of Trials with Complex Treatment Structure Using Multiple Contrast Tests. *Hortscience*. 44(1), 188–195. <https://doi.org/10.21273/HORTSCI.44.1.188>
- Snell, H.G.J., Seipelt, F., & Weghe, H.F.A.V. d. (2003). Ventilation Rates and Gaseous Emissions from Naturally Ventilated Dairy Houses. *Biosystems Engineering*, 86(1), 67–73. [https://doi.org/10.1016/S1537-5110\(03\)00113-2](https://doi.org/10.1016/S1537-5110(03)00113-2)
- Stickstoffwerke Piesteritz GmbH (2020). Retrieved June 14, 2020, from <https://www.skwp.de/en/company/>
- Swierstra, D., Braam, C. R., & Smits, M. C. (2001). Grooved Floor System For Cattle Housing: Ammonia Emission Reduction And Good Slip Resistance. *Applied Engineering in Agriculture*. (17), 85–90. <https://doi.org/10.13031/2013.1929>
- Tergast, H., Schickramm, L., Lindena, T., Ellßel, R., & Hansen, H. (2019) Steckbriefe zur Tierhaltung in Deutschland: Milchkühe, 1–17. Retrieved August 24, 2020 from [https://literatur.thuenen.de/digbib\\_extern/dn061460.pdf](https://literatur.thuenen.de/digbib_extern/dn061460.pdf)
- Trenkel, M. E. (2010). *Slow- and Controlled –release and stabilized fertilizers: An option for enhancing nutrient use efficiency in agriculture*. International fertilizer industry association (IFA). Paris, France.

- UBA [Umweltbundesamt] (2018a). Überschreitung der Belastungsgrenzen für Eutrophierung. Umweltbundesamt. Retrieved August 28, 2018, from <https://www.umweltbundesamt.de/daten/flaeche-boden-land-oekosysteme/land-oekosysteme/ueberschreitung-der-belastungsgrenzen-fuer-0>
- UBA [Umweltbundesamt] (2018b). Ammoniak-Emissionen. Umweltbundesamt. Berlin, Germany. Retrieved January 22, 2019, from <https://www.umweltbundesamt.de/daten/luft/luftschadstoff-emissionen-in-deutschland/ammoniak-emissionen#textpart-1>
- Varel, V. H., Nienaber, J. A., & Freetly, H. C. (1999). Conservation of nitrogen in cattle feedlot waste with urease inhibitors. *Journal of Animal Science*, 77(5), 1162. <https://doi.org/10.2527/1999.7751162x>
- Varel, V. H. (1997). Use of urease inhibitors to control nitrogen loss from livestock waste. *Bioresource Technology*, 62(1-2), 11–17. [https://doi.org/10.1016/S0960-8524\(97\)00130-2](https://doi.org/10.1016/S0960-8524(97)00130-2)
- Wu, W., Zhang, G., & Kai, P. (2012). Ammonia and methane emissions from two naturally ventilated dairy cattle buildings and the influence of climatic factors on ammonia emissions. *Atmospheric Environment*, 61, 232–243. <https://doi.org/10.1016/j.atmosenv.2012.07.050>
- Zähner, M., & Schrade, S. (2020). Laufflächen mit 3 % Quergefälle und Harnsammelrinne in Laufställen für Milchkühe. *Agroscope Merkblatt*, Nr. 80/2020. Retrieved April 24, 2020, from <https://www.bauernzeitung.ch/media/113005382/lauffla-chen-ammoniak.pdf>
- Zhang, G., Strøm, J. S., Li, B., Rom, H. B., Morsing, S., Dahl, P., & Wang, C. (2005). Emission of Ammonia and Other Contaminant Gases from Naturally Ventilated Dairy Cattle Buildings. *Biosystems Engineering*, 92(3), 355–364. <https://doi.org/10.1016/j.biosystemseng.2005.08.002>

## General discussion

### *Aim and content*

The main aim of this study was to quantify the reduction potential of inhibitor type K in general and investigate the chronological development of the response in particular. The previous chapters (Chapters 3 to 5) described and discussed the individual investigations. In this chapter, the experiments, together with the experiences gained and the recommendations for future studies, are discussed in a combined and comprehensive way. This general discussion comprises five subsections. The first subsection focuses on the different experimental test facilities. The inhibitor was tested in respiration chambers, naturally ventilated dairy barns and in mechanically ventilated units. The benefits and limits of the different test facilities are discussed and recommendations for possible upcoming studies are briefly provided. The second subsection addresses the criteria for the different datasets selected for the analysis. It was observed that the data selected has a great impact on the estimated reduction potential. This chapter discusses previous assumptions and provides information for future studies. The third subsection focuses on the efficiency of the inhibitor type K. The chapter addresses the results of the chronological development. Based on the investigations of previous studies, together with the findings of the time slot analysis, the functioning of the inhibitor and its limits are discussed. The fourth subsection addresses the factors influencing the efficiency of the urease inhibitor and its reduction potential. Possible factors and their impact on the inhibitor are discussed. The fifth subsection compares the reduction potential of the urease inhibitor type K with other mitigation methods. Previously tested mitigation measures are discussed and compared with the reduction potential in this study. A general overview of the individual costs of the different measures is given as well as a brief discussion on the prospects of the application technique.

#### *1. Test facilities*

In order to achieve the predefined aims of the experimental investigations, different experimental approaches and test facilities were used. The general advantage of the three test facilities is the opportunity they provide to measure the ammonia emissions directly. Previous inhibitor studies used indirect urease activity measurements (Hagenkamp-Korth, Haeussermann, & Hartung, 2015; Leinker, 2007). The investigations discussed in Chapter 3 were carried out in four respiration chambers. The inhibitor could be tested under a controlled environment with a focus on the ammonia emissions response. Moreover, in addition to the laboratory framework, realistic circumstances could be achieved by using test animals (dairy cows) inside the chambers. Previous inhibitor studies used floors with artificially fouled surfaces (Leinker, 2007) or

applied artificial urine (Reinhardt-Hanisch, 2008). This setup, however, can be considered comparable to tie stall housing, since the animal inside the chamber was unable to walk or turn around. Due to these circumstances, the duration of stay inside the chambers was limited to a maximum of three days. The air temperature inside the chamber could be set to a range between 0 °C to 35 °C (Derno, Elsner, Paetow, Scholze, & Schweigel, 2009). Pereira, Misselbrook, Chadwick, Coutinho, and Trindade (2012) investigated the temperature effect on the ammonia emissions under laboratory conditions. They noted that higher temperatures significantly increased ammonia emissions. In this study, however, the air temperature was fixed at 15 °C. Influences due to temperature variation could therefore be excluded and no statements could be made based on possible temperature effects. In general, different air flow rates inside the chamber were also been possible (Metges, Kuhla & Derno, 2014). In this study, however, the value was fixed at 30 m<sup>3</sup> h<sup>-1</sup>. The experiences gained and the statements provided are therefore based on a consistent, selected airflow. The DIN18910 (2017) recommends an air volume flow of 108 m<sup>3</sup> h<sup>-1</sup> for a 600 kg dairy cow. The air flow inside the chamber was lower than the requirements of the DIN18910 (2017), and therefore the ammonia emissions measured need to be interpreted with caution and cannot be directly compared with ammonia emissions in naturally ventilated housings. However, the main aim was to investigate the urease inhibitor reduction effect and not compare measured ammonia emission data. In addition to the fixed parameters, additional data could be provided in the chambers, such as information on animal weight, standing/lying position, water and feed consumption and a 24-hour camera control (Metges, Kuhla & Derno, 2014). In order to address unexplained variations in the ammonia emissions, several side parameters could be consulted. A possible disadvantage of the chambers was the “clean” conditions in the chamber prior to the test. The test animals were placed inside previously cleaned chambers. In order to estimate the inhibitor effect, feasible reference values were needed for comparison with the measured values after inhibitor application. However, in general, a stable emissions level was measured after around 24 hours inside the chamber. Brose (2000) measured ammonia emissions inside respiration chambers and found an increase in emissions over time. The investigations in Chapter 3 include a pretest with two applications over the three days of the experimental duration. However, it was observed that the stay of the animals differed and exactly three days were not always provided. Therefore, the second application was excluded and the focus was on a stable emissions level before inhibitor application. Although the experimental setup inside the chambers is not identical to realistic field conditions inside naturally ventilated dairy housings, the aim of this investigation could be achieved by focusing on the chronological development of the inhibitor K. However, as noted above, the

respiration chambers do not fully simulate the environment of a naturally ventilated dairy barn, and since the inhibitor is to be used in the dairy sector, it is important to test the inhibitor under field conditions.

For this reason, Chapter 4 discusses the testing of the inhibitor under practical conditions in two mechanically ventilated units. The experiments were conducted in two small-scale units with a mechanically ventilated “hybrid-system” (wind nets, curtains and doors between the units). Although nowadays most commercial dairy barns are naturally ventilated for animal welfare reasons, the advantage of the test facility was the opportunity to perform two experimental designs based on the Verification of Environmental Technologies for Agricultural Production (VERA) (International VERA Secretariat, 2018) test protocol: a direct “case-control approach” and a “case-control in time approach”. A detailed description of the experimental setup of the two methods can be found in the *Material & methods* section (*4. Verification of Environmental Technologies for Agricultural Production test protocol*). Although both units were identically constructed, small differences between the measured ammonia emissions were observed. These differences were considered in the statistical analysis. During feeding and milking times, all doors and curtains between the units were opened and the incoming and outgoing air was mixed. This might have had an impact on the measured ammonia emissions and subsequently on the reduction potential. However, the test facility provided the opportunity to test the inhibitor under field conditions and thus use two different experimental approaches.

