POSTER PRESENTATIONS

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Hopping mad – an abnormal gait investigation

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What is it?

Within Cancer Research UK (CRUK) Cambridge, we house a colony of mice in which we see a particular phenotypical characteristic that is colourfully referred to as a 'bunny-hop'. These mice are part of the Gli1 pathway of Hedgehog signalling, which is very important in embryonic development.

What do we do?

We carry out a few precautions to ensure we alleviate any potential suffering or discomfort when this genotype is suspected.

- We ensure that extra nutritional supplements are available to the post weaned mice, this is as the Gl1 knock out (KO) pups are generally smaller so this gives them an extra boost after weaning.
- We perform biopsies for genotyping at 10 days rather than 14 days so we make sure potential GLi1KO pups have everything they need to thrive sooner.
- We carry out post-wean checks on all animals to reduce the risk of health concerns being missed.

When did this happen?

Approximately two years ago, it was observed that some mice displayed this hopping characteristic. As it became more apparent in several mice, it was found that this 'bunny-hop' coincided with the genotype of the mice being Gli1KO. These mice were initially labelled as 'VIP'.

Weight comparisons

Within the Gli1s, the Gli1KOs tend to be smaller in size than the wild type (WT) or heterozygous (Het) Gli1s. Although we do not know the extent of this, or why it happens, it is interesting to see the weight trends (Figure 1).

What happens next?

We decided to investigate possible reasons why these mice displayed the 'bunny-hop' characteristic. One potential theory was a shortening of the femur and

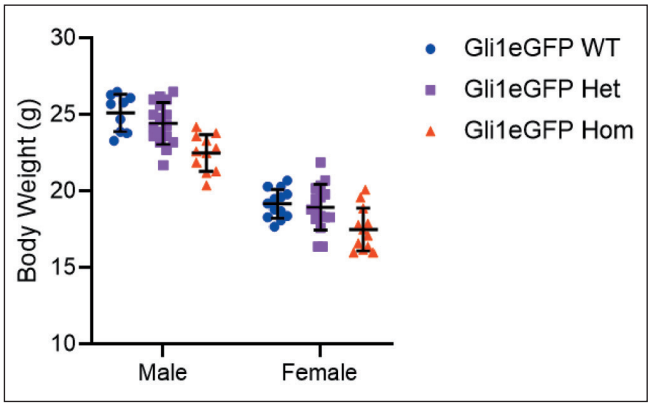

Figure 1. Graph showing weights of Gli1WT, Gli1Het and Gli1 homozygous (Hom) (KO) 6-8 weeks old.

humerus causing this unusual gait, for that reason this was our focus.

With the help of the de la Roche laboratory, we performed computerised tomography (CT) scanning of Gli1WT and Gli1KO mice to investigate the bone structure to see if this provided the evidence to back up the theory.

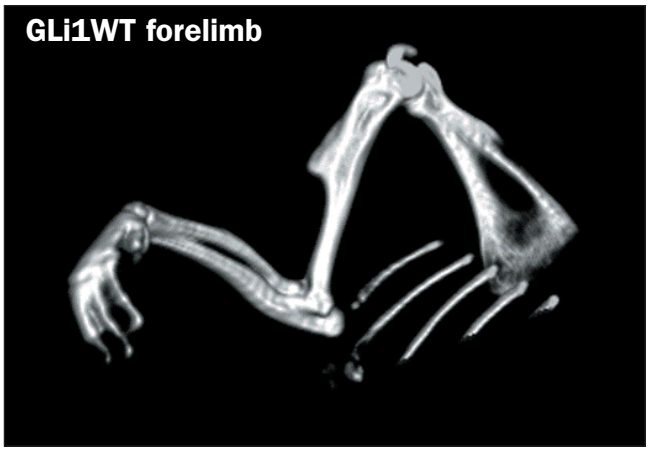

Figure 2.

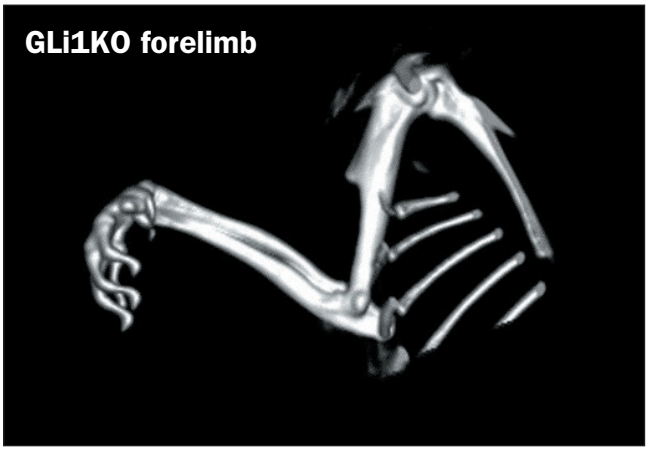

Figure 3.

Figures 2 and 3 show the difference between the GLi1WT forelimb and the GLi1KO forelimb (humerus) that appears to be slightly shorter than the WT at 6 weeks old.

