JUNE 2026

Projekt

From wood chips to Biobed: Impact on Behavior and Indoor Climate in Nilgai enclosures

Student: Stephanie Maria Lanchow
Advisor: Louise Flemmer Nielsen

School: Hansenberg 4. Core Program in Animal Care with a Specialization in Zoos
Internship location: Givskud Zoo - Zootopia

Project Description

For the final exam project in the Zoo Animal Keeper Education

Student's name: Stephanie Maria Lanchow

Project title:

From Wood Shavings to Biobed: Impact on Behaviour and Indoor Air Quality in the Nilgai Stable

Background of the project:

The project is being carried out at GIVSKUD ZOO – ZOOTOPIA and was initiated due to persistently elevated levels of gases, dust and particles in the nilgai stable, to which the animals are exposed on a daily basis.

Against this background, there was a need to explore alternative bedding solutions. Based on experience from other zoological institutions as well as GIVSKUD ZOO's own primate facility, Biobed was chosen as a relevant alternative.

It was therefore considered professionally relevant to investigate the effect of Biobed on the animals' behaviour and the air quality in the antelope stable.

Purpose of the project:

The purpose of this project is to investigate how changing the bedding material from wood shavings to Biobed affects the antelopes' behaviour and the indoor air quality in the stable.

The study is based on systematic behavioural observations conducted using surveillance cameras over a period of 46 days, divided into four months. Scan sampling is used as the observation method, as this method is well suited for studies of groups and the recording of behavioural states, which are central to this investigation.

To assess the indoor air quality, an air quality monitor and an ammonia meter are used for ongoing measurements in the stable.

Since optimising the animals' working environment is also part of the project, the work procedures involved in changing the bedding material will also be examined.

Theoretical areas on which the project is based:

- Behaviour, behavioural understanding & training
- Feeding
- Welfare
- Communication
- Facilities, adaptations and technology **(X)**
- Health and diseases
- Breeding and reproduction

Table of Contents

1. Method and Theory

The study was designed as a before-and-after study to enable a direct comparison of behaviour and indoor climate following the replacement of the bedding substrate. This section reviews the theoretical foundation of the project as well as the methodological considerations behind the behavioural observations.

1.1. Behavioural Observations

When studying animal behaviour, several observation methods are available. The choice depends on the research objective and the number of animals being observed. In this project, scan sampling with recordings every 15 minutes was selected. This method is well suited for herd animals, as several individuals can be observed simultaneously, providing an overview of the herd's overall behavioural patterns. Observations were conducted between 2:30 PM and 7:30 AM to minimise human influence, as nilgai are known to be highly adaptable to human presence. This made it easier to exclude behaviour affected by external factors. The observation period was also chosen based on the nilgai's natural activity patterns, consisting of foraging in the morning and evening and resting during the middle of the day. Following the development of an ethogram (Appendix 1), three behavioural states were selected: Standing in the stable, Lying in the stable, Staying outside the stable. The criteria for these categories are also presented in Appendix 1 (Martin & Bateson, 2007; Dawkins, 2007; Schaller, 1967; Dinerstein, 1980).

1.2. Air Quality

Animals in zoological parks often spend a significant amount of time in indoor stable areas, where environmental conditions differ from those outdoors. Stable air is influenced by factors such as temperature, ventilation, dust, and gases, which can be divided into climatic, physical, and chemical conditions. These factors have a major impact on animal health and welfare.To identify potential changes in air quality throughout the project, measurements were taken for PM2.5, PM10, TVOC, HCHO, and ammonia. PM2.5 and PM10 are airborne particles measuring 2.5 and 10 micrometres (µm), respectively. PM2.5 particles can penetrate deep into the lungs, while PM10 primarily affects the upper respiratory tract. In stable environments, particles originate from bedding material, feed residues, sand, and soil carried by the animals. High concentrations may lead to respiratory problems, affect the cardiovascular system, and increase stress levels in animals.TVOC and HCHO describe chemical pollution. TVOC refers to the total concentration of volatile organic compounds in the air, while HCHO (formaldehyde) is one specific compound. These substances may originate from urine, animal respiration, and plastic equipment in warm stables. HCHO is often measured separately because it is particularly harmful to health and may be carcinogenic with long-term exposure. Elevated levels of TVOC and HCHO can cause irritation of the eyes and respiratory tract and may indicate inadequate ventilation. CO_2 was also measured as an indicator of ventilation efficiency; however, as no changes were made to the ventilation system during the project, this parameter was not included further in the study.

[1] PM står for Particulate Matter og dækker over luftbårne partikler af forskellige størrelser (WHO, u.å.).
[2] TVOC står for Total Volatile Organic Compounds, som er en fællesbetegnelse for en stor gruppe kemiske stoffer og gasser i luften (DCCEEW, 2022).
[3] HCHO kaldes også formaldehyd og er en farveløs gasart med stærk lugt (WHO, u.å.).

Figure 1 shows the air-quality monitor used in the project (Kic et al., 2024; DCCEEW, 2022; Palmgren, 2018; WHO, n.d.; Hansenberg, 2023).

Picture 1. Air-quality monitor

Picture 2. Gastec pump

Regarding ammonia, it is produced through the breakdown of urine and faeces, as illustrated by the following equation:

$$(NH_2)_2CO + H_2O \Rightarrow 2NH_3 + CO_2$$

The first component in the equation is urea from faeces. Microorganisms convert urea into ammonia, which is released into the stable air. Ammonia concentration depends on several factors, including temperature, humidity, and stable use.

Ammonia is lighter than atmospheric air and therefore tends to rise. For this reason, concentrations were measured at two different heights during the project to investigate possible differences. High ammonia concentrations can cause irritation of the eyes and respiratory tract, headaches, and an increased risk of respiratory diseases following prolonged exposure. Consequently, ammonia levels should be monitored closely in stable environments for the benefit of animal health.

Figure 2 shows the Gastec pump used for the measurements in this project (Zhao et al., 2014; Bist et al., 2023).

1.3. Biobed

When it comes to flooring substrates, bedding material has a significant impact on both animal comfort and the stable environment. A biobed is a substrate based on the decomposition of organic material by microorganisms.To maintain a functional biobed, it is important to monitor temperature, moisture, and pH, as these factors influence microbial activity. An optimal environment for microorganisms requires a moist substrate; however, it must not become waterlogged, as this reduces oxygen availability.

Additionally, the pH level should remain close to neutral (pH 7), where most microbial processes function best. Microorganisms operate most efficiently at temperatures between 25°C and 40°C. Activity generally decreases at lower temperatures, while higher temperatures may damage the organisms.

Because substrate depth affects the establishment of a microbial environment, a minimum depth is required depending on animal density in the stable. Based on this theory, moisture, pH, and temperature were measured throughout the project to achieve optimal substrate conditions following the conversion. Picture 3 and 4 show the equipment used for substrate testing (Ny Vraa, n.d.a; Masebo et al., 2025).

