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Basic studies on the effects of novel urease inhibitors in livestock husbandry

Ammonia emissions from agriculture in Germany derive mostly from livestock husbandry. Therefore the aim of this research project is to test the effectivity of different novel urease inhibitors in order to reduce ammonia emissions from animal facilities and to compare them to the commercially available urease inhibitor N-(n-butyl)thiophosphoric triamide (NBTPT). A standardised laboratory measuring system was used to detect the inhibitor with the best reduction potential and to study the dose-effect relationships of the inhibitors under laboratory conditions. In general, some novel urease inhibitors showed a reduction of ammonia release and of the conversion of urea-N to NH_3-N and NH_4-N, but the level of the reduction was different and influenced by substrate temperature and concentration of the inhibitor. By the use of the novel urease inhibitor D was obtained an explicit higher reduction of the ammonia release and of the conversion of urea-N to NH_3-N and NH_4-N as by the use of the well-known NBTPT inhibitor. So for instance, after the use of novel urease inhibitor D (concentration: 0,1 % of Total Kjeldahl Nitrogen, substrate temperature: 15 °C) the emission reduction potential is estimated to be rather promising. A remarkable lower portion of only 15 % of the urea-N was transferred to NH_3-N and NH_4-N compared to NBTPT with 76 %. The novel urease inhibitor D was also effective in substrates of different dairy barns, in different layer thicknesses of slurry and in pig slurry.

Keywords
Ammonia, ammonia emission, ammonia reduction, urease, urease inhibitors

Abstract
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A major source of ammonia emissions from livestock farming [2] is the hydrolysis of urea. Urease inhibitors inhibit the hydrolysis of the urea and thus the formation of ammonia [1, 5]. Despite several studies on the use of urease inhibitors in animal husbandry [4, 7, 8, 11, 12] a practical application has not yet been implemented. The aim of the project was therefore to develop a principle solution to reduce ammonia emissions from livestock farming by the use of novel urease inhibitors. In order to simulate the experiments under the conditions of a cowshed a wind-tunnel and water-bath measuring system [3] was used. Preconditions for the experiments were derived from studies in a water-bath measuring system. Major objectives of these studies were the selection of the best urease inhibitor, the quantification of factors influencing the efficacy of the selected urease inhibitors, information about the fate of the injected urea nitrogen, as well as indications for long-term effects of the selected urease inhibitors on the formation of biogas and the effect of urease inhibitors in pig manure.

Materials and Methods

The water-bath measuring system [9, 10] consisted of 28 con-

Tab. 1

Percentage conversion of applied urea nitrogen after application of urease inhibitors at different inhibitor concentrations (cattle slurry, substrate temperature 15 °C)

Ureaseinhibitor (UI) *urease inhibitor (UI)*	**Variante** ***treatment***	Umsatz des Harnstoff-N zu NH_3-N [%] *conversion of urea-N to NH_3-N [%]*					Umsatz des Harnstoff-N zu NH_3- und NH_4-N [%] *conversion of urea-N to NH_3- and NH_4-N [%]*				
		n	**Mittelwert** ***mean***	Minimum *minimum*	Maximum *maximum*	Standard-abweichung *standard deviation*	n	**Mittelwert** ***mean***	Minimum *minimum*	Maximum *maximum*	Standard-abweichung *standard deviation*
-	**B**	52	**6**	3	11	2,2	41	**90**	62	127	11,2
C	**C3**	16	**6**	4	9	1,7	12	**91**	70	101	8,6
C	**C4**	32	**3**	2	6	1,1	29	**76**	38	101	16,4
D	**D3**	47	**1**	0	3	0,8	29	**33**	-3	89	20,5
D	**D4**	20	**1**	0	1	0,2	12	**15**	7	22	4,5
E	**E3**	8	**4**	3	5	0,6	8	**91**	60	111	14,7
E	**E4**	8	**3**	3	4	0,3	8	**89**	70	100	10,9
F	**F3**	4	**4**	3	4	0,4	4	**66**	57	81	11,1
F	**F4**	3	**2**	2	3	0,6	3	**29**	28	30	0,8
G	**G3**	4	**8**	6	9	1,6	4	**79**	72	82	5,1
G	**G4**	4	**6**	4	7	1,3	4	**76**	62	91	11,6