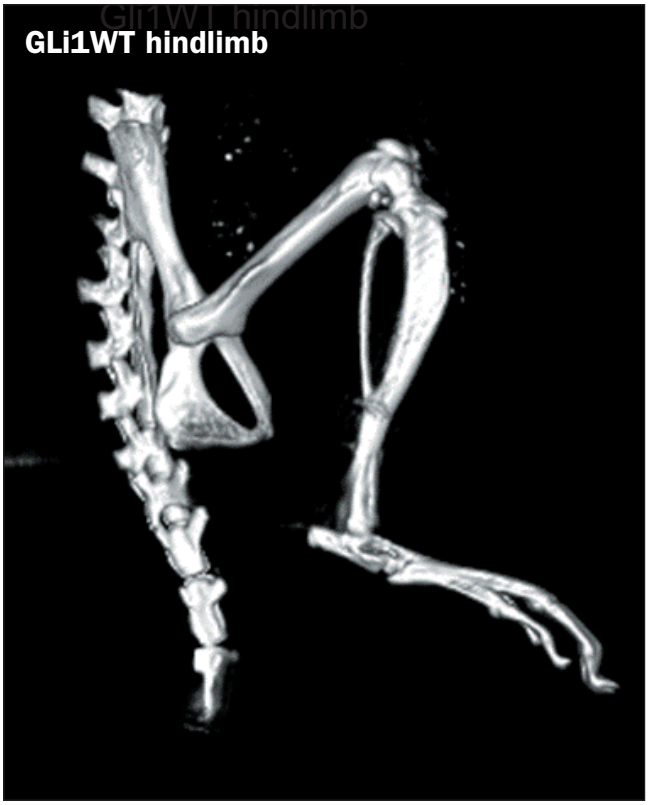

Figure 4.

Figure 5.

Figures 4 and 5 show the difference between the hindlimb (femur) of the Gli1WT and the Gli1KO mice. The Gli KO is slightly shorter with the pelvis a different shape at 6 weeks old.

Summary

To summarise our scans, by observing the images it appears that differences in bone structure and size are evident when comparing the two genotypes side by side.

Conclusion

This could be the primary cause of the unusual gait of the mice.

Acknowledgements

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Does environmental colour matter to Zebrafish

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Aim

To determine if environmental colour affects the Zebrafish (*Danio rerio*) performance metrics of survival, growth rates, fecundity and fertility.

Introduction

It is our responsibility to continually improve the husbandry conditions of animals used in research. Zebrafish (*Danio rerio*) are a widely used biomedical model as they reach sexual maturity quickly and have high fecundity, (Lee 2019).[1] In addition, their eggs are externally fertilised, enabling easier gene manipulation and the creation of new lines. Understanding how environments can affect the animals is crucial to their husbandry in research. Where possible, factors that can be shown to be beneficial to Animal Welfare should be implemented to maintain high standards of husbandry and to improve the quality of data collected. We wanted to understand how colour can affect Zebrafish. Some research has been conducted into the colour preferences of Zebrafish and other similar fish species with varying and conflicting results. Previous research has mainly been preference testing but we wanted to look at the direct results of different colours on performance metrics as stated in the aim.

Method

To create tanks of different colours, the exterior of 3 litre (L) and 8L tanks were spray painted. Triplicates of each tank size were painted green (Hex 08B29), blue (Hex 1050A8) and black (Hex 0E0E10). With clear tanks being used as the control. 3L tanks were used for rearing fish in the nursery, from 4 days post fertilisation (d.p.f.) until 28 d.p.f. and the 8L tanks were used for juvenile and adult fish post 28 d.p.f. Each tank started with a population of 30 UCL (ABxTL) hybrid Zebrafish per tank.

All colours had red, green and blue (RGB) components. The control was the room lights that have a lot of blue and decreasing towards red. Green tanks have a large portion of blue, green and red in almost equal portions (Figure 1). It should be noted that the room light affects how the colour is viewed and how the light emitting-diode (LED) light's emissions will be reflected and absorbed by the paint.