Picture 3. Soil meter for pH and moisture. **Picture 4. Soil thermometer.**

Finally, substrate type plays an important role in the comfort and health of hoofed animals. Hard surfaces can place stress on joints, making it important to provide varied substrates that allow both relief and natural hoof wear.

In addition, hoofed animals should not spend extended periods in wet environments, as this can promote infections. Therefore, monitoring moisture levels in the biobed is highly relevant, as excessive watering may negatively affect both hoof health and microbial activity.

Furthermore, slippery surfaces may cause animals to lose their footing, which is why alternative solutions were considered throughout the project to avoid this issue (Ibid; Kwiatkowska-Stenzel et al., 2016).

Overall, this theoretical framework formed the basis for converting the substrate in the nilgai stable at Givskud Zoo from wood shavings to a biobed system.

2. The conversion work, including materials

2.1. Before the conversion

The foundations for this project were laid at the end of October, when I got in touch with Morten, the product manager at Ny Vraa, to enquire about the possibility of a collaboration, to which he agreed. In consultation with our animal keepers and biologist, we decided on the nilgai enclosure. Before the conversion, Figures 5 and 6 show that the base layer consisted of a thin layer of wood shavings with two ‘beds’ marked out by 45 x 100 mm battens, where there was a thicker layer of wood shavings. Appendix 2 shows a floor plan of the barn, where these beds are marked with dotted lines – labelled A and B. The battens form an edge that the animals must cross. However, this is not considered a safety issue, as nilgai are accustomed to moving across uneven terrain due to their natural habitat including woodland areas. It was decided to retain one of the sleeping areas, designated ‘A’, in the small room and to enlarge the area in the large room so that it included both areas B and C in Appendix 2, rather than just area B as was the case prior to the reorganisation. The expanded area with biobeds was created to provide the animals with a larger area in which to lie down, as the facility houses a population of 1,811 individuals (Dinerstein, 1980; Simonsen, 2026; Frisk, 2026).

Pictures 5 & 6. The stable with wood shavings before the refurbishment: the small pen on the left and the large pen on the right. The bedding area in the large pen was extended to include the rear section right up to the wall.

In November, I set up cameras to film the animals’ behaviour. Cameras were chosen as a cost-effective method that allowed for longer observation periods, which was relevant to a project focusing on permanent changes to the barn environment. At the same time, this method reduced human interference, as the observations could be recorded without the need for a physical presence in the barn. One camera was mounted on a pole at a height of 3.9 metres to cover both rooms in the barn. To ensure a complete overview, two smaller cameras were also installed: one at the entrance and one in a corner at a height of 2.3 metres. From the end of November, I carried out 23 days of observations prior to the reorganisation. In addition, I placed an air quality monitor at a height of 2 metres in a corner of the barn. I chose this location because the area was less affected by draughts from the door and was also situated in the large area where the animals spent most of their time. The positions of the cameras and the air quality monitor are shown in appendix 2

The behavioural observations were recorded in a spreadsheet, noting the date, time and behavioural state. Here, I noted the number of calves and adults at each observation, after which the activity percentages were calculated to compare the distribution of behaviour before and after the conversion (Appendix 3). Air quality measurements of HCHO, TVOC, PM2.5 and PM10 were also recorded over 15 days to provide an overall picture of the trends. Following the conversion, the number days since watering were also recorded to investigate the correlation between watering and air quality (Appendix 4).

During the same period, I took ammonia samples on 10 occasions using a Gastec pump with an accompanying indicator tube in order to obtain results that were as accurate as possible. I carried out the measurements in the morning, when the animals had been in the barn for some time. Measurements were taken both at floor level (approx. 25 cm) and at the height of the nilgai's head (approx. 150 cm) to reflect the areas where the animals spent most of their time.

I decided to take the samples in the middle of the large room, which corresponded to the animals' preferred area. The results were recorded in a spreadsheet, noting the date, location and value (Appendix 5). Watering days were also noted following the changeover to investigate the relationship between watering and ammonia concentration. The measurement points are shown in Appendix 2.

To calculate the amount of biobed required, I sought advice from Ny Vraa and drew on the experience of my colleagues who were already using the material for animals such as rhinos, giraffes and pygmy hippos. According to Ny Vraa's guidelines, a base layer of 20–25 cm is recommended for animals weighing between 100 and 500 kg, whilst a minimum of 15 cm is recommended for horses. As nilgai weigh approx. 200–270 kg, I based my calculations on a layer 20 cm thick. The site plan in Appendix 2 shows the areas for biobed zones A, B and C. The calculations below show the quantity of biobed required to create a 20 cm layer (Ny Vraa, n.d.-a; Ny Vraa, n.d.-b; Dinerstein, 1980).

$$\text{Total area: } 17,75\, m^2 + 18,00\, m^2 + 22,50\, m^2 = 58,25\, m^2$$

$$m^3 \text{ at a height of } 20\, cm\text{: } 58,25\, m^2 \cdot 0,2\, m = 11,65\, m^3$$

For financial reasons, we as a company decided to order a whole container to be shipped home, as this saved us a number of costs, since we would then have enough to supply other stables in the long term.

2.2. The Convertion and the period that followed

At the beginning of February, the substrate was replaced and looked as shown in Figures 7 and 8.

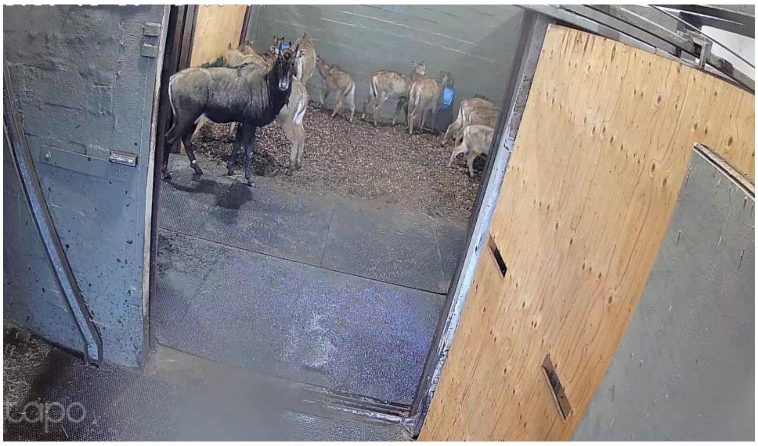
Picture 7 & 8. The enclosure with biobed after the conversion.

I decided to scatter a little Biobed outside the beds because of the slippery floor, until a different solution could be found.

