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Zusammenfassung der kumulativen Dissertation

## **Conditionability of micturition behaviour in cattle**

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## Summary

The focus of this work is on the conditionability of urination behaviour in cattle.

Cattle urinate and defecate apparently randomly in terms of time and place. In animal-friendly loose housing, this leads on the one hand to soiled walking and lying areas, which involve a health risk, and on the other hand to the release of environmentally harmful ammonia when faeces and urine are mixed. Due to the negative consequences of ammonia emissions, many countries have committed themselves to reducing them. So far, there are mainly technical solutions on the market that either prevent the contact of faeces and urine or prevent/disrupt the enzymatic reaction for ammonia formation. In this thesis, a behavioural approach is taken in which cattle are taught to use a latrine. The thesis is divided into three chapters.

**Chapter 1** includes the general introduction, which starts with the elimination behaviour of cattle and discusses the resulting problems for the environment and the animals. It then explains the legal framework for regulating emissions and nitrate occurrences in groundwater. This is followed by an overview of the more technical approaches to solutions, including their reduction potential. The first approaches for behaviour-based solutions are also discussed. The last section describes the objectives and hypotheses of the study.

**Chapter 2** presents the three published studies of this thesis.

Study 1 is a literature review that investigated whether cattle meet the neurophysiological and cognitive requirements for latrine training and what approaches exist for toilet training in both cattle and humans. It was found that the anatomy and physiology of the bladder and rectum in cattle does not differ significantly from that of other mammals. The voluntary and reflexive behaviours of latrine training were then elaborated. Other studies showed that both voluntary and reflexive behaviours can be conditioned in cattle. In toilet training with humans, operant procedures are used that start with either conditioning the voluntary behaviours or conditioning the reflexive behaviours.

Based on the results of Study 1, a training protocol was developed in experimental Study 2 in which the voluntary behaviours were trained first. Then the reflexive behaviours were superimposed into the behavioural chain. The voluntary behaviours were quickly learned by all the experimental calves. In 95% of the urinations in the alley, the calves inhibited or interrupted when they received the vibration signal, and in 65% of the cases they subsequently urinated in the latrine. In 63% of the urinations in the latrine, the calves were already oriented towards the exit before the reward. This indicates an association between urination behaviour and reward.

In experimental Study 3, reflexive behaviours were first trained for improved place conditioning. In the first training phase, 10 out of 16 calves showed rapid reward orientation and 11 out of 16 calves reached the final training phase. At the end of the training, the calves independently visited the latrine for  $\frac{3}{4}$  of all urinations. This indicates that calves have interoceptive awareness.

**Chapter 3** contains an overall discussion of the three studies. This includes a section on the placement in the learning theory background. The results are then classified in terms of their potential impact on the environment, cattle and farmers. Finally, an outlook on subsequent studies with regard to practical application is given and a final conclusion is drawn.

In this work, a training protocol for latrine use in cattle was developed and successfully demonstrated for the first time. The results pave the way for toilet training that can also be applied on farms to avoid ammonia production while improving animal health and welfare. As the use of a toilet leads to cognitive environmental enrichment, this offers animals the opportunity to actively influence their environment and avoid boredom.

## **Zusammenfassung**

Der Fokus dieser Arbeit liegt auf der Konditionierbarkeit von Miktionen bei Rindern.

Rinder urinieren und koten scheinbar willkürlich hinsichtlich Zeit und Ort. In tiergerechten Laufställen führt dies zum einen zu verschmutzten Lauf- und Liegeflächen, welche ein gesundheitliches Risiko bergen, zum anderen wird umweltschädliches Ammoniak freigesetzt, wenn Kot und Urin vermischt werden. Aufgrund der negativen Folgen von Ammoniakemissionen, haben sich viele Staaten dazu verpflichtet, diese zu reduzieren. Bislang sind vor allem technische Lösungen auf dem Markt verfügbar, die entweder den Ansatz verfolgen, den Kontakt von Kot und Urin zu unterbinden oder die enzymatische Reaktion zur Entstehung von Ammoniak verhindern/beeinträchtigen. In dieser Arbeit wird ein verhaltensbasierter Ansatz verfolgt, bei dem die Rinder lernen sollen eine Latrine zu nutzen. Die Arbeit ist in drei Kapiteln unterteilt.