In Chapter 5, the testing of the urease inhibitor inside two naturally ventilated dairy barns is discussed. Naturally ventilated barns represent a housing system that strongly supports the animal’s welfare and provides the most realistic circumstances, since most dairy cows in Germany are kept in freestalls (Tergast, Schickramm, Lindena, Ellbel, & Hansen, 2019). However, only the “case-control in time approach” of the VERA test protocol (International VERA Secretariat, 2018) could be conducted. A direct “case-control approach” couldn’t be performed since the dairy barns used couldn’t be divided into two similarly-equipped barns. Although the inhibitor type K had already been tested inside a cubicle housing system (Hagenkamp-Korth et al., 2015), the reduction potential was additionally estimated by measuring urease activity, which represents an indirect method of measuring ammonia emissions. However, a method for the direct measurement of ammonia emissions was used in this investigation.

In summary, the different test facilities were used to test the inhibitor in a stepwise manner. The first experiment was conducted under laboratory conditions and the last experiment was carried out under practical conditions on naturally ventilated farms. All measurements were carried out between the middle of 2016 and the beginning of 2018. In order to include every season in the

field experiments, the measurements were conducted almost simultaneously. The same urease inhibitor formulation was used and thus the reduction results discussed in Chapters 3 to 5 could be compared. The laboratory investigation in Chapter 3 provided a controlled environment and specific questions relating to the chronological inhibitor response could be explained. Laboratory tests are useful for investigating selected parameters in advance in small-scale experiments. The investigations conducted under field conditions by Hagenkamp-Korth et al. (2015) and Parker et al. (2016) were based on indirect ammonia emission measurement methods. This study, however, used direct ammonia emission measurement methods, which increased the robustness of the results. Moreover, a variety of different dairy cow housings were used to test the inhibitor. The use of the international VERA test protocol (International VERA Secretariat, 2018) was a first step in the direction of a standardized measurement.

Finally, the different test facilities provided the opportunity to not only estimate the reduction, but to investigate for the first time the chronological development of the inhibitor 1) under laboratory conditions and 2) under field conditions.

## 2. Selected database

In calculating the reduction, it was observed that the selected dataset of the reference and application values had, in general, a strong impact on the estimated reduction potential in all cases (Chapters 3 to 5).

In Chapter 3, the reference values were based on the four hours before the application occurred. The application took place, in general, after 24 hours, since the ammonia emissions needed time to stabilize. Brose (2000) observed a similar slow increase in ammonia emissions inside the respiration chambers at the beginning of the experiment. Only the four hours before the application point could be used in the reference values, since the previous data included a continually evolving emissions level. Therefore, the application could only be carried out after 24 hours. The duration of the stay of the cows inside the chambers was limited to three days and by excluding 20 hours at the beginning, only part of the measured data could be used. The values of the application phase were divided into 4-hour time slots in order to show the different reductions for each time frame. Pretests attempted to detect a breaking point (see Material & methods, *Chapter 3: Investigating the chronological reduction potential of a urease inhibitor in respiration chambers*, Figure 11) after inhibitor application. The basic idea of this approach was to provide a manually selected time frame (two hours) and observe the calculated decrease (breaking point) of the urease inhibitor K. However, no robust results were found, possibly due

to strong data variation, and a general time slot analysis with four-hour time slots was performed.

In Chapter 4, the database for the reference phase was chosen to have the same length as the values of the application phase. This was the case for both approaches (case-control approach and case-control in time approach). The application phase of the case-control in time approach, however, was based on only two application days rather than the original three days. The first day of the application phase was excluded because the application was carried out in the morning, meaning the previous night hours (no effect) would have been included in the reduction estimation. The outcome would therefore be a possible underestimation of the inhibitor effect. Future studies should use hourly values rather than full days in order to include more feasible data. In the case-control approach, however, the full three application days and full three reference days could be used. Even though all the application days could be used, only three of the four measurement periods were credible. Due to a time overlap of remaining inhibitor effect, no case-control approach could be provided. In order to prevent this in future studies, the length of the experimental schedule should be extended.

The database in Chapter 5 is based on two reference and two application days. As for the case-control in time approach in Chapter 4, only two of the three application days could be used, since the night hours where there is no effect needed to be excluded. Therefore, future studies should preferably use hourly values to avoid the exclusion of one full day.

In summary, the databases selected are of great importance in all three chapters. There should be a stable emissions level in the respiration chambers (Chapter 3) for at least 24 hours before the application. The case-control in time approach in Chapters 4 and 5 should focus on using hourly values rather than eliminating the first full application day. In so doing, more of the measurements could be used. The time slot analysis of the case-control approach in Chapter 4 could focus on extending the time slots. Rather than 24 hours, a reduction potential of 48 hours could be investigated. Moreover, the previously selected 4-hour time slots could be expanded to 6-hour time slots. The classification of the 4-hour time slots was based on pretests. Different length time slots could provide further information on the working of the inhibitor. The database in Chapter 4 was derived from the measurements in a mechanically ventilated dairy barn (*see the above subsection, 1. Test facilities*). Based on the duration of feeding and milking, pretests excluded four hours of every 24-hour measurement day. However, the reductions for the case-control in time approach were only 1–2 % higher when compared to reductions that included this data.

Finally, the decision to use all data in the analysis was based on observations, even though the original four hours of feeding and milking varied greatly from day to day. In order to exclude certain hours, the exact time of measurement needs to be recorded.

### 3. *Efficiency of the urease inhibitor type K*

The efficiency of urease inhibitors has been the subject of many discussions. A previous study by Hagenkamp-Korth et al. (2015) confirmed the general efficiency of urease inhibitor type K. The reduction potential was calculated based on urease activity. No direct ammonia emissions were measured and therefore only assumptions relating to ammonia emissions reduction could be made. In order to provide information on the chronological course of the efficiency, ammonia emissions were measured directly and the inhibitor was tested based on a time slot analysis. The time slot analysis with the same four-hour time slots is discussed in Chapters 3 and 4. Both chapters separately discuss the course of the analysis in detail. The general agreements and differences will be discussed in this subsection.

Although different experimental setups were used, an almost identical chronological reduction pattern was observed. The reduction potential appeared to increase in a linear pattern followed by an almost linear decrease after reaching the reduction peak in the 12–16 hour time slot. The highest peaks had a reduction of 37 % (Chapter 3) and 18 % (Chapter 4). Although a faster decrease effect was observed, the reduction effect remained until the 24-hour time slot. The analysis in both chapters was based on datasets that were limited to the 24-hour time slot of the application phase. In order to provide more information on the effect, it would be interesting to extend the time slot frame.

Comprehensive knowledge concerning the different inhibitor substance groups, the working processes of inhibition, the enzyme kinetic of inhibitors in general and the different kinds of inhibition was provided by Leinker (2007) and Reinhardt-Hanisch (2008). A reversible inhibitor was described as causing a consistent, instant effect, whereas irreversible inhibition is due to a time-dependent effect (Reinhardt-Hanisch, 2008). However, whether urease inhibitors have a reversible or irreversible effect is still unknown. Reinhardt-Hanisch (2008) tested different urease inhibitor types in a water tank measurement system. However, the measurement interval was 24 hours and therefore a shorter chronological effect couldn't be measured. A retrospective deduction by Reinhardt-Hanisch (2008) was made for the investigations conducted by Leinker (2007), where a delayed evolving effect of the inhibitor (type D) over 4-hour time periods could be detected and an irreversible effect was presumed. However, both test systems have been not



appropriated to define the characteristic of urease inhibitors in the correct manner. The chronological investigations in this study (Chapters 3 and 4) were based on 4-hour time slots and a similar delayed evolution of the urease inhibitor effect was observed. It is likely that the inhibitor first needs to be converted before resulting in an effective bond with the enzyme, however, this behaviour might not be directly linked to an irreversible effect. Instead, the urease inhibitor effect might be classified as defined, time-dependent (Personal communication, PD Dr Schilling, Head of Protein and Drug Biochemistry Unit, Fraunhofer Institute for Cell Therapy and Immunology, (09.07.2020)). To finally classify whether the inhibitor effect is irreversible or reversible, more research on enzyme kinetics and the enzymology is necessary.