To begin with, I put on an FFP3 mask and goggles, as removing the shavings was a very dusty process. I cleared the area using a snow shovel and an electric wheelbarrow to make the heavy work a bit easier. After 1.5 hours, all the topsoil had been removed, and the area was swept before I received help from the technical department to bring in the biobed. Whilst I stood there spreading the new biobed myself, our gardener worked it in with the machine. Two hours later, all the Biobed had been brought in, and the studs were put back in place in the large room, as I'd had to unscrew them to make room for the machine – they're fixed to the walls with angle brackets. Below are some photos from the day the barn was cleared out and the Biobed brought in.

Picture 9 & 10. The enclosure on the day of conversion.

As the litter was already damp, it was not watered that day. To gain a better understanding of the litter's impact on the indoor climate and behaviour, a couple of days passed before observations and measurements began. I then carried out observations for a further 23 days to establish the same baseline as before the change. In mid-February, I got in touch with TDJ in Holstebro and requested a quote for rubber mats, as the concrete floor was very slippery and we wanted to avoid other types of bedding that might affect the biobed. We'd had good experience with the rubber mats in our savannah barn, which is why, in consultation with my colleagues, I considered this to be a good solution. In the last week of February, one of our technicians spent half a day helping me lay the mats, as the work was physically demanding and he also had experience of similar tasks from the giraffe barn. Figures 11 and 12 below show the mats in the barn, whilst Appendix 2 illustrates the distribution of Biobed and mats in the area. The following weekend, I decided to wash down the entire barn to gain a better insight into how the bedding affected the barn environment, and at the same time create a more consistent starting point for the work ahead. As the cleaning had to be carried out using a high-pressure washer in the tall barn, the work was both dirty and laborious. I therefore wore rainwear, a mask, ear defenders and safety goggles throughout the process (Simonsen, 2026; Karmark, 2026; Frisk, 2026).

Picture 11 & 12. The rubber mats in the stable on the day of washing.

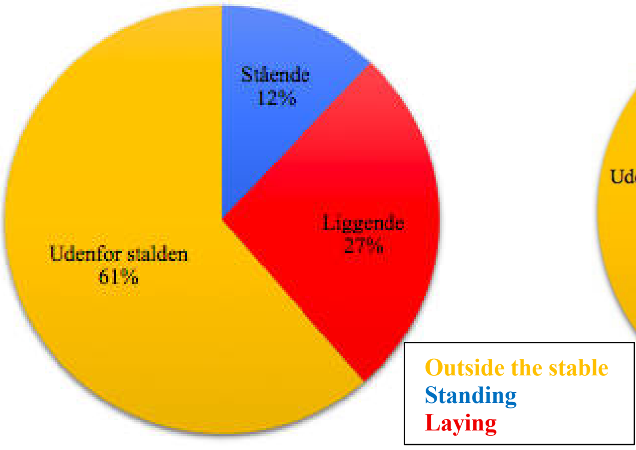
After the conversion and cleaning, I continued my behavioural observations using the cameras as before. At the same time, I began testing the bedding to identify the most optimal conditions for a microbial environment. Using camera footage, I also documented the workflow in the barn in order to draw up a work plan that combined an effective bedding system with a practical daily routine for the animal keepers. I recorded the start and end times for the same 23 days during which the animals' behaviour was observed, and at the same time noted the days on which watering took place, in order to investigate the relationship between working hours and watering (Appendix 6).
Samples of the substrate were taken on watering days immediately before watering in order to assess the optimal interval between waterings. The samples were taken at the same location in the centre of the two biobed areas to provide an accurate picture of the areas as a whole and to enable a comparison of developments between the rooms. The locations of the samples are shown in the appendix 2. pH and moisture content were measured using a combined soil tester, which was placed approximately halfway down into the topsoil and read after 10 minutes. pH was measured according to the standard pH scale, whilst moisture content was assessed on a scale of 1–10, where 8–10 was classified as wet, 4–7 as normal and 1–4 as dry. Temperature was measured using a soil thermometer placed in the same location and at the same depth as the soil tester to assess the subsoil's ability to support a microbial environment. The results were recorded in a spreadsheet, broken down by watering on the 2nd, 3rd and 4th days, with seven measurements in each category (Appendix 7).

Finally, I took ammonia measurements over 10 randomly selected days, using the same method, equipment and locations as before the change, in order to make a direct comparison of the results before and after.[4]

[4] All samples and observations were taken during a period when the average daily temperature did not exceed 10°C, in order to ensure the most accurate basis for comparison.Resultater

3.1. Behavioural observations

Data from behavioural observations revealed significant changes in both adults and calves following the conversion. Figures 1 and 2 show that the proportion of time calves spent lying down in the barn increased from 27% to 47%, whilst the proportion of calves standing increased marginally from 12% to 13%. In contrast, the proportion of calves outside the barn fell from 61% to 40% following the change. The results indicate that, following the change, the calves spent more time in the barn and devoted more time to resting than before.



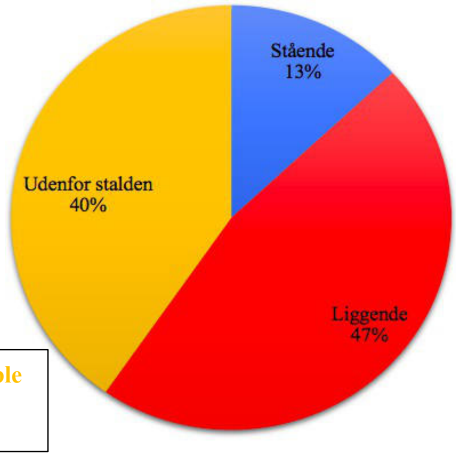
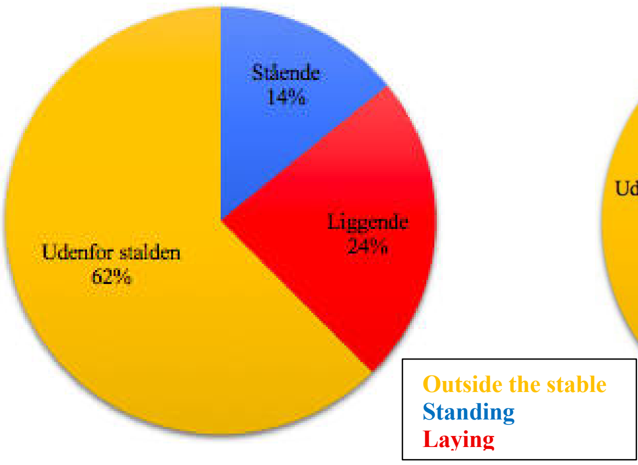


Figure 1. Percentage distribution of the calves' behaviour BEFORE the switch to organic bedding.

Figure 2. Percentage distribution of the calves' behaviour AFTER the switch to organic bedding.