UI-Konzentration 3: Soll-Konzentration 0,01% von Total Kjeldahl Nitrogen
concentration 3 of UI: target value 0.01% of Total Kjeldahl Nitrogen

UI-Konzentration 4: Soll-Konzentration 0,1% von Total Kjeldahl Nitrogen
concentration 4 of UI: target value 0.1% of Total Kjeldahl Nitrogen

n: Anzahl der Wiederholungen
n: number of repetitions

tainers filled with 2 l of cattle or pig manure, respectively. The system worked according to the basic principle of a dynamic chamber. The use of the water-bath measuring system resulted in a precise and constant temperature of the substrates. All tests were carried out at 5, 15 and 25 °C, respectively. Each experiment was done with randomised repetitions of the different treatment variants. Ammonia concentrations (non-dispersive infrared spectroscopy) and the substrate temperatures in the respective container were measured every 45 min. The composition, mass, and pH of the slurry were recorded at the beginning and the end of each experimental period.

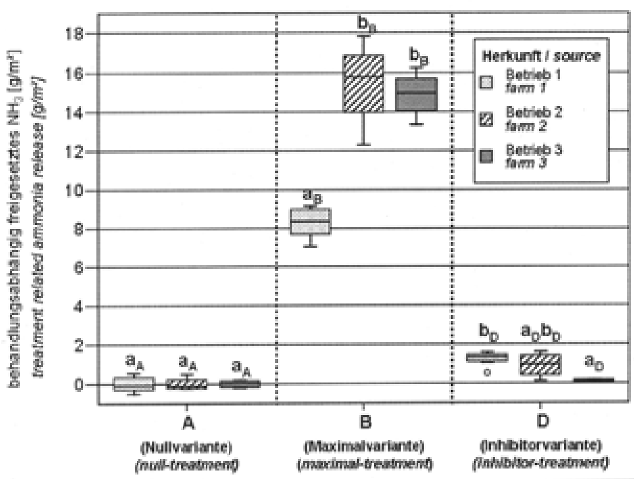
A standard experiment took a total of one week. After a single initial application of urease inhibitor solution (UIL) on day 0 (=Monday), the addition of 100 ml urea solution (HSL; concentration: 20 g/l) followed every 24 h (Monday to Friday). A total of five different urease inhibitors were investigated (variants C, D, E, F, G). The inhibitors D, E, F and G were actually developed by the project partners SKW Stickstoffwerke Piesteritz GmbH [6]. NBTPT (AGROTAIN®), a drug already introduced as an urease inhibitor in plant production was used as a reference (variant C). For the null-variant (variant A) no inhibitor and no urea was used, only water was added in same amounts. A non-inhibited variant B was included by adding water instead of inhibitor solution and adding urea solution like in the other treatments.

The concentration of the respective urease inhibitor solutions was calculated according to the Total Kjeldahl Nitrogen (TKN) in the slurry and the specified percentage (% of TKN). All data were collected with the software DASYLab, imported into an Excel spreadsheet and checked for plausibility. The statistical processing and analysis of the data was made with the program system SPSS 12.0.

Results

The five urease inhibitors differed clearly with regard to their effect on the urea hydrolysis and thus on the release of ammonia **(Table 1)**. The best achieved mitigation effect was determined by using the novel developed urease inhibitor D. For example, at a substrate temperature of 15 °C and an inhibitor concentration of 0.1% of TKN on average only 15% of the added urea were converted to ammonia-N and ammonium-N. By using the reference product NBTPT at the same concentration the urea hydrolysis was much higher with an average of 76%. The effect of urease inhibitor D depends on the substrate temperature: in order to achieve a significant reduction, at a substrate temperature of 25 °C a higher dosage with 0.1% of TKN had to be applied, whereas at lower substrate temperatures (5 and 15 °C) lower inhibitor concentrations (only 0.01% of TKN) were necessary for the same effect. Furthermore, for the urease inhibitor D also a significant dose-effect relationship was proven: with increasing concentration of the inhibitor, the urea hydrolysis and hence the ammonia release decreased.