The amount and concentration ( $2.5 \text{ mg m}^{-2}$ ) of inhibitor K used was based on the investigations by Hagenkamp-Korth et al. (2015) and Leinker (2007). Leinker (2007) investigated different urease concentrations and different frequencies. The results showed that lower inhibitor concentrations ( $2.5\text{--}10 \text{ mg m}^{-2}$ ) with a higher application frequency are more efficient than higher concentrations ( $3\text{--}300 \text{ mg m}^{-2}$ ) in a single application over a period of four days. The time slot analysis in this study gave a similar result. The inhibitor decreased after reaching a peak with the reduction effect clearly diminishing after 24 hours. Because Leinker (2007) used a different inhibitor, a higher concentration of the inhibitor type K in combination with a daily application frequency might result in higher reduction effects. Nevertheless, higher concentrations might also not result in a longer-lasting mitigation effect, since Leinker (2007) tested higher dosages of the inhibitor and found that the inhibitor effect only lasted for one day. The additional  $50 \text{ ml m}^{-2}$  of water added should not be a limiting factor, since an even distribution of the inhibitor is necessary in order to fully achieve its potential (Leinker, 2007). A higher reduction potential and a slower decrease in the effect was observed under the laboratory conditions (Chapter 3). In contrast, a lower reduction potential and a faster decrease in the inhibitor effect was observed in the open field experiment in Chapter 4. One reason for this might be the influence of temperature. The air temperature inside the respiration chambers was fixed at a constant  $15 \text{ }^\circ\text{C}$ . The time slot analysis in Chapter 4, however, was conducted over all seasons with the inhibitor being exposed to a variety of different temperatures. Investigations by Jansen (2016) confirmed that the inhibitor disappeared in liquid cattle manure over the storage duration of the liquid manure. The degradation of the inhibitor K was more significant in liquid cattle manure with a higher content of dry matter and total nitrogen as well as a storage temperature of  $20 \text{ }^\circ\text{C}$  compared to temperatures between  $2$  and  $8 \text{ }^\circ\text{C}$ . The inhibitor was degraded by an average of  $50 \%$  over eight and 1.5 days at temperatures  $2\text{--}8 \text{ }^\circ\text{C}$  and  $20 \text{ }^\circ\text{C}$ , respectively. These observations highlight the fact that daily inhibitor application is necessary, especially during warmer

periods, for a consistent effect.

The annual reduction scenario discussed in Chapter 5 provides information on the remaining reduction potential when it is not possible to apply the inhibitor during winter and both transitional periods. The annual reduction is addressed and discussed with several mitigating methods in the subsection numbered 5 (Classification of the urease inhibitor type K) below. The urease inhibitor consistency is strongly influenced by temperature. The inhibitor formulation is based on pyrrolidone and changes into a solid state at temperatures  $\leq 5$  °C. Follow-up investigations should examine the time slot analysis at different temperatures in order to find possible differences over the seasons. Further information should provide conclusions on the concentration and frequency of inhibitor application over the different seasons.

Finally, it is important to mention that the time slot analysis discussed in Chapter 3 began at the time of application. The database used in Chapter 4, however, includes the application time but does not use it as the starting point. The definition of the application point is more precise in Chapter 3 than in Chapter 4.

#### *4. Influences on reduction efficiency*

Reduction efficiency can be affected by different parameters. Possible influencing factors could include climate conditions, experimental circumstances, inhibitor characteristics, application technique, size of the treated area and measurement technique. The outside air temperature and relative humidity have a great impact on the microclimate inside naturally ventilated dairy barns (Saha et al., 2014). Higher inside temperatures affect inhibitor efficiency; if the manure reaches temperatures of 20 °C, a rapid degradation of the inhibitor over eight to ten days was observed under laboratory conditions. In contrast, the decomposition time of the inhibitor was extended to 42 days at temperatures between 2 °C and 8 °C (Jansen, 2016). A reaction to higher temperatures by the urease inhibitor was also observed in Reinhardt-Hanisch (2008). It was noted that a lower reduction potential was a result of the higher temperatures. A similar situation was observed in the field investigations in this study (Chapters 4 and 5). The highest reduction potential in summer ranged between 23 % (Chapter 4) and 54 % (Chapter 5) and the highest reduction in winter between 31 % (Chapter 4) and 68 % (Chapter 5). Moreover, air temperature has a large impact on the pyrrolidone-based formulation of the inhibitor. During the field investigations discussed in Chapters 4 and 5, it was found that the pyrrolidone-based formulation of the inhibitor reacted sensitively to temperatures  $\leq 5$  °C. This fact was not noted in previous studies, since the inhibitors were supplied in a powder form (Hagenkamp-Korth et al., 2015; Leinker, 2007). A water bath could be used for the dialysis of the inhibitor, but this was time-

consuming and challenging under practical conditions. However, this change into the solid state is well known and should be taken into consideration in future scenarios. However, in order to not interrupt the ongoing investigations, the decision was made to continue working with the same inhibitor formulation in all three experiments.

Furthermore, unplanned occurrences during the experimental periods could lead to a false interpretation of the efficiency. An unscheduled manure removal (Chapter 4) resulted in lower values for the reference phase and higher values for the application phase. Although initial assumptions might be based on the malfunctioning of the inhibitor, previous circumstances must be taken into account. Such unwanted interferences could be avoided by frequently checking the pit level (e.g. underneath the slatted floor) and by carefully planning in advance. At least two measurement periods per season should be conducted to avoid the risks of questionable data, technical problems or sudden data loss.

Moreover, the ammonia emission measurements inside naturally ventilated barns are challenging due to the large openings (Fiedler & Müller, 2011). Previous inhibitor studies used urease activity, an indirect measurement, to quantify the reduction potential of different inhibitors (Hagenkamp-Korth et al., 2015; Leinker, 2007). This study used direct ammonia emission measurements (Chapters 4 and 5). The measurement setup must be able to meet different demands. An even distribution of the sampling points inside the housing is crucial for measuring the heterogeneous distribution of the emission sources. Moreover, background sampling points are considered necessary for identifying potential additional emission sources. An overview of the status quo of the different measurement techniques is provided by Ogink, Mosquera, Calvet, and Zhang (2013). A correct ammonia emissions measurement should ensure that the reductions are not over or underestimated.

Finally, Leinker (2007) demonstrated that the even distribution of the inhibitor on the floor surface area was necessary for inhibitor efficiency. These estimations were based on ammonia emissions ( $\text{mg NH}_3 \text{ m}^{-2}$ ) over four hours. When the inhibitor was applied to only half (50 %) of the area, a mitigation of 18 % was achieved, whereas an application to the full area (100 %) resulted in a 47 % reduction potential. The application technique contributes greatly to the correct distribution. The application systems used in this study were adaptations of those used in the investigation by Hagenkamp-Korth et al. (2015). Pretests found that a nozzle type that generates fine droplets leads to an even distribution in a cow housing system. In the experiment in Chapter 3, a small commercial hand sprayer (three bar pressure) with a nozzle attached to the front was used. The space inside the chamber was limited and only the far end of the rubber mattress could be reached. However, this area was presumed to be the area of the chamber with

the highest emissions, since the cow was fixed in the front and was unable to turn around. Most of the faeces and urine were secreted at the end of the stanchion. The application techniques discussed in Chapters 4 and 5 were based on an automatic backpack sprayer, which was placed on a mobile undercarriage. During pretests, nozzle types, application heights of the spray bar, spraying patterns and the speed and amount of the solutions being applied were investigated. The length of the spray bar was also modified. The automatization of the application system and its improved mobility meant the application process was faster, easier and more controlled. The pretests were conducted in different dairy barn locations. However, this application technique was very time-consuming and required human resources. A second co-worker was needed at one farm (Chapter 5) where the cows were milked by an automatic milking system and the application took place while the cows were present. The liquid remaining in the applicator was 2–3 % for all experiments under practical conditions.

The above-mentioned investigations by Leinker (2007) found that coverage of the entire area is necessary to achieve the inhibitor's potential. However, in the experiment in Chapter 3, approximately 85 % of the emitted area could be applied with the urease inhibitor. The applicator could not be applied to the funnel at the end of the mattress and the front of the mattress was assumed to be a non-emitting area. Considering, that 85 % of the emissions area resulted in a 37 % reduction (highest reduction). The reduction will be divided by the applied area. The result will be multiplied by 100. By doing so, the reduction potential of 100 % of the area could be calculated. Finally, the reduction result of the whole area minus the result of the 85 % of the reduction, result in the missing reduction. Hereby, in Chapter 3), 15 % of the area was missing and this would add up to 6.5 % of the theoretical reduction potential. In the dairy barns discussed in Chapters 4 and 5, the cubicles were considered to be the only areas where it was not necessary to apply the inhibitor. Therefore, it was assumed that approximately 98 % of the emissions area was covered with the inhibitor. The highest reduction potential values were 31 % (winter, Chapter 4) and 68 % (winter, Chapter 5). Thus, if 2 % of the area was missing in both cases, the extra reduction would be 0.6 % (Chapter 4) and 1.4 % (Chapter 5), respectively. These additional reduction values are negligible, since over 50 % of the emissions area was covered with the inhibitor. Leinker (2007) demonstrated the difference between an application of 50 % of the area or 100 %, but the efficiency of the inhibitor is indeed highly dependent on the area covered and should be taken into account.

### 5. *Classification of the urease inhibitor type K*

In addition to the implementation of good agricultural practice guidelines, several investigations have been carried out in order to reduce ammonia emissions on dairy livestock farms. A detailed overview of these mitigation methods are given in Leinker (2007), Monteny and Erisman (1998) and Ndegwa, Hristov, Arogo, and Sheffield (2008). In order to classify the results in this study, the annual reduction calculated in Chapter 5 should be compared to a variation of selected mitigation methods. The annual reduction for the urease inhibitor type K in the two naturally ventilated dairy farms (A and B) ranged between 57–58 %.

Despite the reduction comparison, a very general cost calculation of the selected mitigation measure is presented (Table 1). In order to implement the urease inhibitor application in practice, more information concerning the costs need to be provided. Comparisons between other measures will be helpful. The selected measures were divided into laboratory and open field experiments.