A similar trend was observed in the adult animals, as shown in Figures 3 and 4, where the proportion of lying behaviour increased from 24% to 47%, whilst the proportion of standing behaviour fell from 14% to 13%. The proportion of behaviour outside the barn fell from 62% to 40%. This indicates that the adult animals are using the barn more for rest than before the conversion, which may be seen as a sign of increased comfort with the new bedding.



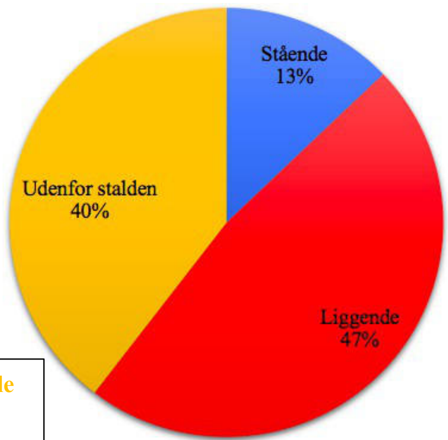


Figure 3. Percentage distribution of adult animals' behaviour BEFORE conversion to Biobed.

Figure 4. Percentage distribution of the behaviour of adult animals AFTER conversion to organic farming.

Based on these data and figures, it can be concluded that, following the conversion, the animals spend more time lying down in the barn across all age groups. It is noteworthy that the difference in behaviour between calves and adult animals has decreased following the conversion. Another change in the animals’ behaviour is evident from Figures 13 and 14, which show that the distribution of the animals within the barn is different. Before the conversion, the animals tended to be concentrated in the centre of the barn, whereas after the conversion they are distributed more evenly across the entire floor area.

Picture 13. Video footage before the conversion.

Picture 14. Video footage AFTER the conversion.

The results suggest that the biobed has a positive effect on the nilgai’s use of the barn. Increased lying behaviour and distribution within the barn may indicate greater comfort in the barn, as resting behaviour and distribution are usually signs of a sense of security (Masebo *et al*., 2025; Kwiatkowska-Stenzel *et al.,* 2016).

3.2. Air Quality

The air quality measurements show significant reductions in the various concentrations following the changeover. Figure 5 shows that the average concentration of HCHO fell from 0.435 mg/m³ to 0.041 mg/m³, which is a reduction of 90.5%, whilst the total TVOC concentration fell from 1.112 mg/m³ to 0.138 mg/m³, corresponding to 87.6%. This suggests that Biobed helps to reduce the accumulation of these compounds in the barn, thereby bringing the concentration of HCHO below the 8-hour limit value of 0.37 mg/m³ (Appendix 8).

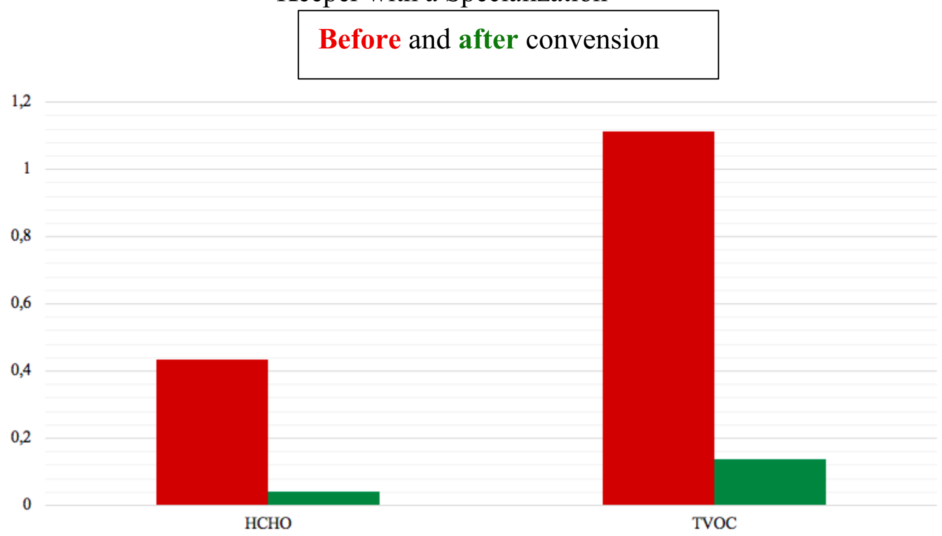


Figure 5. Average change in TVOC and HCHO, measured in mg/m³.

Particle levels fell at the same time as the conversion, as shown in Figure 6. PM2.5 fell from 247 µg/m³ to 37 µg/m³, i.e. by 85 per cent, whilst PM10 fell from 293 µg/m³ to 53 µg/m³, corresponding to 81.9 per cent. However, both types remain just above the annual average limit values of 25 µg/m³ and 40 µg/m³ respectively (Appendix 8). Nevertheless, it can be seen that biobed reduces airborne particulate matter more effectively than wood shavings.

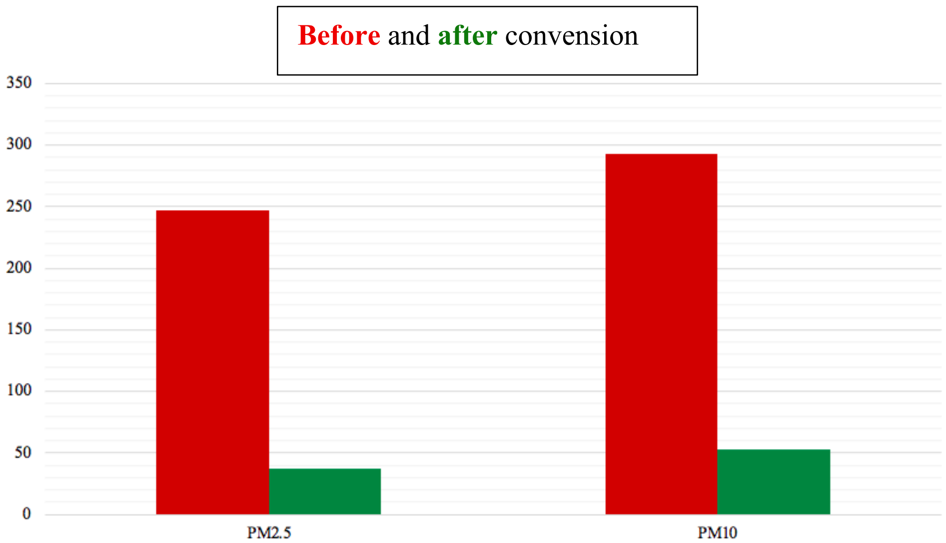


Figure 6. Average change in PM2.5 and PM10 measured in ug/m³.