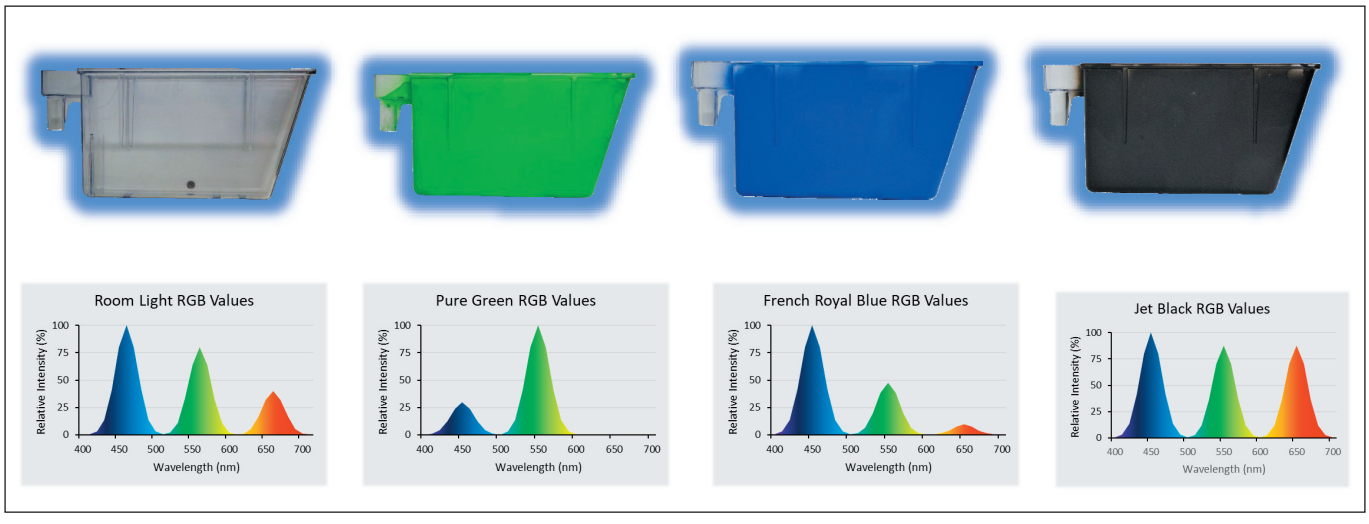

Figure 1. Coloured tanks and their corresponding RGB spectral graphs.

Lux readings were measured using a Seneye light meter (Figure 2) to determine the intensity of light in each tank. The light meter was positioned in the base of the tank pointing directly upwards.

Figure 2. Seneye device used for measuring light intensity.

The fish were photographed in groups of approximately 6 fish from above at 28, 56 and 84 d.p.f (Figure 3). Standard length was measured from these photos using Image J.

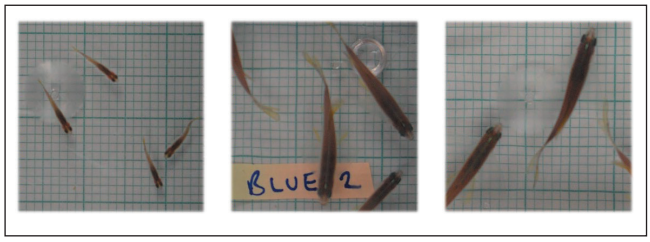

Figure 3. Photographs of fish taken at 28 (left) 56 (centre) and 84 (right) d.p.f.

After 84 d.p.f. embryos were collected from each tank twice per week. This was achieved by leaving breeding trays (Figure 4) inside each tank overnight and collecting embryos the following day. The number laid was calculated by their volume. We took a sub-sample from each collection that we left in an incubator overnight and the following day, the ratio of fertilised to unfertilised eggs was counted to determine fertilisation rates.

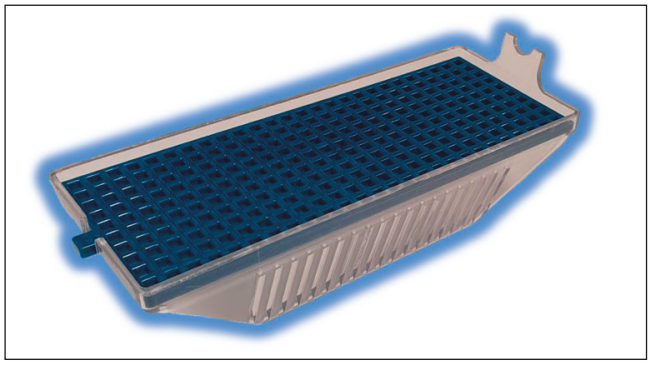

Figure 4. Breeding trays used for collecting embryos from the trial tanks.

Results

It should be noted that light intensity was not constant in each of the tanks (Figure 5).

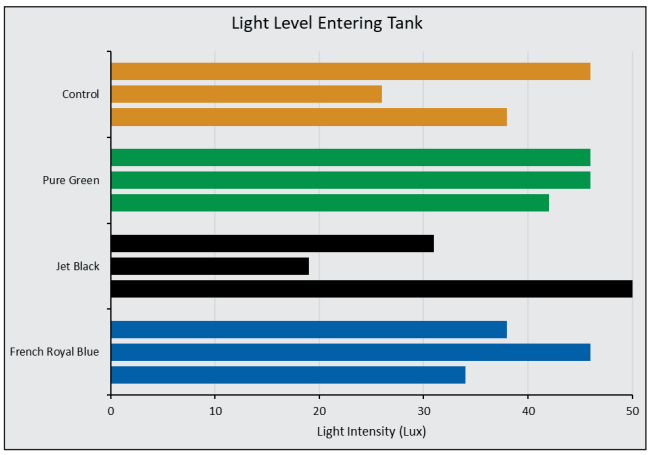

Figure 5. Light intensity entering each trial tank from above.

Placement on the racking alters the amount of light coming into each tank. Tanks towards the sides and top rows of the racking generally are better lit than tanks towards the middle and bottom rows. We attempted to keep this variable as controlled as possible by keeping everything on the same racking level and recording the lighting differences.

Fish in black and control tanks produced a more consistent growth rate compared to the green and blue tanks, which were more variable. All of which can be seen in the size of the error bars in Figure 6.

At 84 d.p.f. all fish were sexually mature and were mated. There were significant differences in production of embryos between the coloured tanks.