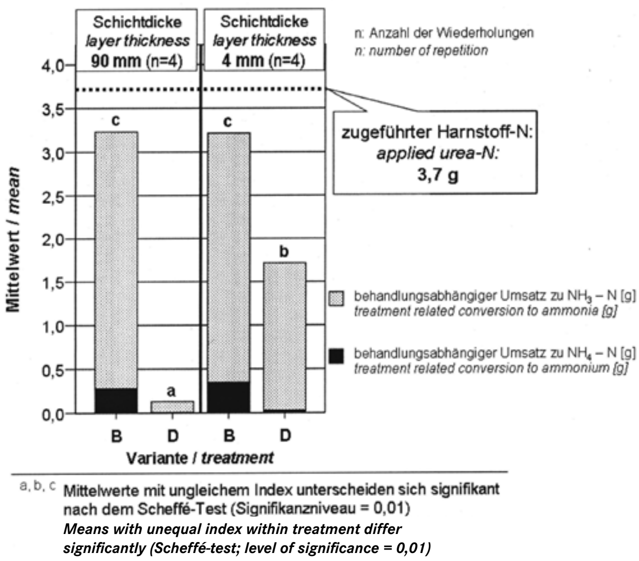
Fig. 1



a, b Mittelwerte mit ungleichem Index innerhalb einer Variante unterscheiden sich signifikant nach dem Scheffé-Test (Signifikanzniveau = 0,01)
Means with unequal index within treatment differ significantly (Scheffé-test; level of significance = 0,01)

Treatment related ammonia release after application of urea solution on slurry of different sources (target value of urease inhibitor concentration: 0.1% of Total Kjeldahl Nitrogen; substrate temperature 15 °C; cattle slurry)

Fig. 2



Treatment related conversion of nitrogen at different layer thickness of slurry (target value of urease inhibitor concentration: 0.1% of Total Kjeldahl Nitrogen; substrate temperature 15 °C; cattle slurry)

The relations between the quantity and frequency of urease inhibitor applications and resulting reduction effects were also analysed. Under laboratory conditions 8 mg of the urease inhibitor D had the same reduction effect independent of the type of application (1x8, 2x4, and 4x2 mg respectively on consecutive days).

For the studies of the effect of urease inhibitor D on cattle slurry (liquid manure) from different sources slurry was used from three different farms. The urease inhibitor D showed a significant reduction effect regardless of the origin of the slurry. Both the treatment-dependent release of ammonia as well as the conversion of the urea-N to ammonia-N and ammonium-N decreased, although the ammonia release from the untreated slurry (variant B in **Fig. 1**) differed according to its origin.

The studies of the effect of urease inhibitor D at different layer thickness of the slurry showed that the use of the inhibitor could significantly inhibit the urea hydrolysis and hence the release of ammonia, at a thickness of 4 mm as well as at a thickness of 90 mm. But differences between the B and D variants were observed (**Fig. 2**). For example, after using the urease inhibitor D with a layer thickness of 4 mm on average 46% of urea were hydrolyzed to ammonia-N and ammonium-nitrogen. At a larger layer thickness with 90 mm this amount was only 4% on average.

For calculating the nitrogen balance, the theoretically remaining level of Total Kjeldahl Nitrogen (TKN) was calculated at the end of a trial based on the experimental data and was then compared to the laboratory analysis of TKN. Both TKN values were almost identical. The differences were generally within the analytical error. So the occurrence of other nitrogen losses during the trials – such as by the release of nitrous oxide – can be excluded.

In further preliminary studies related to the urease inhibitor D, there were no adverse effects of the urease inhibitor D on biogas formation. According to the use of urease inhibitor D in pig manure, similar relationships could be identified like in cattle slurry.

Conclusion and outlook

With the novel developed urease inhibitor D, the urea hydrolysis could significantly be reduced under laboratory conditions. Because of the temperature-dependency and the dose-response relationship of the inhibitor D, the application of appropriate concentrations is necessary; particularly at higher substrate temperatures (> 20 °C) a higher inhibitor concentration is required for a good reduction effect. Under laboratory conditions the reduction effect of the inhibitor was – when applied at same total amounts - independent of the temporal distribution of individual smaller portions. Nevertheless it might be useful in practical farms to apply smaller amounts of the inhibitor more frequently, by that overcoming negative effects of animal movements and of the contamination of the treated area with fresh faeces. Observations in a practical farm [3] confirm these conclusions.