The own calculations (Table 1) were based on previous cost calculations by Leinker (2007). Hereby, Leinker (2007) assumed a dairy barn with 65 cows, with each cow having 5 m<sup>2</sup> space and a walking area size of 325 m<sup>2</sup> as a base for the calculations. The calculation formula by Döhler et al. (2002) was modified to calculate the costs of 1 % of the individually represented ammonia reduction. Due to copyright reasons, no costs for the urease inhibitor type K could be provided. Instead, the cost calculation of the current study is based on the price of one NBPT commercial product according to Leinker (2007). Further details, calculation methods, equation (Eq 2) and more information is provided in the *Appendix* (Table A3).

**Table 1** General overview of the costs for the individual mitigation methods. The foundation for the cost calculation of the “cost per cow (€ a)” and the “cost of 1 % of the NH<sub>3</sub> reduction” were based on own calculations. (see *Appendix*, Table A3).

Author	Mitigation method	Reduction (max, %)	Cost per cow (€ a)	Cost of 1 % of the NH <sub>3</sub> reduction
Ogink and Kroodsma (1995) (practical scale)	(a) 20 L water cow day <sup>-1</sup> ;	(a) 14 %	(a) € 11	(a) € 0.8
	(b) 4 g of formaldehyde per L and 20 L water cow day <sup>-1</sup>	(b) 50 %	(b) € 59.4 (b) € 1129.3	(b) € 1.2 (b) € 22.6
Shi, Parker, Cole, Auvermann, and Mehlhorn (2001) (laboratory scale)	(a) Aluminium sulphate*	(a) 91.5 %	(a) € 588.5	(a) € 6.1
	(b) Aluminium sulphate*	(b) 98.3 %	(b) € 1120.6	(b) € 11.4
	(c) Calcium chloride	(c) 71.2 %	(c) € 456.3	(c) € 6.4
	(d) Calcium chloride	(d) 77.5 %	(d) € 919.8	(d) € 11.9
	(e) Brown humate	(e) 67.6 %	(e) € 1711.9	(e) € 25.3
	(f) Black humate	(f) 60.2 %	(f) € 1711.9	(f) € 28.4
	(g) NBPT*	(g) 64.1 %	(g) € 36.5	(g) € 0.6
	(h) NBPT*	(h) 65.6 %	(h) € 73.0	(h) € 1.1
Zähner and Schrade (2020)** (practical scale)	Solid floor with a slope of 3 % and a urine-collecting gutter	46 %	(a) € 307 (b) € 437	(a) € 15.4 (b) € 21.9
Current study (practical scale)	Urease inhibitor (type K) 2.5 mg m <sup>-2</sup> and 50 ml m <sup>-2</sup>	58 %	€ 20.8	€ 0.4

NBPT by Leinker (2007)

\*two different amounts were used, see a detailed description in the *Appendix*; \*\*costs reflect the investment costs per cow and (a) and (b) represent the cost range, depending on the barn

One option for reducing ammonia emissions is to use water in a “flushing effect” (Kroodsma, Huis in 't Veld, & Scholtens, 1993; Ogink & Kroodsma, 1995). Ogink and Kroodsma (1995) investigated the effect on ammonia emissions using a combination of a scraper and water (20 L cow day<sup>-1</sup>). A reduction potential of up to 14 % was observed. In Chapter 5 of this study, Farm A had an average of 374 dairy cows. When considering the amount of water used in Ogink and Kroodsma (1995), a daily water amount of 7480 L would be needed. Water use should be restricted for sustainability reasons. Moreover, additional water would increase the volume in the manure pits. Ogink and Kroodsma (1995) found an increase in slurry volume of 45 %. The

annual mitigation costs were € 11 per cow and year, assuming water costs of € 1.5 m<sup>3</sup> by the Statistisches Amt für Hamburg und Schleswig-Holstein, (2020). The cost of 1 % of the total 14 % reduction were € 0.8 per cow (Table 1). In the current study (Chapters 3 to 5), additional water was mixed with the inhibitor formulation in order to evenly apply the inhibitor to the floor. The laboratory experiments (Chapter 3) used an amount of 100 ml m<sup>-2</sup> and the field investigations (Chapters 4 and 5) used 50 ml m<sup>-2</sup>. For the laboratory experiments, 1 L water per respiration chamber (one cow) per day was used. The open field investigations used around 4 L for 16 cows (Chapter 4), 86 L for a herd size of around 374 cows (Chapter 5, Farm A) and 15 L for an average herd size of 52 cows (Chapter 5, Farm B). However, due to the different dimensions of the cow pathways and a previously set spraying radius, a small corridor had an overlapping application, but this has already been taken into account in the above-mentioned daily water consumption. Due to the small amount of water used compared to Ogink and Kroodsmas (1995), it can be assumed that the additional used water in this study had no deminishing effect. Further studies investigated the use of amendments. Shi et al. (2001) investigated the effect of different amendments to a beef cattle feedlot surface under laboratory conditions in order to reduce ammonia emissions. The reduction potential ranged from 26.4 % to 98.3 %. However, the costs of the amendments (without the spreading) by Shi et al. (2001) varied between € 0.10 (\$ 0.12) per head (cow) and € 4.69 (\$ 5.53) per head (cow) (currency data retrieved August 24, 2020, 15:08 UTC). The different amendments and their individual reductions are shown in Table 1 (excluding two commercial amendments). The annual cost per cow ranged between € 36.5 and € 1711.9. The lowest cost of 1 % of the ammonia reduction was for the urease inhibitor NBPT (1 kg ha<sup>-1</sup>) and amounted for € 0.6. Shi et al. (2001) calculated that the benefit-cost ratio was greater than 1.0 only for NBPT, meaning that the other amendments cost more in relation to the benefits resulting from the reduced ammonia emissions. However, these results were obtained in laboratory investigations. The amendments might react differently in field conditions and the repercussions on the environment and animal and human health have not yet been evaluated.

Another added amendment investigation was conducted by Ogink and Kroodsmas (1995), where 4 g of formaldehyde combined with 1 L of flushing water (20 L per cow day<sup>-1</sup>) was used under field conditions. Up to 50 % of the ammonia emissions could be reduced and annual costs were calculated to be between € 59.4 (Melktechnik-Discount, 2020) and € 1129.3 (Merck, 2020) meaning that the cost of a 1 % reduction would vary between € 1.2 and € 22.6 (Table 1). The huge price differences depend on the used formalin origin.

However, the adverse effect of formalin/formaldehyde in dairy housing on both animals and

humans were not fully investigated by Ogink and Kroodsma (1995) and the amount of water is still an issue in environmental sustainability.

The limiting factor in the hydrolysis reaction appears to be the urea concentration in the urine of the cows. In order to overcome this, there should be a rapid segregation of the urine and faeces. To achieve this, floor surface design offers some potential alternatives. Several studies have been conducted over the years. By investigating a one-sided 3 % sloped floor, Braam, Ketelaars, and Smits (1997a) found a 21 % reduction potential. Furthermore, Braam, Smits, Gunnink, and Swierstra (1997b) investigated a double-sloped solid floor with a “V”-shaped urine gutter and found a 50 % reduction potential compared with slatted floors. Swierstra, Braam, and Smits (2001) were able to reduce ammonia emissions by 46 % with precast concrete floors with grooves. Zähler et al. (2017) combined several floor surface modifications. A solid floor with a slope of 3 % and a urine-collecting gutter was investigated and an ammonia reduction of 20 % was found. In general, the different floor surface modifications have a reduction range of between 20 % and 50 %. However, new floors or scraping equipment require additional investment. The additional costs can vary greatly depending on the different floor plans, number of cubicles, etc. Zähler and Schrade (2020). An example calculation for a solid floor with a slope of 3 % and a urine-collecting gutter (Zähler et al., 2017) is given in Zähler and Schrade (2020). The extra costs for a dairy barn with a herd size of 60 cows ranged between € 307 (CHF 330) up to € 437 (CHF 470) per cow (currency data retrieved from August, 24, 2020, 15:24 UTC). The cost of a 1 % reduction ranged between € 15.4 and € 21.9 (Table 1). However, in addition to the financial investments, the barn also needs to be empty and the cows have to be relocated for the duration of the renovation. Such circumstances can be very challenging given the current economic situation. Moreover, the daily milk yield is a calculated profit factor. In this study, each square meter was applied with 2.5 mg of the urease inhibitor type K formulation in combination with the 50 ml of water. Due to copyright reasons, the cost calculations (Table 1) of the inhibitor K were performed with the price of a NBPT commercial product and the same side parameters of the example dairy barn in Leinker (2007). The annual reduction in this study was used to calculate the cost of a 1 % of the ammonia reduction. The cost per cow and year were € 20.8, which resulted in costs of € 0.4 for a 1 % reduction (Table 1).

Similar to Leinker (2007), a cost calculation (Table 1) was performed. A very general overview of the used material (e.g. amendments) costs was provided. However, strong distinctions could be observed between the different measures. The costs varied between € 11 and € 1711.9 per cow and year. Furthermore, the observation was made, that the origin/manufacturer of the material impacts the costs. Ogink and Kroodsma, (1995) investigated the use of formaldehyde as a



possible mitigation measure inside dairy housings and costs varied between € 59.4 and € 1129.3 per cow and year. Such high prices might be inconvertible in practice. However, in order to figure out a practicable cost limits, it is of importance to collect further information. Therefore, the calculation of the application technique costs, hours of labour, etc., should be included. In order to create a future cost calculation scenario, the next steps should be the selection of a convenient application technique. A scraping robot might be one way to apply the inhibitor, however, the different manufactures and the variation of prices needs to be considered. In summary, the represented cost calculation (Table 1) provides a general overview of different mitigation methods and the expected costs. However, future studies should extend further economical information in order to provide exact calculations.