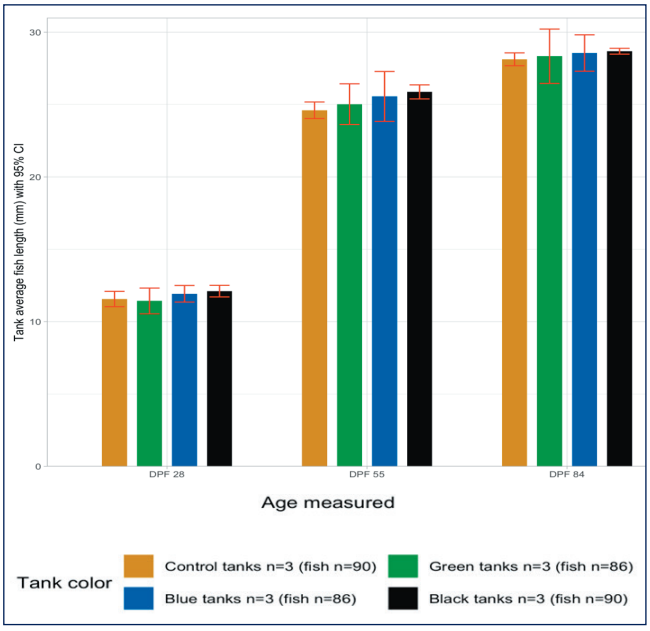

Figure 6. Average fish lengths (with 95% C.I.) from different coloured tanks at 28, 56 and 84 d.p.f.

Fish in blue tanks had the greatest capacity for egg production responsible for almost 50% of embryos (Figures 7 and 8) while still having a comparable fertility rate to the control (Stevens 2021).[2]

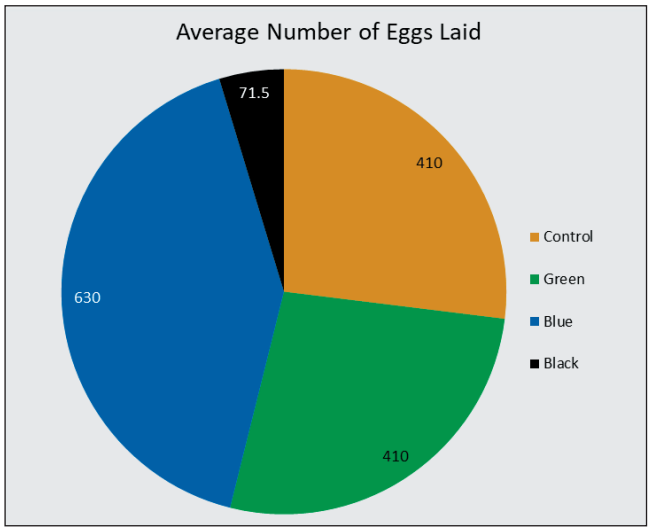

Figure 7. Average number of eggs laid in different coloured tanks.

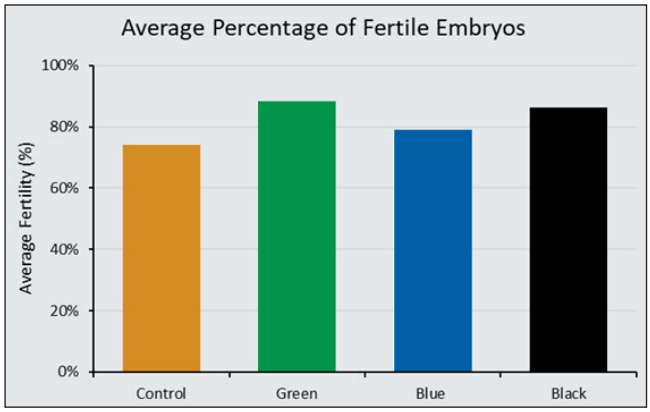

Figure 8. Average percentage of fertile eggs from clutches collected from different coloured tanks.

Both the control and green tanks produced eggs at a similar rate but eggs from the green tank had a slightly higher fertility rate.

Although fertility rates were good from the black tanks, the fish laid the least amount of eggs.

Discussion

Light intensity is highly variable across the rooms and it should be assumed this will be non-standard across different rooms and facilities depending on individual lighting and room set ups. While we tried to standardise this in the trial, further work would be required to determine the true effect of lighting on growth, fertility and fecundity.

In normal circumstances fish are grown and bred in transparent tanks. However this trial suggested that more consistent growth may be achieved by growing in different coloured tanks, in this case black. Similarly, fish may lay significantly more fertilised embryos in a blue tank than any other colour tank, including the transparent tanks (Stevens 2021).[2]

The results of this trial suggest different coloured tanks may be advantageous to growing and breeding fish. Although more studies are required, this work could represent a significant refinement in Zebrafish welfare and husbandry.

Concept

Further to this work, we would alter the tanks with fronts as seen in figures 10 and 11. This would allow health checking and legally required observations of the fish to be carried out easily and not compromise the study.