Finally, Figure 7 shows the results of ammonia measurements, where the average concentration at floor level fell from 19 ppm to 6 ppm – a reduction of 68.4 per cent – whilst at head height it fell from 22 ppm to 7 ppm, i.e. a reduction of 68.2 per cent (Appendix 5). This demonstrates improvements across the entire barn area where the animals are kept. Measurements also show that the ammonia concentration begins to rise at the 8th–10th measurement, where watering takes place every 4 days rather than every 2 or 3 days. In light of these data, regular watering appears to have an effect on reducing ammonia levels.

Before and **after** conversion

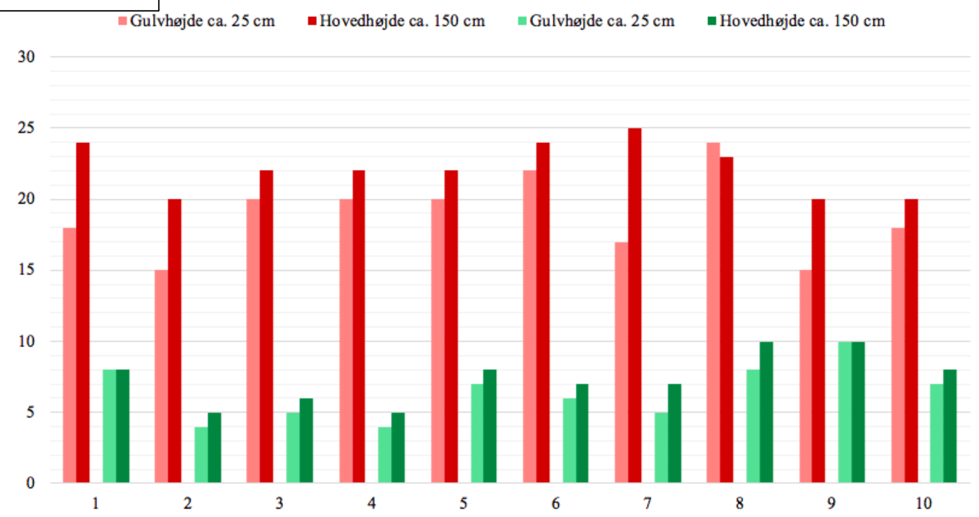


Figure 7. Ammonia measurements taken 10 times before (red) and after (green) the changeover, measured in ppm.

Overall, the results of the air quality measurements show that switching from wood shavings to bio-beds has a significant impact on air quality in the barn.

3.3. Workflow

With a view to optimising the animal keepers' workflow, a study was carried out into daily care routines and time spent in the barn. The aim was to develop a working method that both ensures a well-functioning bedding system and is practical in day-to-day operations. The data in Figure 8 show that the average working time in the barn rose from 16.61 minutes to 21.30 minutes, corresponding to an increase of 4.69 minutes or 28.2 per cent. The fluctuations in the graph clearly reflect the days on which the bedding is watered (days 1, 3, 5, 7, 10, 13, 16, 19 and 22). However, the difference compared with the remaining days is only a few minutes.

Before and **after** conversion

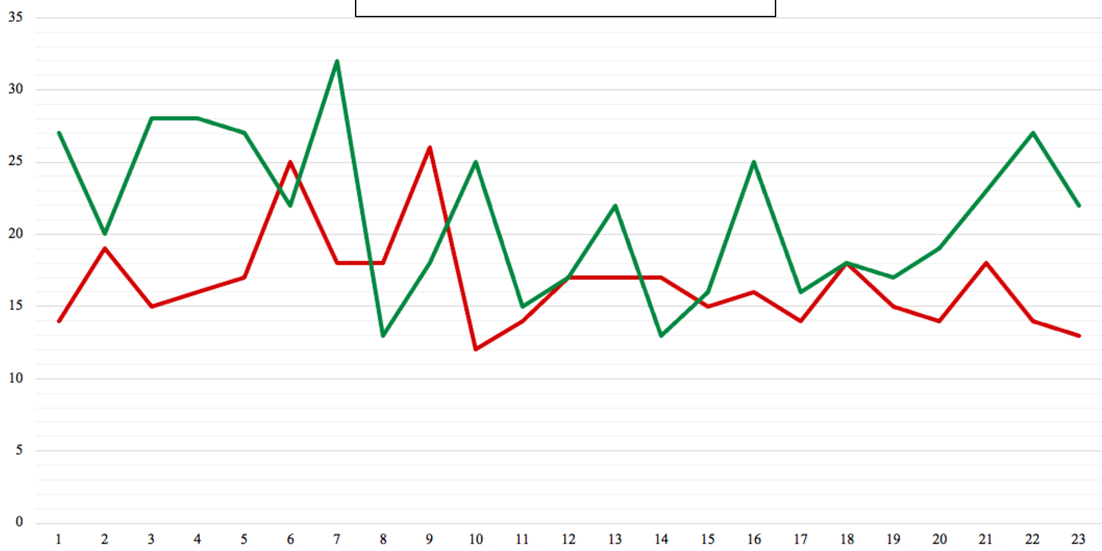


Figure 8. Daily workflow in minutes, broken down over 23 days before and after the changeover.

To provide a basis for a practical work plan for the animal keepers, a series of samples were taken from the substrate to determine the optimal conditions. Figure 9 shows the changes in pH and moisture content at three different watering intervals.