Aside from the investment, one of the most important questions is whether the selected measurement method is limited to new buildings or could be retrofitted inside older housings. The measurements by Ogink and Kroodsmas (1995) were based on an application system with nozzles inside the housing, with storage devices outside the building. Although most of these facilities could be retrofitted into the existing barn, the permanent fixing of nozzles inside the barn could be extremely time-consuming. Similar assumptions can be made for applying the amendments in Shi et al. (2001). It could be possible to retrofit new floor surfaces in older barns, as discussed in Braam et al. (1997a), Braam et al. (1997b), Swierstra et al. (2001), Zähler and Schrade (2020), however, the construction could be extremely complex from a logistical and management perspective. The question is whether this effort would be worthwhile. Floor systems are more commonly implemented in new buildings for housing and retrofitting is unlikely.

Finally, the urease inhibitor application process in this study can be conducted in newly-built housings and can also be adapted to already-existing dairy housing systems. The test facilities described in Chapters 4 and 5 include a large variety of different cow housings. The herd sizes ranged from 16 cows up to 374 cows. Milking was conducted with an automatic milking system as well as at the milking parlour. The milking times differed between two to three times during the day. The floor surfaces of the test facilities included slatted as well as solid floors. The inhibitor could be applied in all the experiments conducted. Based on the experiences gained, it could be observed that no new construction is necessary for the application process. The housing system is not a limiting factor. Instead, the application process needs to be adapted to the different housing types. Although the inhibitor was applied manually in this study, follow-up studies should consider using automated, autonomous ways to apply the inhibitor. Scraping robots could be a commercial way of applying the inhibitor. However, a scraping robot might

be not always be available. Therefore, future investigations should examine the existing farm equipment and possible ways to include a suitable application system.

## References

- Braam, C. R., Ketelaars, J. J. M. H., & Smits, M. C. J. (1997a). Effects of floor design and floor cleaning on ammonia emission from cubicle houses for dairy cows. *Netherlands Journal of Agricultural Science*. (45), 49–64. <https://doi.org/10.18174/njas.v45i1.525>
- Braam, C. R., Smits M. C. J., Gunnink, H., & Swierstra, D. (1997b). Ammonia Emission from a Double-Sloped Solid Floor in a Cubicle House for Dairy Cows. *Journal of Agricultural Engineering Research*. (68), 375–386. <https://doi.org/10.1006/jaer.1997.0215>
- Brose, G. (2000). *Emission von klimarelevanten Gasen, Ammoniak und Geruch aus einem Milchviehstall mit Schwerkraftlüftung*. Ph.D. Thesis. Forschungsbericht Agrartechnik des Arbeitskreises Forschung und Lehre der Max-Eyth-Gesellschaft Agrartechnik VDI-MEG Nr. 362.
- Derno, M., Elsner, H.-G., Paetow, E.-A., Scholze, H., & Schweigel, M. (2009). Technical note: A new facility for continuous respiration measurements in lactating cows. *Journal of Dairy Science*, 92(6), 2804–2808. <https://doi.org/10.3168/jds.2008-1839>
- DIN 18910 (2017). *Thermal insulation for closed livestock buildings - Thermal insulation and ventilation - Principles for planning and design for closed ventilated livestock buildings*: Beuth Verlag. <https://dx.doi.org/10.31030/2680106>
- Döhler, H., Dämmgen, U., Eurich-Menden, B., Osterburg, B., Lüttich, M., Berg, W., ... & Brunsch, R. (2002). Anpassung der deutschen Methodik zur rechnerischen Emissionsermittlung an internationale Richtlinien sowie Erfassung und Prognose der Ammoniak-Emissionen der deutschen Landwirtschaft und Szenarien zu deren Minderung bis zum Jahre 2010. *Abschlussbericht im Auftrag von BMVEL und UBA. UBA-Texte*, 5(02). (No. UBA-FB 000249). Berlin, Germany.
- Fiedler, A. M., & Müller, H.-J. (2011). Emissions of ammonia and methane from a livestock building natural cross ventilation. *Meteorologische Zeitschrift*, 20(1), 59–65. <https://doi.org/10.1127/0941-2948/2011/0490>
- Hagenkamp-Korth, F., Haeussermann, A., & Hartung, E. (2015). Effect of urease inhibitor application on urease activity in three different cubicle housing systems under practical conditions. *Agriculture, Ecosystems & Environment*, 202, 168–177. <https://doi.org/10.1016/j.agee.2015.01.010>

- International VERA Secretariat (2018). VERA test protocol for Livestock Housing and Management Systems: Version 3:2018-09, 1–60. Retrieved January 21, 2019 from [https://www.vera-verification.eu/app/uploads/sites/9/2019/05/VERA\\_Testprotocol\\_Housing\\_v3\\_2018.pdf](https://www.vera-verification.eu/app/uploads/sites/9/2019/05/VERA_Testprotocol_Housing_v3_2018.pdf)
- Jansen, C. (2016). *Final study report Determination of the Storage Stability of Phenyl Phosphorodiamidate in Liquid Manure* (No. Study number IF-16/03690887).
- Kroodsma, W., Huis in 't Veld, J.W.H., & Scholtens, R. (1993). Ammonia emission and its reduction from cubicle houses by flushing. *Livestock Production Science*, 35(3-4), 293–302. [https://doi.org/10.1016/0301-6226\(93\)90099-4](https://doi.org/10.1016/0301-6226(93)90099-4)
- Leinker, M. (2007). *Entwicklung einer Prinziplösung zur Senkung von Ammoniakemissionen aus Nutztierställen mit Hilfe von Ureaseinhibitoren*. Ph.D. Thesis, Martin-Luther-University, Germany: Forschungsbericht Agrartechnik VDI-MEG Nr. 462.
- Melktechnik-Discount (2020). Formalin 37-40% | 21 kg [x]. Retrieved August 21, 2020 from <https://www.melktechnik-discount.de/klaupflege/klauehygiene-klaueinfektion/formalin-37-40-21-kg-x.html>
- Merck (2020). Paraformaldehyde for synthesis. CAS 30525-89-4, chemical formula (CH<sub>2</sub>O): 3338 Bestellnummer: 8187151000. Synonyme: Polyoxymethylen, Formalin-Tabletten. Retrieved August 13, 2020, from [https://www.merckmillipore.com/DE/de/product/Paraformaldehyde-3340hyde,MDA\\_CHEM-818715#anchor\\_orderingcomp](https://www.merckmillipore.com/DE/de/product/Paraformaldehyde-3340hyde,MDA_CHEM-818715#anchor_orderingcomp)
- Metges, C. C., Kuhla, B., & Derno, M. (2014). Chapter 7: Large and Laboratory Animal Respiration Facilities, Leibniz Institute for Animal Biology, Dummerstorf, Germany. In C. Pinares & G. Waghorn (Eds.), *Technical Manual on Respiration Chamber Designs* (pp. 107–119). <https://doi.org/10.13140/RG.2.1.4511.6244>
- Monteny, G. J., & Erisman, J. W. (1998). Ammonia emission from dairy cow buildings: a review of measurement techniques, influencing factors and possibilities for reduction. *Netherlands Journal of Agricultural Science*. (46), 225–247. <https://doi.org/10.18174/njas.v46i3.481>
- Ndegwa, P. M., Hristov, A. N., Arogo, J., & Sheffield, R. E. (2008). A review of ammonia emission mitigation techniques for concentrated animal feeding operations. *Biosystems Engineering*, 100(4), 453–469. <https://doi.org/10.1016/j.biosystemseng.2008.05.010>
- Ogink, N. W. M., & Kroodsma, W. (1995). Reduction of Ammonia Emission from a Cow Cubicle House by Flushing with Water or a Formalin Solution. *Journal of Agricultural Engineering Research*. (63), 197–204. <https://doi.org/10.1006/jaer.1996.0021>