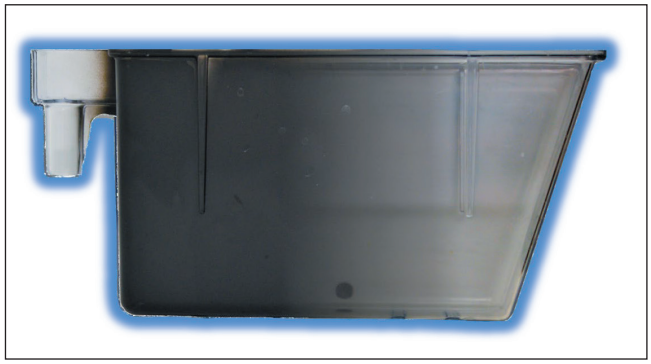

Figure 10. Proposed tank with graduation from black colour to clear.

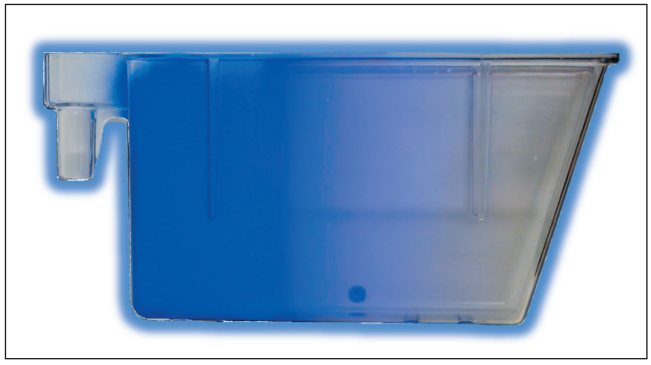

Figure 11. Proposed tank with graduation from blue colour to clear.

To implement this into practical applications we suggest, based on these findings, that fish are reared in black tanks to optimise consistent growth in the population during early development. This will mean that a population growing at a uniform rate is likely to all breed at the same time and produce a similar number of embryos.

Speculatively this may reduce levels of aggression in tanks as fish of similar size are less likely to be aggressive to each other.

Although this work is currently a concept, we hope to explore this more as a method of refinement of housing and with the aim of reducing the number of animals required.

Acknowledgements

Many thanks to the UCL Fish Facility staff: Paul Barwood, Heather Callaway, Karen Dunford, J. Hakkestegg, Elise Hitchcock, Joseph Upstone, Joe Warmsley.

References

1 **Lee, C.J., Paull, G.C., & Tyler, C.R.** (2019). Effects of environmental enrichment on survivorship, growth, sex ratio and behaviour in laboratory-maintained Zebrafish (*Danio rerio*). *Journal of Fish Biology,* 94(1), 86-95.

2 **Stevens, C.H., Reed, B.T., & Hawkins, P.** (2021). Enrichment for laboratory Zebrafish – a review of the evidence and the challenges. *Animals,* 11(3), 698.

Not everyone likes bubbles! Trials and tribulations of fumigating a new life sciences facility using hydrogen peroxide

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Introduction

Following the deep clean and fumigation of our new bio facility, numerous patches of bubbled/blistered paintwork (Figure 1) were observed throughout the building. The paint had been selected as it was known to be used in other facilities regularly fumigated using hydrogen peroxide (H_2O_2) in vapour form. But upon investigation we discovered the formulation of the paint recently changed to remove volatile organic compounds (VOCs). The data sheet had been revised listing only 3 compatible H_2O_2 fumigation systems – the system we had used was not listed.

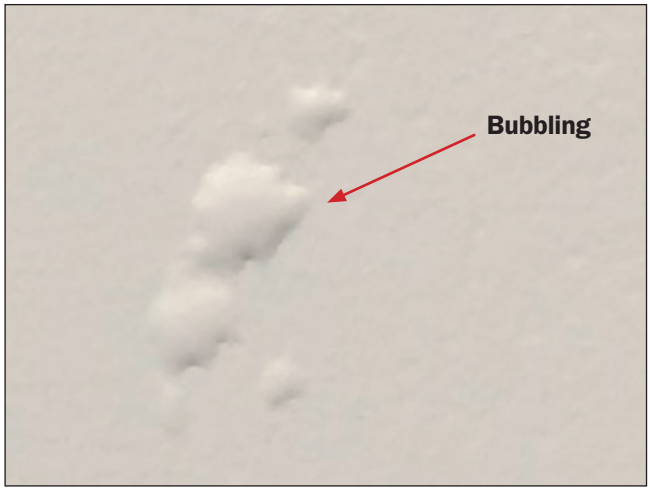

Figure 1. Shows characteristic blistering of paint after fumigation with hydrogen peroxide.

The damaged paintwork was repaired and after a period longer than the recommended curing time the rooms were re-fumigated using a different system; one listed by the paint manufacturers. Although there was less bubbling a small number of patches occurred. This was particularly problematic within the Containment Level 3 (CL3) facility which would need regular routine fumigation.

Meetings were held to discuss the issue:
Do we repaint the entire CL3 with a new paint product or install sheet wall covering? Both of which would involve long-term closure of the facility and complete removal of the existing paint or investigate alternative fumigation methods?

We decided on the latter which was fortunate as COVID-19 was just around the corner and our CL3 facility would be required!