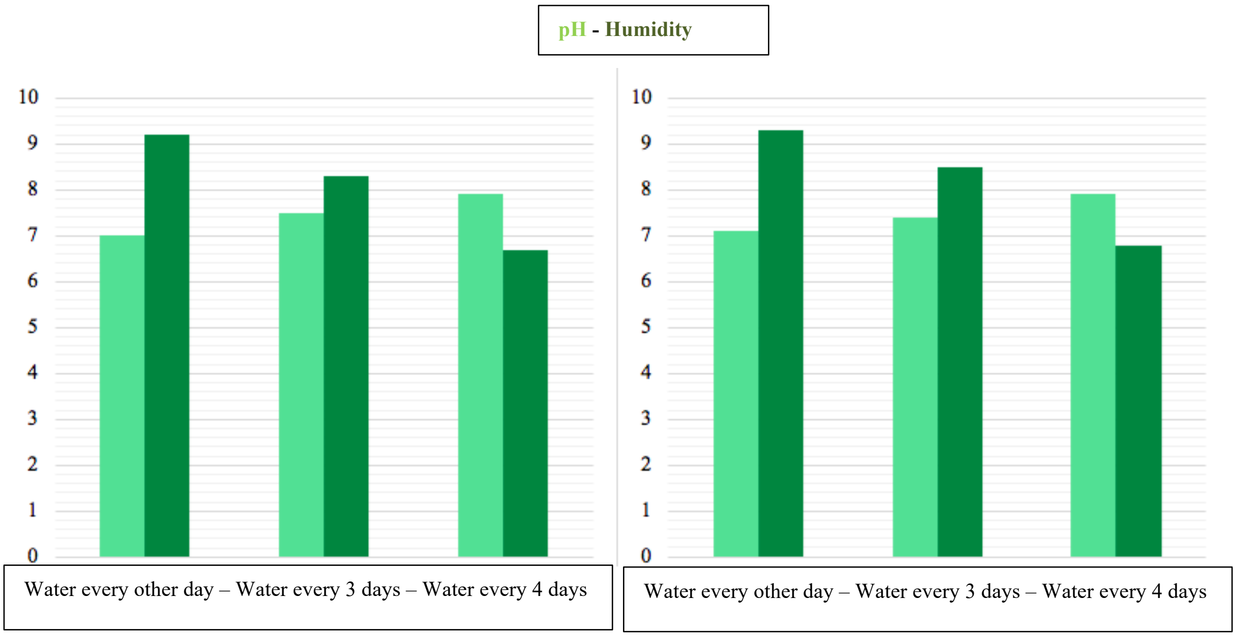


Figure 9. pH and humidity in a small room (left) and a large room (right).

The figure shows a correlation between the number of days between watering and the moisture content and pH of the topsoil. Initially, the moisture content was relatively high at just over 9 when watering every other day, which is categorised as ‘wet’. In this context, the pH remained stable around the neutral level of 7. When watering every 4 days, the moisture content fell to around 6.7 in both rooms – which is at the drier end of the scale – whilst the pH rose to around 7.9. In contrast, the pH remained at 8.3–8.5 when watering every 3 days, with a pH of 7.4–7.5, i.e. closer to the neutral range. Watering every 3 days therefore provides the optimal combination of pH and moisture levels.

4. Data processing

Based on the results, a number of improvements can be seen in both the air quality in the barn and the animals’ use of the barn and their lying behaviour. The conversion has led to a clear reduction in particles and chemical compounds in the barn air. The concentration of HCHO is just below the limit value, whilst the particle count is approaching the limit value – though it remains slightly above it, as shown in Figures 5 and 6 (Appendix 8).

Data show that the ammonia concentration is affected by the irrigation interval, as the activity of the microorganisms depends on the oxygen conditions in the bottom layer (Figure 7; Appendix 5). The microorganisms in the new substrate will, through nitrification, convert ammonia into nitrite and then nitrate, which can subsequently be converted into free nitrogen through denitrification. As free nitrogen constitutes the majority of the air in the atmosphere, this is not considered to pose a risk to the animals. The reduction in ammonia is regarded as particularly relevant in terms of both animal welfare and the working environment. The measurements also show that regular watering has a positive effect on air quality, as the particle concentration has fallen following the conversion (Figure 6; Appendix 4). The significant reduction in particulate matter can probably be explained by the removal of wood shavings, which were assessed as being the main cause of the dusty indoor environment. However, the level of particulate matter remains above the Ministry of the Environment's guidelines and the WHO's recommendations (Appendix 8). The WHO's recommendations are stricter than those of the Ministry of the Environment, but they are relevant to include in the assessment of health-related factors – particularly during periods when the animals spend more time indoors (Kic et al., 2024; Bist et al., 2023).

The optimal balance in the substrate was recorded when watering was carried out every 3 days, with a moisture content of approximately 8.5 and a pH of 7.5 (Figure 9). These are considered the most favourable conditions for the microorganisms, as they thrive best at a neutral pH and a moisture content that allows sufficient oxygen in the substrate. The results make it possible to draw up guidelines for animal keepers. Working hours increased by just under 5 minutes per day, primarily due to watering (Figure 8). Daily work continues to consist of feeding, removing straw bedding and cleaning the communal toilet areas that nilgai are known to establish. New tasks include watering and, where necessary, replenishing the bedding. It is therefore important to strike a balance between improved animal welfare and a slight increase in working hours when using biobeds (Dinerstein, 1980; Ny Vraa, n.d.-a).

To accommodate both animal carers and the animals, adjustments were made on an ongoing basis. As described in section 2.1, the edges of the beds were moved to create larger lying areas. In addition, the use of slats was regularly evaluated in collaboration with the animal carers. Experience from a trial period without them shows that the slats are beneficial, as they help the biobed to hold together better. During the sampling, the air quality monitor was also moved from the small room to the large room, as the animals were mainly kept there, thereby making the measurements more representative. All the samples used were taken after the monitor had been moved. Finally, the observations reveal changes in the animals' use of the barn. The photographs in section 3.1 show that, following the conversion, the animals are more spread out within the barn, which may indicate a greater sense of security. The increased lying behaviour may also suggest improved comfort with the new bedding. As the observations were made in early spring, it will be relevant to monitor developments further to investigate how the animals use the barn in the longer term.

5. Conclusion

This project documented that nilgai spent more time in the barn following the switch from wood shavings to biobed. It was observed that the animals lay down more whilst indoors and, at the same time, were more spread out within the barn. Furthermore, air quality measurements showed a clear reduction in both the amount of particulate matter and the concentration of gases in the air, with a decrease of over 50 per cent in all measurements. With regard to the animal keepers' work processes, it emerged that daily tasks took slightly longer on average following the changeover. It is therefore important to assess both the improved air quality and the changes to work processes as a whole, in order to weigh up the pros and cons of the transition from wood shavings to biobed.

6. Bibliografi

6.1. Litterature and documents

- Bist, R. B., Subedi, S., Chai, L. & Yang, X. (2023): *Ammonia emissions, impacts, and mitigation strategies for poultry production: A critical review*. Journal of Environmental Management.
- Dawkins, M. S. (2007): *Observing animal behaviour.* Oxford University Press.
- Dinerstein, E. (1980): *An ecological survey of the Royal Karnali-Bardia Wildlife Reserve, Nepal.* Biological Conservation, vol. 16, issue 4.
- Hansenberg (2023): *Klimakompendium.* Hansenberg.
- Kic, P., Wohlmuthová, M. & Starostová, L. (2024): *Effects on the Indoor Environment in a Stable for Horses in Winter: A Case Study.* Agriculture, vol. 14, issue 8.
- Kwiatkowska-Stenzel, A., Sowińska, J. & Witkowska, D. (2016): *The effect of different bedding materials used in stable on horses behavior*. Journal of Equine Veterinary Science.
- Martin, P. & Bateson, P. (2007): *Measuring Behaviour: An Introductory Guide.* Cambridge University Press, 3rd ed.
- Masebo, N. T., Benedetti, B., Mountricha, M., Lee, L. & Padalino B. (2025): *A Literature Review on Equine Bedding: Impacts on Horse and Human Welfare, Health, and the Environment.* Animals, vol. 15, issue 5.
- Ny Vraa (u.d.-a): *General biobed information: How zookeepers can maintain a healthy bioactive substrate.* Ny Vraa ApS.
- Ny Vraa (u.d.-b): *Biobed vejledning A4 - heste.* Ny Vraa ApS.
- Palmgren, F., Ellermann, T., & Hertel, O. (2018): *Luftkvaliteten.* Miljøets Fodspor nr. 2. Miljøstyrelsen.
- Schaller, G.B. (1967): *The Deer and the Tiger: A Study of Wildlife in India.* University of Chicago Press.