- Ogink, N.W.M., Mosquera, J., Calvet, S., & Zhang, G. (2013). Methods for measuring gas emissions from naturally ventilated livestock buildings: Developments over the last decade and perspectives for improvement. *Biosystems Engineering*, *116*(3), 297–308. <https://doi.org/10.1016/j.biosystemseng.2012.10.005>
- Parker, D. B., Rhoades, M. B., Baek, B. H., Koziel, J. A., Waldrip H. M., & Todd, R. W. (2016). Urease Inhibitor for Reducing Ammonia Emissions from an Open-Lot Beef Cattle Feedyard in the Texas High Plains. *Applied Engineering in Agriculture*, *32*(6), 823–832. <https://doi.org/10.13031/aea.32.11897>
- Pereira, J., Misselbrook, T. H., Chadwick, D. R., Coutinho, J., & Trindade, H. (2012). Effects of temperature and dairy cattle excreta characteristics on potential ammonia and greenhouse gas emissions from housing: A laboratory study. *Biosystems Engineering*, *112*(2), 138–150. <https://doi.org/10.1016/j.biosystemseng.2012.03.011>
- Reinhardt-Hanisch, A. (2008). *Grundlagenuntersuchungen zur Wirkung neuartiger Ureaseinhibitoren in der Nutztierhaltung*. Ph.D. Thesis, University of Hohenheim, Germany: Forschungsbericht Agrartechnik VDI-MEG Nr. 471.
- Saha, C. K., Ammon, C., Berg, W., Fiedler, M., Loebstin, C., Sanftleben, P., . . . Amon, T. (2014). Seasonal and diel variations of ammonia and methane emissions from a naturally ventilated dairy building and the associated factors influencing emissions. *The Science of the Total Environment*, *468-469*, 53–62. <https://doi.org/10.1016/j.scitotenv.2013.08.015>
- Shi, Y., Parker, D. B., Cole, N. A., Auvermann, B. W., & Mehlhorn, J. E. (2001). Surface amendments to minimize ammonia emissions from beef cattle feedlots. *American Society of Agricultural Engineers*. (Vol. 44 (3)), 677–682. <https://doi.org/10.13031/2013.6105>
- Statistisches Amt für Hamburg und Schleswig-Holstein (2020). Wasser- und Abwasserentgelte in Schleswig-Holstein 2019. Retrieved July 03, 2020, from [https://www.statistik-nord.de/fileadmin/Dokumente/Statistische\\_Berichte/verkehr\\_umwelt\\_und\\_energie/Q\\_I\\_6\\_3j\\_S/Q\\_I\\_6\\_3j\\_19\\_SH.pdf](https://www.statistik-nord.de/fileadmin/Dokumente/Statistische_Berichte/verkehr_umwelt_und_energie/Q_I_6_3j_S/Q_I_6_3j_19_SH.pdf)
- Swierstra, D., Braam, C. R., & Smits, M. C. (2001). Grooved Floor System For Cattle Housing: Ammonia Emission Reduction And Good Slip Resistance. *Applied Engineering in Agriculture*. (17), 85–90. <https://doi.org/10.13031/2013.1929>
- Tergast, H., Schickkramm, L., Lindena, T., Ellßel, R., & Hansen, H. (2019) Steckbriefe zur Tierhaltung in Deutschland: Milchkühe, 1–17. Retrieved August 24, 2020 from [https://literatur.thuenen.de/digbib\\_extern/dn061460.pdf](https://literatur.thuenen.de/digbib_extern/dn061460.pdf)

Zähner, M., Poteko, J., Zeyer, K., & Schrade, S. (Eds.) (2017). *Laufflächengestaltung: Emissionsminderung und verfahrenstechnische Aspekte - erste Ergebnisse aus dem Emissionsversuchsstall Tänikon*. Irdning: Höhere Bundeslehr- und Forschungsanstalt für Landwirtschaft Raumberg-Gumpenstein; Österreich.

Zähner, M., & Schrade, S. (2020). Laufflächen mit 3 % Quergefälle und Harnsammelrinne in Laufställen für Milchkühe. *Agroscope Merkblatt, Nr. 80/2020*. Retrieved April 24, 2020, from <https://www.bauernzeitung.ch/media/113005382/lauffla-chen-ammoniak.pdf>

## General conclusions

The aim of this thesis was to quantify the reduction potential of the urease inhibitor type K under laboratory and field conditions. Moreover, knowledge of the chronological development of the urease inhibitor was investigated. The investigations were conducted in laboratory and field experiments. The experimental setups were based on the international Verification of Environmental Technologies for Agricultural Production test protocol. In order to estimate the reduction, different statistical analyses were performed. The general conclusions are divided into five separate subsections below and include recommendations for future studies.

### *1. Test facilities*

The urease inhibitor was tested under laboratory and field conditions using different test facilities. The laboratory investigations were conducted in respiration chambers (Chapter 3). A predefined environment with constant conditions was provided by fixing the air temperature at 15 °C and the airflow at 30 m<sup>3</sup> h<sup>-1</sup>. Thus, any variation in climate conditions over the course of a day were negligible. Moreover, the animal inside the chamber was limited in its movements and could only lie down or stand up. At the beginning of the experiment, a continuous increase in ammonia emissions was detected. However, the ammonia emissions stabilized after the animal had been inside the chambers for around 24 hours. Future studies should take this “build-up time” into account in order to generate realistic and reliable reference data. The stay inside the chambers was limited to a maximum of three days, therefore, a clear experimental time structure is essential to provide sufficient time to estimate the effects after the application. The estimated reduction potential was based on predefined animal groups. Although only group A received supplements, similar feed components should be provided to exclude any potential bias resulting from nutrition, since the amount of ammonia emissions could be influenced by the feed. Due to the limited characteristics of respiration chambers, this laboratory setup should only be used for investigating the chronological development of the urease inhibitor.

The first field experiment (Chapter 4) was carried out in two identically constructed units in a mechanically ventilated dairy building. This housing system provided an opportunity to test the inhibitor using two different approaches (the in time and the case-control approach). However, the curtains and doors between the units were opened during feeding and push-up times. This resulted in the mixing of the inside and outside air, which might have impacted the reduction potential. Future investigations should observe and record these opening and closing periods. Switching to a naturally ventilated system with a measurement method such as the CO<sub>2</sub> balance could take the openings of the curtains and doors into account and the housing system would

represent the status quo of animal husbandry in the dairy sector.

The second field investigation (Chapter 5) was carried out in two naturally ventilated dairy housings. The inhibitor was tested on two different farms (Farm A and Farm B), with different housing sizes, animal number and management procedures. Dairy husbandry of this nature represents the most realistic scenario. The large herd size of Farm A was divided into four groups. During the milking process (three times a day), each cow group left the barn separately. However, the calculations were performed on a full herd size over the course of the day since the correct animal number inside the barn could not be determined due to the milking process. Future studies should aim to collect data from the hourly herd size. In order to verify the results from this investigation, further studies should be conducted in naturally ventilated dairy housings.

## *2. Selected database*

The selected database had a large impact on the estimated reductions. The laboratory investigation (Chapter 3) used the last four hours before the application process as reference values. It was observed that the ammonia emissions needed at least 24 hours to build up to an almost constant level. Therefore, the beginning values could not be used in the reduction calculations. Moreover, the application point was pooled over time “0” for all animals. Future studies should focus on the same hourly starting point. The statistical analysis of the in time approach (Chapters 4 and 5) was based on two days for the reference phase and two days of three for the application phase. The first application day could not be used since the night times included data before the application point. In order to prevent the exclusion of one full day and to extend the database for the reduction calculation, hourly values should be used. The statistical time slot analysis (Chapters 3 and 4) divided the 24 hours of the application phase into six 4-hour time slots. A residual reduction potential at the 24-hour time slot was observed. In order to investigate the decreasing inhibitor effect, the application phase data should be extended to possibly double the duration of the experiment (48 hours). The data from the statistical time slot analysis in the field investigation (Chapter 4) was based on only three of the four experimental periods per season. Overlapping residual effects potentially resulted in biased values. Therefore, future studies should focus on a clear decrease in the inhibitor effect by extending the experimental length.

### 3. *Efficiency of the urease inhibitor type K*

The efficiency of the urease inhibitor type K was estimated based on the values of direct ammonia emission measurements as well as urease activity measurements. The laboratory investigations in the respiration chambers (Chapter 3) found a reduction potential of up to 37 % and an increasing mitigation effect until the 12-hour time slot. The first field investigation (Chapter 4) estimated the reduction potential of the two different approaches. In the in time approach, a seasonal reduction potential of 17–23 % in summer, 10–31 % in winter and 22 % in spring was calculated. For the case-control approach, a time slot analysis was conducted where reductions of up to 18 % and an increasing reduction trend until the 16-hour time slot were observed. The second field investigation (Chapter 5) calculated a reduction potential in all seasons. For Farm A and Farm B respectively, 65 % and 68 % were estimated in winter, 64 % and 54 % were detected in the transition period (spring/fall) and 40 % and 53 % were calculated in summer. On both farms, annual reductions of 58 % (Farm A) and 57 % (Farm B) were observed.

In general, a reduction effect using directly-measured ammonia emissions was confirmed. In the field investigations, the highest reduction was observed in winter and the lowest in summer. It is likely that there is a temperature dependency between the ammonia emissions and the use of the urease inhibitor. The chronological development of the urease inhibitor was investigated under both laboratory and field conditions. Earlier and higher reductions were observed in the laboratory experiment. A possible explanation might be the artificially-maintained constant environment. Further studies should be conducted to verify the urease inhibitor efficiency results. Urease activity was also measured and values decreased over the course of both field investigations. In the first field experiment (Chapter 4), urease activity was reduced by 69 % in both units and for all seasons. In the second field investigation (Chapter 5), urease activity was reduced by 83 % in winter, 79 % in spring and 113 % in summer on both farms. A general reduction in urease activity of the urease inhibitor K was confirmed in the field experiments since a general connection between decreasing urease activity and decreasing ammonia emissions was confirmed. Urease activity proved to be a reliable backup measurement for further investigations.