Das **Kapitel 1** beinhaltet die generelle Einleitung und startet mit der Betrachtung des Ausscheidungsverhalten von Rindern und den daraus resultierenden Problemen für die Umwelt und die Tiere. Anschließend wird der gesetzliche Rahmen zur Regulierung von Emissionen und Nitratvorkommen im Grundwasser erläutert. Dann folgt eine Übersicht der eher technischen Lösungsansätze inklusive deren Minderungspotenziale. Ebenso wird auf bereits erste Ansätze zu verhaltensbasierten Lösungen eingegangen. Im letzten Abschnitt werden die Ziele und die aufgestellten Hypothesen zur Arbeit beschrieben.

**Kapitel 2** stellt die drei publizierten Studien dieser Arbeit vor.

Bei der ersten Studie handelt es sich um eine Literaturübersicht, in der herausgearbeitet wurde, ob Rinder die neurophysiologischen und kognitiven Voraussetzungen für ein Latrinentraining erfüllen und welche Ansätze es bei Rindern, als auch bei Menschen zum Toilettentraining gibt. Es konnte festgestellt werden, dass sich die Anatomie und Physiologie der Blase und des Rektums bei Rindern nicht wesentlich von anderen Säugern unterscheidet. Anschließend wurden die freiwilligen und reflexiven Verhaltensweisen des Latrinentrainings herausgearbeitet. Andere Studien zeigten, dass sowohl freiwillige als auch reflexive Verhaltensweisen bei Rindern konditioniert werden können. Beim Toilettentraining mit Menschen werden operante Verfahren verwendet, die entweder mit der Konditionierung der freiwilligen Verhaltensweisen oder mit der Konditionierung der reflexiven Verhaltensweisen beginnen.

Basierend auf den Erkenntnissen von Studie 1 wurde in der experimentellen Studie 2 ein Trainingsprotokoll entwickelt, in dem zuerst die freiwilligen Verhaltensweisen des Toilettentrainings erlernt wurden. Danach wurden die reflexiven Verhaltensweisen in die Verhaltenskette integriert. Die freiwilligen

Verhaltensweisen wurden von allen Testkälbern schnell gelernt. In 95 % der Miktionen im Gang, haben die Kälber diese aufgehalten oder unterbrochen, wenn sie das Vibrationssignal bekamen und in 65 % der Fälle haben sie anschließend in der Latrine uriniert. Bei 63 % der Miktionen in der Latrine haben sich die Kälber bereits vor der Belohnungsgabe zur Ausgabe orientiert. Das deutet, auf eine Assoziation zwischen Miktion und Belohnung hin.

In der experimentellen Studie 3 wurden die reflexiven Verhaltensweisen des Toilettentrainings, mit der Absicht einer besseren Platzkonditionierung, zuerst trainiert. In der ersten Trainingsphase zeigten 10 von 16 Kälbern eine schnelle Belohnungsorientierung und 11 von 16 Kälber erreichten die letzte Trainingsphase. Am Ende des Trainings suchten die Kälber bei  $\frac{3}{4}$  aller Miktionen selbstständig die Latrine auf. Dies deutet darauf hin, dass Kälber über eine interozeptive Wahrnehmung verfügen.

**Kapitel 3** beinhaltet eine übergreifende Diskussion der drei Studien. Diese umfasst einen Abschnitt über die Einordnung in den lerntheoretischen Hintergrund. Anschließend werden die Ergebnisse hinsichtlich ihrer potentiellen Wirkung für die Umwelt, die Rinder und die Landwirte eingeordnet. Zum Schluss wird ein Ausblick über nachfolgende Studien hinsichtlich des Praxiseinsatzes sowie ein abschließendes Fazit gegeben.

