Refinements in diabetic research – a technician led initiative

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Background

A genetically altered diabetic mouse model (Ins2akita) was introduced to the facility two years ago. From information available, it was expected that heterozygous animals would develop hyperglycaemia, hyperinsulinemia, polydipsia and polyuria by 3 to 4 weeks old with males exhibiting a more advanced phenotype in comparison to females. As the colony grew and the animals aged, we as Animal Technicians noticed subtle behaviours and identified ways to refine our husbandry practices to better support their welfare.

Challenge one: Water consumption

We knew the animals' water intake would increase as diabetes progressed but the extent was unclear.

After monitoring, we found that two Ins2akita mice consume 150 to 200mls of water in 24 hours and so to avoid risk of them running out of water we sourced 300ml bottles and replace them daily.

We also observed that the mice spent most of their time drinking, which could reduce time spent eating. To address this we scatter diet pellets across the cage floor and provide soaked diet in a small pot for easier access.

Challenge two: Excessive urination

Anticipating excessive urination, we knew we would need to modify our standard bedding approach.

Approach 1:

We initially used a more absorbent white paper-based substrate that is known to be more absorbent, but close observation showed that it could not handle the volume of urine produced by group-housed mice.

We also noticed that whilst the substrate absorbed urine, the wetness remained in the upper layer of the bedding, leaving the animals in contact with dampness. The nesting material also became wet, making the animals cold and uncomfortable. This was exacerbated by the worsening phenotype of the model. Left: soaked bedding to the right of the cage (Figure 1).

Figure 1. Soaked bedding to the right of the cage base and clean white paper-based substrate.

Approach 2:

We placed an absorbent cage liner at the base of the cage and added the white paper-based substrate on top, aiming to trap any excess fluid away from the mice. However we found that the urine was absorbed by the substrate before reaching the cage liner, resulting in no improvement.

Approach 3:

During IAT congress, we discussed the issue with one of our suppliers who recommended mixing the white paper-based substrate with our normal aspen cob (Figure 2), as the cob would allow the urine to sink to the lower layers of the bedding.

Figure 2. Aspen corn cob and white paper substrate mixture.

While this approach helped to some extent there was still significant surface wetness, meaning the animals as well as the nesting material and handling tube remained in contact with it.

Approach 4:

After discussing the issue with our NVS, we decided to add a mezzanine layer (Figure 3) to provide the animals with a dry refuge above the bedding.

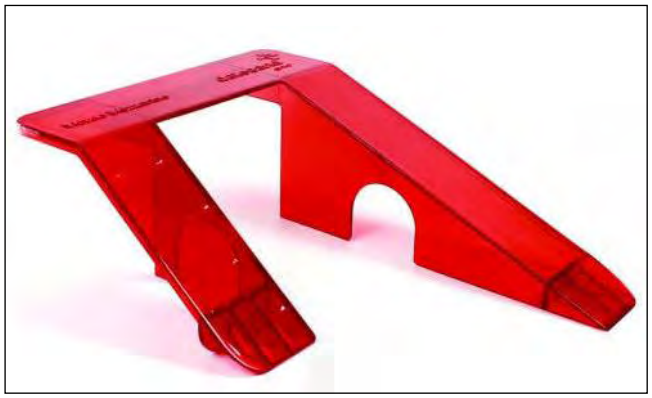

Figure 3. Mezzanine.

However while the mice used the mezzanine, they began urinating on it and then drinking their own urine from the surface. This was of scientific concern as the animals were part of a study where the treatment under investigation was provided in their drinking water. As a result the mezzanine was removed.

Approach 5:

We identified an aspen cob with larger pieces, which was reported to be more absorbent than the smaller aspen chips. The aim was that, when mixed with the white paper-based substrate (Figure 4), the larger chips would help improve drainage to the lower layers, directing moisture away from the mice.

Figure 4. Large aspen cob chips.

While this approach was successful in reducing wetness around the animals, due to the volume of urine, it began pooling in the corners of the cage, despite daily cleaning. We also noticed that the mice were digging in the wet corners and drinking their urine instead of the water from the bottle. Again, this was of scientific concern, as the animals were part of a study where the treatment under investigation was provided in their drinking water.