### 4. *Urease inhibitor type K*

The SKW supplied the urease inhibitor K a phosphorodiamidate, ready-made by a liquid chemical formulation based on pyrrolidone. The same urease inhibitor with a concentration of 2.5 mg m<sup>-2</sup> was used in both the laboratory and field experiments. In order to avoid changes from its liquid state to its solid state at temperatures  $\leq 5$  °C, the inhibitor formulation needs to be and



will be improved. The estimated reduction potential as well as the results of the chronological development in the time slot analysis contribute to a better understanding of the inhibitor characteristics. Based on the evolving reduction pattern in the laboratory investigations (Chapter 3) and the first field investigation (Chapter 4), it could be assumed that the inhibitor reduction potential increases and reaches a maximum at a certain time (12-hour to 16-hour time slot). After reaching a reduction peak of up to 37 % (Chapter 3) and 18 % (Chapter 4), a declining effect was observed. However, at the 24-hour time slot, there is still a remaining effect of up to 32 % (Chapter 3) and 10 % (Chapter 4), suggesting a residual effect of the inhibitor. Upcoming studies should extend the time slots in order to investigate the diminishing effect.

#### *5. Application technique*

Even though different application techniques were used for the laboratory (Chapter 3) and the field investigations (Chapters 4 and 5), the requirements for an even spraying pattern on the floor surface were the same. In order to apply the inhibitor evenly, an amount of 100 ml m<sup>-2</sup> water in the laboratory experiments (Chapter 3) and 50 ml m<sup>-2</sup> water in the field experiments was added to the inhibitor. The water was used as a carrier and proved to be efficient. Due to the limited space inside the respiration chamber under laboratory conditions, a small hand sprayer with a nozzle was used. In the laboratory experiments, it was possible to have an overview of the emissions area, in contrast to the large cow housings. Therefore, in the field investigations, an automatic backpack sprayer with an added set of wheels (rollator) was used. All applications were carried out manually, which proved to be extremely time consuming, especially in the larger barns.

Finally, upcoming studies should evaluate the use of different automatic and autonomously functioning systems. The use of already existing scraping robots might be a possible option for applying the inhibitor without the need for extra investments. The dairy farming sector is already under economic pressure and most farms have little resources available for investing in new application techniques.

## General summary

Dairy farming in naturally ventilated barns is one of Germany's largest ammonia emitters. This husbandry system contributes to animal welfare and represents species-appropriate, modern dairy farming. However, the uncontrolled release of ammonia emissions into the atmosphere can potentially cause problems for human and animal health as well as cause irreparable damage to the environment. In order to avoid further irreversible damages, Germany has agreed to reduce their ammonia emissions ceiling in the long term and has signed national and international conventions to this effect. The project "REDUCE – sustainable, animal and environmentally friendly cattle farming by reducing ammonia emissions using a urease inhibitor" aims to reduce ammonia emissions to achieve this. The project was conducted within the current thesis and is divided into three main chapters. The experiments were based on laboratory and field investigations in order to quantify the mitigation potential of a urease inhibitor (type K). In addition, basic knowledge relating to the chronological mitigation and the inhibitor characteristics were investigated.

The laboratory investigations (Chapter 3) were conducted inside respiration chambers. The aim was to investigate the chronological effect of the inhibitor. The statistical evaluation was performed using a time slot analysis. For this, the measured data (24 hours) after the inhibitor application was divided into four-hour time slots (4, 8, 12, 16, 20, 24 hours) in order to investigate the chronological development of the reduction in detail. In general, an increase in the reduction over the 24-hour period was measured. The highest reduction of 37 % was registered at the 12-hour-time slot. There was a subsequent slight decrease in the reduction, but not a full degradation. Future studies should focus on an extended chronological database to investigate the course of the reduction beyond 24 hours.

The first field investigation (Chapter 4) was carried out inside a mechanically ventilated dairy housing with two identically constructed units. The aim was to quantify the reduction potential and the chronological effect under practical conditions. Due to the identical construction of the units, two different experimental approaches (case-control and case-control in time) could be performed. The statistical analysis was able to estimate the seasonal reduction, while at the same time a time slot analysis could be conducted. The ammonia emissions were reduced by 31 %. In addition, based on the time slot analysis, a reduction of 18 % at the 16-hour time slot was found. These results support the previously conducted laboratory investigations. However, future studies should use hourly data rather than daily data in order to avoid the unnecessary omitting of the first application day.

The second field investigation (Chapter 5) was carried out in two commercial, naturally ventilated dairy housings with different building sizes, animal numbers and management programmes. The aim was to quantify the seasonal inhibitor effect under realistic housing conditions. The experimental setup was based on the case-control in time approach. The statistical analysis estimated a reduction potential for each farm and season. The highest reductions of 68 % were generated in winter and the lowest of 40 % in summer. The annual reduction was calculated at between 57 % and 58 % for both farms. In addition, urease activity was measured under field conditions and confirmed the effectiveness of the inhibitor. The results provide a sustainable mitigation of urease activity after the application of the inhibitor.

In summary, this study presents for the first time an (semi) automatic, modular application technique that can be used in new buildings as well as retrofitted to older housings. However, future studies should focus on fully-automated and autonomously functioning application systems. A modified scraping robot is a possible example. Finally, knowledge concerning the seasonal reduction potential of the urease inhibitor (type K) under field conditions was gained. In addition, the study provided information on the chronological effect using a time slot analysis for the first time in this context. Based on the results from the time slot analysis, a daily temperature-related application of the inhibitor is considered appropriate. The lessons learned in this study should serve further research projects as a basis for the investigation of the potential additional efficiency of the urease inhibitor.

## Zusammenfassung

Die Milchviehhaltung in frei belüfteten Ställen ist eine der größten Ammoniak-Emittenten in Deutschland. Diese Haltungsform trägt allerdings erheblich zum Tierwohlbefinden bei und stellt die artgerechte und moderne Milchviehhaltung dar. Dennoch kann die diffuse Abgabe von Ammoniakemissionen in die Atmosphäre zu schwerwiegenden gesundheitlichen Schäden bei Menschen und Tieren führen. Ebenso wird der natürliche Umweltkreislauf langfristig geschädigt. Um weitere, nicht umkehrbare Schädigungen zu verhindern, hat sich Deutschland im Rahmen nationaler und internationaler Abkommen verpflichtet, den Ammoniakausstoß nachhaltig und langfristig zu senken. Umgesetzt werden soll dies insbesondere mit Hilfe des durchgeführten Projektes „**REDUCE** – Nachhaltige tier- und umweltgerechte Rinderhaltung durch Minderung von Ammoniakemissionen mit Hilfe eines Ureaseinhibitors.“ Dieses Projekt wurde im Rahmen der vorliegenden Dissertation durchgeführt und gliedert sich in drei Hauptkapitel. Die Experimente basieren sowohl auf Laboruntersuchungen als auch auf Praxisversuche, um das quantitative Minderungspotential durch einen Ureaseinhibitor (Typ K) nachzuweisen. Zusätzlich sollten grundlegende Erkenntnisse über den zeitlichen Minderungsverlauf und der Inhibitorcharakteristik erforscht werden.

Die Laboruntersuchungen (Kapitel 3) wurden in Respirationskammern durchgeführt. Ziel war es, den zeitlichen Wirkungsverlauf zu untersuchen. Die statistische Auswertung wurde mit Hilfe einer Zeitfensteranalyse durchgeführt. Hierfür wurden die gesammelten Daten (24-Stunden) nach Zugabe des Inhibitors in Vier-Stunden-Zeitfenster (4, 8, 12, 16, 20, 24 Stunden) eingeteilt, um den zeitlichen Reduktionsverlauf detailliert untersuchen zu können. Hierbei konnte als Kernaussage ein über den zeitlichen Verlauf beobachteter Anstieg der Reduktion gemessen werden. Die höchste Minderung von 37 % konnte im 12-Stunden-Zeitfenster registriert werden. Anschließend konnte ein leichter Reduktionsrückgang, allerdings kein gänzlicher Abbau, festgestellt werden. Im Nachfolgenden kann eine erweiterte zeitliche Datenbasis dazu genutzt werden, den Reduktionsverlauf auch nach 24 Stunden zu untersuchen.

Der erste Praxisversuch (Kapitel 4) wurde in einem mechanisch belüfteten Milchviehstall mit zwei baugleichen Units durchgeführt. Ziel war es, das Reduktionspotential und den zeitlichen Wirkungseffekt unter praktischen Bedingungen zu ermitteln. Aufgrund der identischen Bauweise der Units konnten zwei Versuchsansätze (Fall-Kontroll-Ansatz und Fall-Kontrolle im Zeitverlauf) durchgeführt werden. Mit Hilfe der statistischen Auswertung konnten die saisonalen Reduktionen geschätzt werden und zusätzlich eine Zeitfensteranalyse durchgeführt werden. Die gesamte Ammoniakemission konnte um bis zu 31 % reduziert werden, zusätzlich konnte

durch die Einteilung in Zeitfenster die höchste Reduktion von 18 % im 16. Zeitfenster nachgewiesen werden. Die vorliegenden Ergebnisse bestätigen die vorherige Laboruntersuchungen. Allerdings sollten nachfolgende Studien als Datenbasis Stunden statt Tage verwenden, um ein unnötiges Streichen des ersten Applikationstages zu vermeiden.