6.2. Homepages

- Ministry of Employment (2025): Order No. 1356 on limit values for substances and materials (chemical agents) in the working environment. Leagal Information. https://www.retsinformation.dk/eli/lta/2025/1356 (besøgt 18.04.26)

- DCCEEW (2022): *Total volatile organic compounds.* Department of Climate Change, Energy, the Environment and Water, Australian Government. https://www.dcceew.gov.au/environment/protection/npi/substances/fact-sheets/total-volatile-organic-compounds (visited 20.04.26)
- Miljø- og Ligestillingsministeriet (2017): *Executive Order on the Assessment and Management of Air Quality (Executive Order No. 1472 of 12 December 2017).* Leagal Information. https://www.retsinformation.dk/eli/lta/2017/1472 (visited 26.04.26)
- WHO (2010): *WHO guidelines for indoor air quality: Selected pollutants.* World Health Organization. https://iris.who.int/handle/10665/260127 (besøgt 22.04.26)
- WHO (u.å.): *Air quality, energy and health: Types of pollutants.* World Health Organization. https://www.who.int/teams/environment-climate-change-and-health/air-quality-and-health/health-impacts/types-of-pollutants (visited 22.04.26)
- Zhao, L., Manuzon, R. & Hadlocon, L. J. (2014): *Ammonia emission from animal feeding operations and its impacts.* Ohio State University Extension. https://ohioline.osu.edu/factsheet/AEX-723.1 (visited 28.04.26)

6.3. Personal communication

- Frisk, T. (2026). Dyrepasser, Givskud ZOO - Zootopia. tlf. 21 20 01 40
- Karmark, L. (2026). TDJ Andersen, Holstebro. mail: lene@tdj.dk
- Simonsen, K. S. (2026). Biolog Givskud ZOO - Zootopia. mail: kss@givskudzoo.dk

7. Appendix 1-8

Appendix 1

Behavioural catalogue and excerpt from an ethogram

Behaviour Catalogue (selected behavioural states)

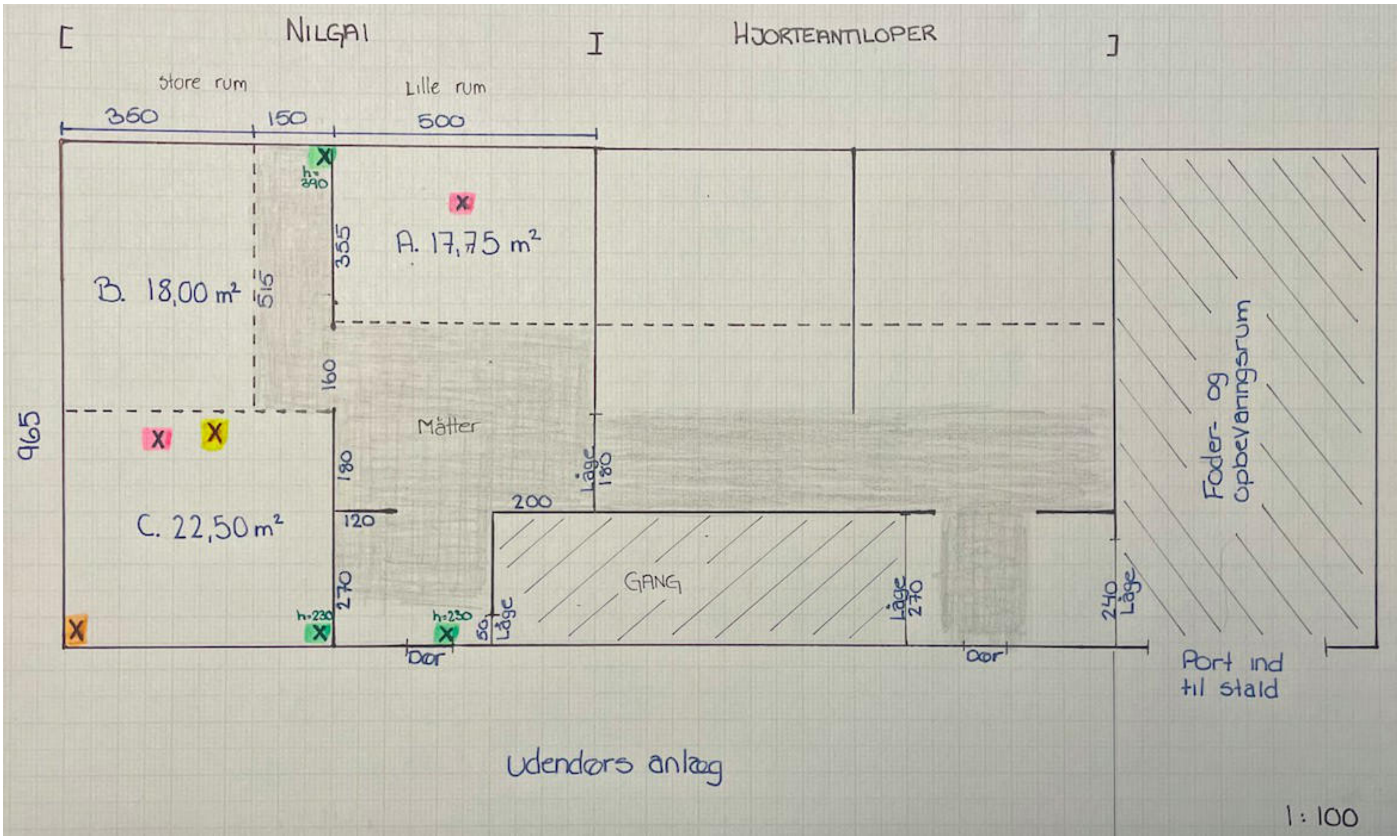
Behaviour element	Abbreviation	Category	Description
Lying in the barn	Lying	State	The animal is included regardless of where in the barn it is lying or what it is doing, but it is lying down. The animal must be lying on the floor – the head may be raised. Animals in the process of lying down are also included.
Standing in the barn	Standing	State	The animal is included regardless of where in the barn it is standing or what it is doing, but it is standing. Animals in the process of getting up are also included.
Time spent outside the barn	Outside barn	State	The animal is outside the barn. All individuals not observed in the barn are included.