We approached one of the other two companies recommended by the paint manufacturer and after a successful test run, the University was offered a generator on loan, to carry out further trials within the CL3 facility. As well as providing the loan equipment the representative company provided all consumables along with continual support and assistance during what turned out to be a lengthy trial period.

The generator heats, ionises and then disperses the fumigant as a fine dry fog into rooms at 80m per second. The generator had a maximum capacity of 1 litre and was able to disinfect rooms from 10 to 1000m^3.

The bio-disinfectant used contained 12% hydrogen peroxide and silver, some other systems use 35% hydrogen peroxide. Figure 2 shows a typical room set up.

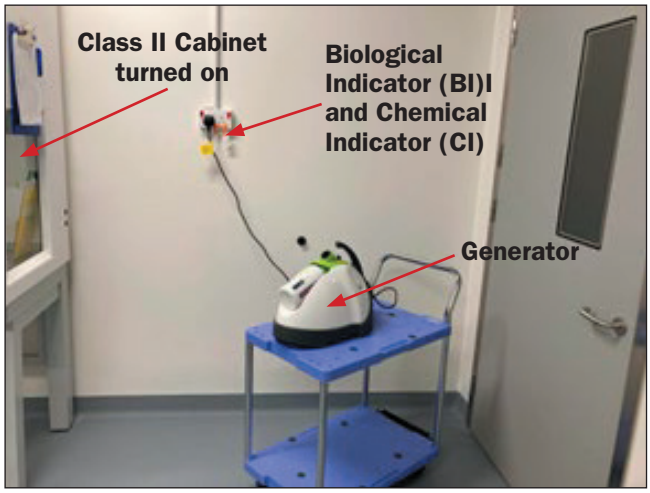

Figure 2. Typical room set up for fumigation.

Method and Results

The volume of the room to be fumigated was calculated and this figure is multiplied by the amount of fumigant per m^3 required; generally 3ml per m^3. The generator was placed in a corner of the room with the dispersal nozzle (venturi) facing upwards towards the opposite diagonal corner and the fumigant level set. The room heating, ventilation and air conditioning (HVAC) was turned off and the doors sealed with acrylic door covers and tape. The Class II Safety Cabinets and room based individually ventilated cage air handling units (IVC AHUs) (if present) remain operational. All caging containing nesting and bedding material was removed.

During each trial we used 10 Biological Indicators (BI) (Figure 3) which use a stainless-steel carrier inoculated with An E6 (6 log) population of *Geobacillus stearothermophilus* spores; the inoculated carriers were placed in a Tyvek® 1 envelope. These 10 Bis were used in association with 10 Chemical Indicators (CI) (Figure 4) that were placed throughout the room at different heights and in challenging locations e.g. within cupboards and under benchtops.

Figure 3. Biological indicator (BI).

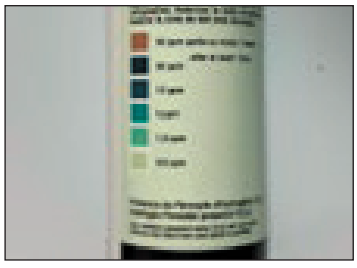

Figure 4. Chemical indicator.

For a cycle to be deemed successful the University required a 6 log deactivation of all Bis and a colour change to the CI (Figure 5) shows CIs before and after fumigation) indicating exposure of >50ppm for at least 1 hour. A control BI was used in conjunction with every test; all Bis was not achieved during the first few trials, but after removing the room circulation fan (the tests indicated this fumigant was most effective with a dwell period with limited air movement), increasing the amount of fumigant used from 3ml to 5ml per m3 and running two cycles consecutively, 3 hours apart (each cycle lasted circa 30 mins), we were able to develop repeated deactivation of all Biological Indicators in the room and thus provide us with validated cycles for both the holding and procedures rooms within the CL3 facility and almost as importantly – with no bubbling of the paintwork.

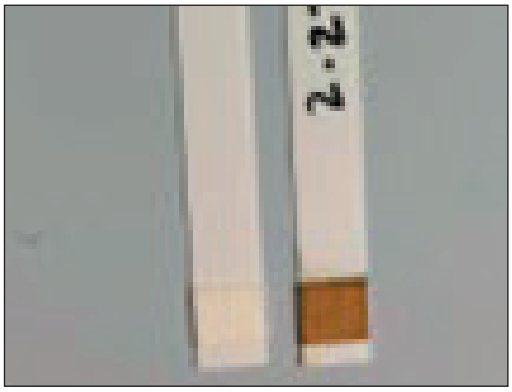

Figure 5. Chemical indicator (CI)

As previously mentioned, the generator is room based, which is fine for decontaminating the actual room but we also needed to decontaminate the exhaust ductwork.