Approach 6:

To avoid the issue with mice drinking urine from the cage corners, the bedding depth was increased allowing most of the urine to be absorbed and preventing the mice from reaching any residual wetness at the base of the cage (Figure 5).

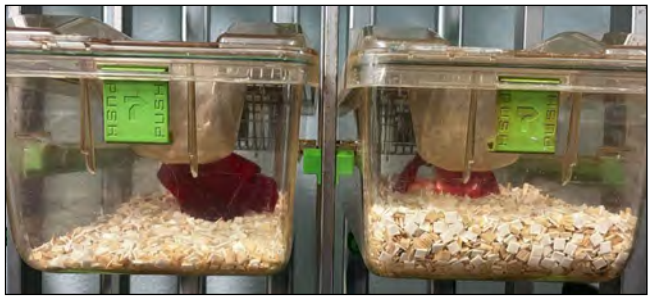

Figure 5. Original bedding depth on the left and increased bedding depth on the right.

Challenge three: Increased cage moisture

Our standard cage setup includes a cardboard handling tunnel, cardboard shelter and rotation of additional enrichment items. However despite resolving the issue with the surface wetness, undertaking daily cleaning and the frequent air changes within the individually ventilated cage (IVC), the overall dampness still posed a risk for growth of mould and bacteria on the cardboard cage items, which could negatively affect Animal Welfare and the science.

To address this, we replaced the cardboard with plastic tunnels and mouse houses. We chose domed plastic houses to prevent urine pooling on flat surfaces due to our previous experience with the mice drinking their urine off the mezzanine floor (Figure 6).

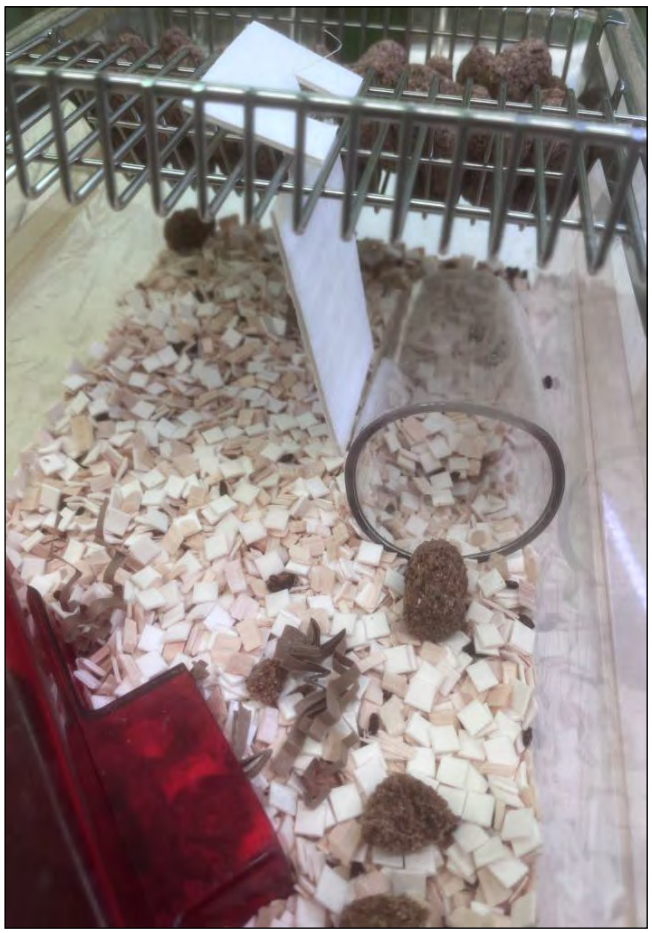

Figure 6. Plastic items and enrichment suspended from cage lid.

Conclusion

It is well known that diabetic animals drink and urinate excessively, so we recognised the need to implement measures that would support the animals during this period and minimise any impact on their welfare. While the initial approaches seemed to improve the home-cage environment, we as trained Animal Technologists working closely with these animals, quickly identified that these improvements were superficial and did not provide real benefits to the animals' welfare. As Animal Technologists, we raised our concerns and leveraged our knowledge and expertise to refine the husbandry approaches, ensuring they offered tangible benefits to the animals in our care. Our methods are now routinely implemented in the care of diabetic animals at the University of Birmingham.