Ethogram (15 min. ad-libitum observation)

Behaviour element	Abbreviation	Category	Description	Remarks
Lying with head on the floor	LI	State	The animal is lying still with its head resting on the floor.	
Lying down and chewing the cud	LG	State	The animal is lying down and ruminating.	Head is raised.
Grooming	GR	Event	The animal performs some form of grooming.	Both allo- and autogrooming.
Eating hay	EH	Event	The animal stands and eats hay.	
Grunting	G	Event	The animal makes a grunt.	The animal may be lying down or standing.
Eating from a feeding trough	EF	Event	The animal eats from a feeding trough.	
Lying down	LÆ	Event	The animal is in the process of lying down.	

Bilag 2

Floor plan of the enclosure



All length measurements are in cm.
Labels: 1. **Cameras**; 2. **Air quality monitor**; 3. **Ammonia measurements**; 4. **Biobed measurements.**

Appendix 3

23/11/25 – Behaviour observations

Time point	Standing – Adults	Standing – Calves	Lying – Adults	Lying – Calves	Outside barn – Adults	Outside barn – Calves
00:00:00	2	1	1	2	7	8
00:15:00	3		1	4	6	7
00:30:00	2		2	2	6	9
00:45:00	1		3	4	6	7
01:00:00			4	3	6	8
01:15:00			4	3	6	8
01:30:00	1	1	4	3	5	7
01:45:00			5	4	5	7
02:00:00	1		4	4	5	7
02:15:00	2	2	1	3	7	6

Bilag 2

Accumulated observations (Condition)

Condition	Animal group	Count
Standing	Adults	2238
	Calves	2085
	Total	4323
Lying	Adults	3724
	Calves	4669
	Total	8393
Outside barn	Adults	9908
	Calves	10703
	Total	20611

The table shows an example of how behavioural observations are recorded and totalled at the end to calculate the activity percentag

Bilag 4

Air quality measurements

Biobed Measurements

Watering every 2nd day	SMALL ROOM		LARGE ROOM		Comments
	pH	Humidity	pH	Humidity	
16/2	6,5	8,5	7	9,5	Temperature (small room) 36°C. Temperature (large room) 32°C.
18/2	7	10	7,5	10	Temperature (small room) 38°C. Temperature (large room) 31°C.
20/2	7	9,5	6,5	8,5	Temperature (small room) 37°C. Temperature (large room) 32°C.
22/2	7,5	9	7	8,5	Temperature (small room) 38°C. Temperature (large room) 34°C.
24/2	6,5	10	7	9	Temperature (small room) 35°C. Temperature (large room) 29°C.
26/2	7	9	7,5	10	Temperature (small room) 36°C. Temperature (large room) 33°C.
28/2	7,5	8,5	7,5	9,5	Temperature (small room) 35°C. Temperature (large room) 32°C.
AVERAGE	**7**	**9,2**	**7,1**	**9,3**	

The table shows an example of how air quality measurements are recorded with values. The values prior to the change are recorded in the same way—though without the column for watering days.

Appendix 5

Ammonia measurements

Before the changeover

Date	ppm – Floor height approx. 25 cm	ppm – Head height approx. 150 cm
22/12	18	24
25/12	15	20
29/12	20	22
31/12	20	22
6/1	20	22
9/1	22	24
14/1	17	25
15/1	24	23
22/1	15	20
28/1	18	20
AVERAGE	19	22

Bilag 4

After the changeover

Date	ppm – Floor height approx. 25 cm	ppm – Head height approx. 150 cm	Days since last watering
18/2	8	8	2 days
24/2	4	5	2 days
28/2	5	6	2 days
2/3	4	5	The entire barn was washed 2 days previously
8/3	7	8	3 days
14/3	6	7	3 days
17/3	5	7	3 days
5/4	8	10	4 days
9/4	10	10	4 days
13/4	7	8	4 days
AVERAGE	6	7	

The table shows a sample of how the ammonia measurements are recorded. The values prior to the change are recorded in the same way—though without the column for irrigation days.

Appendix 6

Work flow

After the changeover

Date	ppm – Floor height approx. 25 cm	ppm – Head height approx. 150 cm	Days since last watering
18/2	8	8	2 days
24/2	4	5	2 days
28/2	5	6	2 days
2/3	4	5	The entire barn was washed 2 days previously
8/3	7	8	3 days
14/3	6	7	3 days
17/3	5	7	3 days
5/4	8	10	4 days
9/4	10	10	4 days
13/4	7	8	4 days
AVERAGE	6	7	

The table shows a sample of the recorded working hours spent in the barn. The same table was created for the workflow prior to the reorganization—though without the "watering" columns.

Bilag 7

Measurements in Biobed

Biobed Measurements

Watering every 2nd day	SMALL ROOM		LARGE ROOM		Comments
	pH	Humidity	pH	Humidity	
16/2	6,5	8,5	7	9,5	Temperature (small room) 36°C. Temperature (large room) 32°C.
18/2	7	10	7,5	10	Temperature (small room) 38°C. Temperature (large room) 31°C.
20/2	7	9,5	6,5	8,5	Temperature (small room) 37°C. Temperature (large room) 32°C.
22/2	7,5	9	7	8,5	Temperature (small room) 38°C. Temperature (large room) 34°C.
24/2	6,5	10	7	9	Temperature (small room) 35°C. Temperature (large room) 29°C.
26/2	7	9	7,5	10	Temperature (small room) 36°C. Temperature (large room) 33°C.
28/2	7,5	8,5	7,5	9,5	Temperature (small room) 35°C. Temperature (large room) 32°C.
AVERAGE	**7**	**9,2**	**7,1**	**9,3**	

The table shows the pH, moisture content and temperature readings taken from the topsoil. The same format has been used for measurements taken during watering every 3rd and 4th day.

Bilag 7
Appendix 8

Limit values for particulate matter and substances in the air

	Limit values
Particles	PM10: <10 µm (can penetrate the respiratory tract) - 40 µg/m³ (annual average) - 50 µg/m (24-hour average) – may be exceeded on a maximum of 35 days per year. According to the Ministry of the Environment and Gender Equality PM2.5: <2.5 µm (can penetrate the lungs) - 25 µg/m³ (annual average) According to the Ministry of the Environment and Gender Equality Note: WHO guideline - 5 µg/m³ (annual average) - 15 µg/m³ (daily average)
Ammonia (NH_3)	According to the Ministry of Employment - 20 ppm (8-hour limit value) - 50 ppm (short-term limit value)
TVOC	HCHO: - 0.37 mg/m³ (8-hour limit value) - 0.74 mg/m³ (short-term limit value) According to the Ministry of Employment There is no statutory limit for TVOC in Denmark. Instead, the Danish Working Environment Authority regulates individual substances (e.g. HCHO).

(Ministry of the Environment and Gender Equality, 2017; Ministry of Employment, 2025; WHO, 2010).