Camlocks had been incorporated into the HVAC ductwork design to allow for plant room-based fumigation of the holding and procedure rooms, see picture 6. To facilitate the movement of fumigant into the exhaust duct whilst

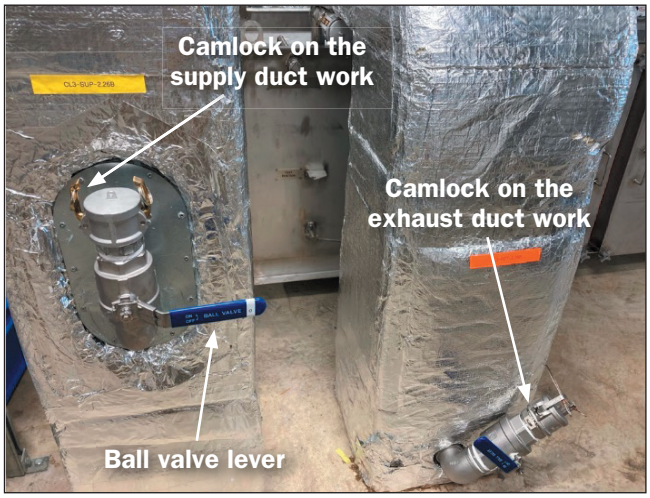

Figure 6. Showing design of camlocks on ventilation system.

using a room based generator, we employed a slightly modified isolator fan to draw fumigated room air into the exhaust duct and back into the room via the supply duct using these camlocks. A BI and CI were placed within the fan unit to demonstrate whether the fumigant had travelled along the entire length of ductwork.

This method worked well for short lengths of ducting but not for longer lengths >7m.

We were uncertain as to the cause of the poor results with the longer lengths of ductwork, the CIs indicated that the fumigant was progressing along the ductwork but the BIs were not being deactivated. Possible reasons considered were that the twists and turns in the long lengths of ductwork were causing airflow issues or that warmed fumigant laden air was condensing upon contact with the cool stainless-steel ductwork. Whatever the issue was, we had a problem!

We decided that to get sufficient fumigant at the right concentration into the exhaust ductwork, a new approach was required. We needed to introduce the fumigant directly into the ducting.

Further discussions were held with the generator representative and they agreed to supply a second generator and suggested an alternative dispensing nozzle which when used with a 3-way connection attached to the camlock on the High Efficiency Air Particulate (HEPA) housing meant that we could introduce fumigant straight into the ductwork nearest to the HEPA filter. The return fan hose was connected to a camlock at the room end of the ductwork so that a semi closed loop was formed with the fumigant being drawn through the duct.

For the first cycles we decided to fumigate the duct and room at the same time so that the entire length of exhaust duct, from inside the room up to the face of the HEPA filter was decontaminated together.

A T-connection was used for this initial trial but unfortunately it was noted that fluid was leaking from the joint during the cycle. As well as the joint not being airtight we surmised that the angle the fumigated air was entering the air flow was causing the fumigant to condense. For the second trial we used a Y connection which allowed the fumigant and air from the fan to mix whilst moving in the same direction. No leakage was