Der zweite Praxisversuch (Kapitel 5) erfolgte in zwei kommerziellen, jedoch in Größe, Tieranzahl und Management unterschiedlichen, frei belüfteten Milchviehställen. Ziel war es, die jahreszeitliche Inhibitorwirkung unter realistischen Haltungsbedingungen zu quantifizieren. Der Versuchsaufbau basierte auf der Fall-Kontrolle im Zeitverlauf. Mit Hilfe der statistischen Auswertung konnte für jeden Stall und jede Jahreszeit ein Reduktionspotential kalkuliert werden. Die höchsten Reduktionen lagen bei 68 % im Winter und die niedrigsten bei 40 % im Sommer. Ergänzend wurde eine jährliche Reduktion ermittelt, die für beide Betriebe zwischen 57 % und 58 % lag. Die zusätzlich in den Freilandversuchen gemessene Ureaseaktivität konnte die Wirksamkeit des Inhibitors bestätigen. Die Ergebnisse zeigten eine nachhaltige Minderung der Ureaseaktivität nach Zugabe des Inhibitors.

Zusammenfassend konnte innerhalb dieser Studie erstmalig eine (halb) automatische, modular aufgebaute Applikationstechnik vorgestellt werden, die unabhängig von Alt- oder Neubauten eingesetzt werden kann. Zukünftige Projekte sollten den Fokus auf eine vollautomatische und selbstagierende Applikationstechnik legen. Dies könnte zum Beispiel durch einen modifizierten Entmistungsroboter der Fall sein. Es wurden Erkenntnisse über den jahreszeitlichen Verlauf des Reduktionspotential des Ureaseinhibitors (Typ K) unter praktischen Versuchsbedingungen gewonnen. Zusätzlich wurden Informationen über den zeitlichen Wirkungsverlauf präsentiert, indem erstmalig eine Zeitfensteranalyse durchgeführt wurde. Basierend auf den gewonnenen Zeitfenster-Ergebnissen sollte eine tägliche, temperaturbezogene Applikation des Inhibitors stattfinden. Die Erkenntnisse dieser vorliegenden Studie dienen weiteren Forschungsprojekten als Grundlage um eine mögliche, weitere Effizienzsteigerung durch den Ureaseinhibitor zu untersuchen.

**Appendix****Tab. A1** Overview of the blank application protocol and the required information.

<b>Date</b>	<b>Start</b>	<b>End</b>	<b>Amount of water/inhibitor</b>	<b>Chamber number</b>	<b>Animal number</b>	<b>Person (Kürzel)</b>	<b>Bemerkung</b>

**Table A2** Overview of the application and urease activity measurement protocol.

Applikationsprotokoll														
Probennehmer:					Wettersituation:			Temp.:			Datum:			
Ort	Datum	Zeit (Anfang/Ende)	Abteil	Wassermenge	Inhibitor	Restmengen Spritze	Messplatz	Zeit	Bodenbeschaffenheit	Temperatur	Höhe des Schmutz	Grad d. Verschmutzung	feucht/nass/trocken	Bemerkung
							MP1							
							MP2							
							MP3							
Spritzendruck:							Substratprobe	Zeit	pH-Wert	Temperature		Bemerkung		
Besonderheiten:							MP 1							
Bemerkungen:							MP 2							
							MP 3							

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$$\mathbf{Eq (1)} \text{ NEL (MJ kg}^{-1}\text{)} = -0.22 + 0.1062 \times \text{Gas24 (ml 200 mg}^{-1}\text{ DM)} + 0.0048 \times \text{XP (g kg}^{-1}\text{ DM)} + 0.0132 \times \text{XA (g kg}^{-1}\text{ DM)}.$$

**Text A1:** Description of the fatty acid supplements.

- (1) Control (CNTR, n = 9): 76 g d<sup>-1</sup> coconut oil (Bio-Kokosöl #665, Kräuterhaus Sanct Bernhard KG, Bad Ditzgenbach, Germany) and 0.06 g d<sup>-1</sup> vitamin E (Covitol®1360, BASF SE, Ludwigshafen, Germany), 1.48 MJ NE<sub>L</sub> d<sup>-1</sup>.
- (2) Essential fatty acids (EFA, n = 9): 78 g d<sup>-1</sup> linseed (DERBY Leinöl #4026921003087, DERBY Spezialfutter GmbH, Münster, Germany) and 4 g d<sup>-1</sup> safflower oil (GEFRO Distelöl, GEFRO Reformversand Frommlet KG, Memmingen, Germany), comprised of 0.06 g d<sup>-1</sup> vitamin E, 1.57 MJ NE<sub>L</sub> d<sup>-1</sup>.
- (3) Conjugated linoleic acid (CLA, n = 10): 38g d<sup>-1</sup> Lutalin (BASF SE, Ludwigshafen, Germany) and 0.06 g d<sup>-1</sup> vitamin E (Covitol 1360, BASF SE, Ludwigshafen, Germany), 0.69 MJ NE<sub>L</sub> d<sup>-1</sup>.
- (4) Essential fatty acids and conjugated linoleic acid (EFA+CLA, n = 10): 78 g d<sup>-1</sup> linseed (DERBY Leinöl #4026921003087, DERBY Spezialfutter GmbH, Münster, Germany), 4 g d<sup>-1</sup> safflower oil (GEFRO Distelöl, GEFRO Reformversand Frommlet KG, Memmingen, Germany) and 38 g d<sup>-1</sup> Lutalin (BASF SE, Ludwigshafen, Germany), comprised of 0.06 g d<sup>-1</sup> vitamin E, 2.26 MJ NE<sub>L</sub> d<sup>-1</sup>.



**Table A3** Broad overview of the cost calculations of different mitigation measures. The calculations are based on the side parameters of an example dairy barn (barn size, cow space, number of cows, NBPT costs) provided by (Leinker, 2007). All costs have been converted into €, since Shi et al. (2001) and Zähler et al. (2017) used different currencies. The cost calculation formula by Döhler et al. (2002) was used and modified to the cost of 1 % of the reductions. The following formula Eq (2) in the Appendix was used.

<b>General side parameters for the cost calculation</b>					
Barn size	325 m <sup>2</sup>				
Cow space	5 m <sup>2</sup>				
Number of cows	65				
Water cost	€ 1.50 m <sup>3</sup> (Statistisches Amt für Hamburg und Schleswig-Holstein, 2020)				
Application frequency	once per day				
Formalin <sup>(a)</sup> (37- 40 %)	€ 1.66 kg (Melktechnik-Discount, 2020)				
Formalin-tablets <sup>(b)</sup>	€ 38.30 kg (Merck, 2020)				
NBPT	€ 45.29 kg (Leinker, 2007)				
<b>Research by Ogink and Kroodsma (1995)</b>					
Reduction method	(a) Flushing with 20 L water cow day <sup>-1</sup> ; (b) Flushing with 4 g of formaldehyde per L and 20 L water cow day <sup>-1</sup>				
Reduction in % (max)	(a) 14 %; (b) 50 %				
Daily water consumption barn	(a) and (b) 1300 L				
Daily water costs barn	(a) and (b) € 1.95				
Daily water costs per cow	(a) and (b) € 0.03				
Daily formaldehyde consumption barn	(b) 5.2 kg				
Daily formaldehyde costs barn(no water)	(b) € 8.6 <sup>(a)</sup> € 199.2 <sup>(b)</sup>				
Daily formaldehyde costs cow (no water)	(b) € 0.13 <sup>(a)</sup> € 3.1 <sup>(b)</sup>				
Annual costs per barn (with water)	(a) € 711.75 (b) € 3862.4 <sup>(a)</sup> € 73405.2 <sup>(b)</sup>				
Annual costs per cow (with water)	(a) € 11 (b) € 59.4 <sup>(a)</sup> €1129.3 <sup>(b)</sup>				
<b>Research by Shi et al., (2001)</b>					
Reduction method	Adding different amendments				
Reduction in % (max)	98.3 %				
Amendment	Amount (kg ha <sup>-1</sup> )	Daily costs head	Daily costs barn	Annual costs head	Annual costs barn
Aluminium sulphate*	4500	€ 1.53	€ 99.45	€ 558.45	€ 36299.25
Aluminium sulphate*	9000	€ 3.07	€ 199.55	€ 1120.55	€ 72835.75
Calcium chloride	4500	€ 1.35	€ 81.25	€ 456.25	€ 29656.35
Calcium chloride	9000	€ 2.52	€ 163.80	€ 919.80	€ 59787.00
Brown humate	9000	€ 4.69	€ 304.85	€ 1711.85	€ 111270.25
Black humate	9000	€ 4.69	€ 304.85	€ 1711.85	€ 111270.25
NBPT*	1	€ 0.10	€ 6.50	€ 36.50	€ 2372.50
NBPT*	2	€ 0.20	€ 13.00	€ 73.00	€ 4745.00

<b>Zähner et al. (2017)</b>	
Reduction method	Solid floor with a slope of 3 % and a urine collecting-gutter
Reduction (max)	20 %
Costs per cow	(a) € 307; (b) € 437
Costs per barn	(a) € 19955; (b) € 28405
<b>Current urease inhibitor study</b>	
Reduction method	Urease inhibitor 2.5 mg m <sup>-2</sup> in combination with 50 ml m <sup>-2</sup>
Reduction (max)	58 %
Daily water consumption barn	16.25 L
Daily water costs barn	€ 0.02
Daily water costs per cow	€ 0,0004
Daily NBPT consumption barn	812.5 mg
Daily NBPT costs barn	€ 3.7
Daily NBPT costs cow	€ 0.06
Annual costs per barn	€ 1352
Annual costs per cow	€ 20.8

$$\text{Eq (2)} \quad \text{Mitigation costs [€ per 1 \% NH}_3 \text{ reduction]} = \frac{\text{Costs per cow and year [€ a]}}{\text{Reduction [\%]}}$$