Erstmalig wurde ein Trainingsprotokoll für die Latrinennutzung entwickelt und erfolgreich demonstriert. Die Ergebnisse ebnen den Weg für ein praxistaugliches Toilettentraining, um die Produktion von Ammoniak zu vermeiden und gleichzeitig die Gesundheit und das Wohlbefinden der Tiere zu verbessern. Da die Benutzung einer Toilette zur kognitiven Umweltanreicherung führt, bietet dies den Tieren die Möglichkeit, ihre Umgebung aktiv zu beeinflussen und Langeweile zu vermeiden.

## Theses

### Objectives of research

In recent decades, dairy cows have increasingly been kept in loose housing, which is preferable to tie stalls for animal welfare and economic reasons. However, the larger space available in these housings also means that the area where cows defecate and urinate is larger. Often these are walking and lying areas, so the cows' claws and udders are constantly in contact with their excreta, which has a negative impact on health. In addition, this results in a large emission surface for the formation of ammonia. Ammonia is a secondary greenhouse gas that contributes, among other things, to the acidification of soils and water bodies, the eutrophication of nutrient-poor ecosystems and the formation of fine dust. We call this trade-off between animal welfare and environmental protection the climate killer conundrum. The solution to this conundrum could be the separation of faeces and urine so that ammonia formation is prevented. So far, there are already numerous technical approaches to achieve separation, but they only start when the two substances have already come into contact and, if necessary, the entire stable area has to be considered. A behavioural approach that allows animals to urinate and defecate either in separate places or only in one place (a toilet), so that the separation techniques would only need to be installed there, might be a better solution. There are already some approaches to this, but it has not yet been possible to reliably train the entire behavioural chain for toilet training. The aim of this work was therefore (1) to check literarily whether cattle in principle fulfil the neurophysiological and cognitive prerequisites for toilet training and (2) to test in a proof-of-concept how the training should be designed.

### Main Findings

In the context of the cognitive and neurophysiological requirements for toilet training, the following findings have been published in *Neuroscience & Biobehavioral Reviews* 115, 5-12 (2020):

- ✓ The somatic and autonomic nervous systems, along with central processes, are involved in the control of excretion.
- ✓ Voluntary control of the urinary and anal sphincters has been trained in various mammalian species by classical or operant conditioning or both.
- ✓ Cattle are capable of performing the voluntary parts of the toilet chain, i.e. remembering rewarding places and the routes to them and going to them in response to a signal.
- ✓ It has also been shown that calves and heifers are aware of their excretions, associate them with a reward and can increase the frequency of their urination in a specific place.

The following findings from the first experimental approach, in which voluntary behaviours were trained first, have been published in *Animals 10 (10), 1889 (2020)*:

- ✓ All test calves learned the voluntary parts of the behavioural chain (leaving the latrine, waiting for a vibration signal, moving to and entering the latrine, waiting in front of a blue light for the reward).
- ✓ Once the reflexive behaviours were superimposed in the chain, the calves stopped urinating outside the latrine as soon as they received the vibration signal in 95% of the cases and started urinating again when they were in the latrine in 65% of these cases.
- ✓ Thirty-one percent of all urinations of the test calves were self-initiated in the latrine.
- ✓ In 63% of all urinations that took place in the latrine, whether they started there or were re-triggered, calves oriented themselves to the reward bowl before a reward was delivered. This supports previous findings that calves can associate their excretions with a subsequent reward.

Finally, the following findings from the second experimental approach, in which the reflexive behaviours were trained first, have been published in *Current Biology 31, R1033-R1034 (2021)*:

- ✓ Calves that associated their urination more quickly with the reward also urinated more in the latrine. It can therefore be assumed that as soon as urination came under the control of the reward, the correct place for excretion was also learned.
- ✓ The control of urinary reflexes was quickly learned by 11 of the 16 calves, so that 77% of their behavioural sequences involved urinating into the latrine.
- ✓ Self-initiated urination into the latrine indicates that calves are able to consciously perceive endogenous signals and adapt their behaviour to them. This ability is called interoceptive perception.

## **Conclusions**

This work has shown not only theoretically but also practically that toilet training in cattle is possible. This paves the way for toilet training that can also be applied on farms, avoiding the production of ammonia while increasing animal health and welfare. In addition, the use of a toilet offers animals the opportunity to actively influence their environment and avoid boredom as it is cognitive environmental enrichment.