In future investigations the entire process chain up to the spreading of slurry in the field must be considered to find optimal technical solutions for the application of the inhibitor in the stable. Indeed, if the urea-N can be kept in the slurry during the entire process chain by inhibiting hydrolysis, environmentally harmful ammonia emissions can be reduced and furthermore a higher fertilizing value of manure will be available.

Literature

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[1] Amtul, Z., A. Rahman, R.A. Siddiqui, M.I. Choudhary (2002): Chemistry and Mechanism of Urease Inhibition. Current Medicinal Chemistry, 2002, Vol. 9, No. 14, p. 1323-1348

[2] ● Hartung, E. (2001): Ammoniak-Emissionen der Rinderhaltung und Minderungsmaßnahmen. In: KTBL-Schrift 406, Hrsg.: Kuratorium für Technik und Bauwesen in der Landwirtschaft e.V. (KTBL), Darmstadt

[3] ● Leinker, M. (2007): Entwicklung einer Prinziplösung zur Senkung von Ammoniakemissionen aus Nutztierställen mit Hilfe von Ureaseinhibitoren. Forschungsbericht Agrartechnik VDI-MEG Schrift 462, Dissertation Martin-Luther-Universität Halle-Wittenberg, ISSN-Nr. 0931-6264, Cuvillier Verlag, Göttingen, ISBN-Nr. 978-3-86727-460-9

[4] Ludden, P.A., D.L. Harmon, B.T. Larson, D.E. Axe (2000): Influence of the novel urease inhibitor N-(n-butyl) thiophosphoric triamide on ruminant nitrogen metabolism: I. In vitro urea kinetics and substrate digestion. J. Animal Science, 2000, Vol. 78, p. 181 – 187

[5] McCarty, G.W., J.M. Bremner, J.S. Lee (1990): Inhibition of plant and microbial ureases by phosphoramides. In: Plant and Soil, 127, p. 269-283, Kluwer Academic Publishers in the Netherlands

[6] Niclas, H.J., H.J. Michel, C. Schuster (2003): Ureaseinhibitoren zur Senkung der Ammoniakemissionen aus der Landwirtschaft. Antrag auf BMBF-Fördermittel im Rahmen der Fördermaßnahme „Integrierter Umweltschutz in der Wirtschaft“, SKW Stickstoffwerke Piesteritz GmbH (SKWP), Wittenberg-Piesteritz, Mai 2003

[7] Panetta, D.M., W.J. Powers, J.C. Lorimor (2005): Management strategy impacts on ammonia volatilization from swine manure. Journal of Environmental Quality, 2005, Vol. 34(3), p. 1119 – 1130

[8] Parker, D.B., S. Pandrangi, L.W. Greene, L.K. Almas, N.A. Cole, M.B.

Rhoades, J.A. Koziel (2005): Rate and Frequency of Urease Inhibitor Application for Minimizing Ammonia Emissions from Beef Cattle Feedyards. Transactions of the ASAE, ISSN 0001-2351, Vol. 48(2), p. 787 – 793

[9] Reinhardt-Hanisch, A. und E. Hartung (2006): Comparison of novel urease inhibitors to reduce ammonia emissions under laboratory conditions. Proceedings of the CIGR World Congress “Agricultural Engineering for a Better World”, Bonn 03. bis 07.09.2006, VDI-Verlag GmbH, Düsseldorf, 2006, VDI-Berichte Nr. 1958, Archive Nr. 440119580469, ISBN 3-18-091958-2, Abstract S.469-470

[10] ● Reinhardt-Hanisch, A. (2008): Grundlagenuntersuchungen zur Wirkung neuartiger Ureaseinhibitoren in der Nutztierhaltung. Forschungsbericht Agrartechnik VDI-MEG Schrift 471, Dissertation Universität Hohenheim, ISSN-Nr. 0931-6264, im Internet veröffentlicht unter: http://opus.ub.uni-hohenheim.de/volltexte/2008/302/

[11] Todd, C.R. & Z.F. Ming (2001): Efficacy of various microbial urease inhibitors on controlling ammonia emission from swine manure slurry (Study I – III). Project final report to Ontario Pork HEMS Program Canadian Pork Council

[12] Varel, V.H., J.A. Nienaber, H.C. Freetly (1999): Conservation of Nitrogen in Cattle Feedlot Waste with Urease Inhibitors. J. Animal Science, 1999, Vol. 77, p. 1162 - 1168

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