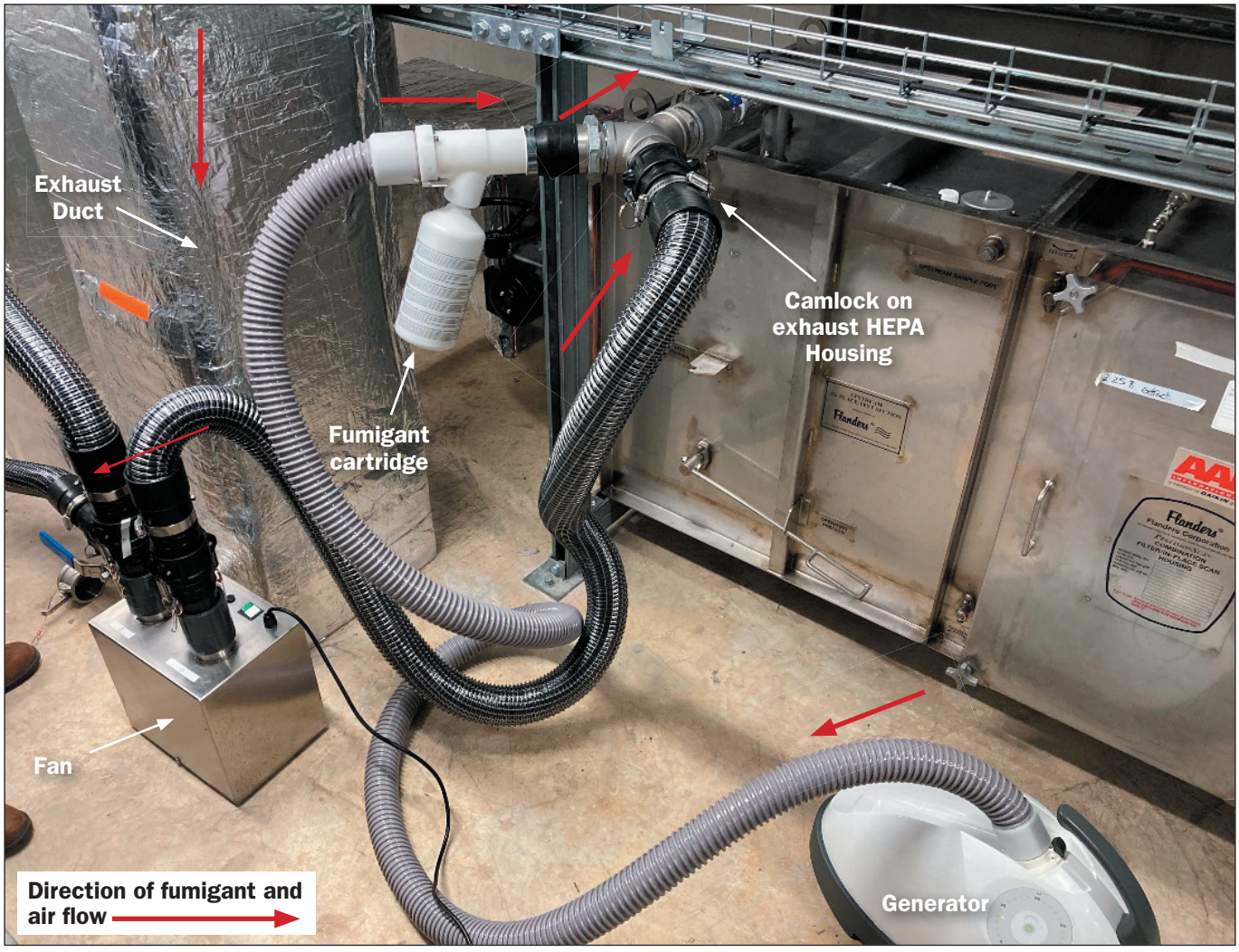

Figure 7. Showing equipment set up for ensuring ductwork fumigated correctly.

noted during the cycle (no condensate was found in the hoses).

However although the results from the second trial were good, we believed them to be inconclusive. The CI in the fan had a good colour change and the BI was deactivated but the positioning of the fan meant that we could not state that the changes to the BI and CI were due to the fumigant that had travelled along the duct rather than the fumigant coming directly from the room. Another change of approach was required. For this we fumigated the ductwork independently of the room fumigation. The generator was set up as before but the room was not fumigated at the same time, this meant that any CI colour change and deactivation of the BI was due solely to fumigant that had been drawn through the entire length of duct.

Due to the design of the ductwork, complete separation of the exhaust duct and the room was not possible. We therefore had to accept that there would be some leakage of fumigant into the room and that some 'fresh' room air would be drawn into the duct, to counteract this we increased the amount of fumigant to 7.5ml per m^3. It was decided that each cycle would consist of a 4 minute 30 second injection of fumigant on the hour for four hours followed by an overnight (12 hour) dwell period. (It is acknowledged that this amount of fumigant is probably excessive and the cycle will be refined in due course.) A second full cycle with fresh CI and BI was repeated the following day and the BIs sent for culturing. The colour change to the CIs was very good and the results received 6 days later showed no growth to the BIs. We had now demonstrated that the room and exhaust ductwork up to the HEPA filter could be successfully decontaminated.

Conclusion

Having had over 20 years experience of using various H_2O_2 dispensing systems I must admit to being rather sceptical of the isonised hydrogen peroxide system and the results that would be achieved BUT –

Firstly, the equipment was very easy to use:

1. Calculate the volume of the room and multiply by the amount per m^3 to be used (e.g. 3ml per m^3).
2. Then, set the dial to the resulting amount.
3. Turn off the room ventilation.
4. Seal the room.
5. Press start on the remote control.

It is also extremely portable, being light and about the same size as a microwave oven.

Secondly and most importantly we obtained repeatable results with 100% deactivation of all spore strips (BIs) placed in the room and with **NO BUBBLES TO THE PAINTWORK!**

Future Plans

Following the successful trials within the CL3 facility we have looked at other applications for the ionised H_2O_2 generator. Initially the method of delivery appeared to restrict its use to the room fumigation however following the addition of the recirculation fan additional uses looks possible including fumigation of Class II Safety Cabinets and Isolators where connections for a closed loop H_2O_2 system had been fitted.

Discussion

Fumigation using H_2O_2 in vapour form is now one of the primary methods of decontamination within the life science field. Over the past few years several new methods to dispense hydrogen peroxide have been developed but uptake of these new technologies appears to be slow within the UK Life Sciences industry whereas they are widely used by our contemporaries in Europe and America and are also routinely used in other UK sectors such as Public Health. However, after our experiences with reformulated paint it may be time to give these new approaches a chance within our industry, not only have we found that they work, but they are also cheaper too!

Acknowledgements

For many reasons, not least the Coronavirus pandemic, the development of validated cycles for use within our CL3 facility have been a lengthy process and we would like to thank the following companies for their time, support, encouragement, patience and above all their generosity.
Oxy'Pharm Ltd
Surrey Diagnostics Ltd
North Kent Plastics (NKP) Ltd

Green Clay: not just for a pretty face

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Introduction

Green clay can be bought and used to improve impurities of the skin. It is expected to have various nutrients to assist healing and re-energising. This has been used at the Cancer Research UK Cambridge Institute (CRUK CI) to alleviate skin lesions in mice at our facility.

Background

Mice can be prone to skin lesions for various reasons. Some theories as supported by Burkholder *et al. (2012)*[1].

- Dermatitis (ulcerative, muzzle (furunculosis), contact ((see Figure 1)).
- Scratching and itching as an inflammatory response.
- Fight wounds commonly seen in group housed male mice.
- Barbering and overgrooming.

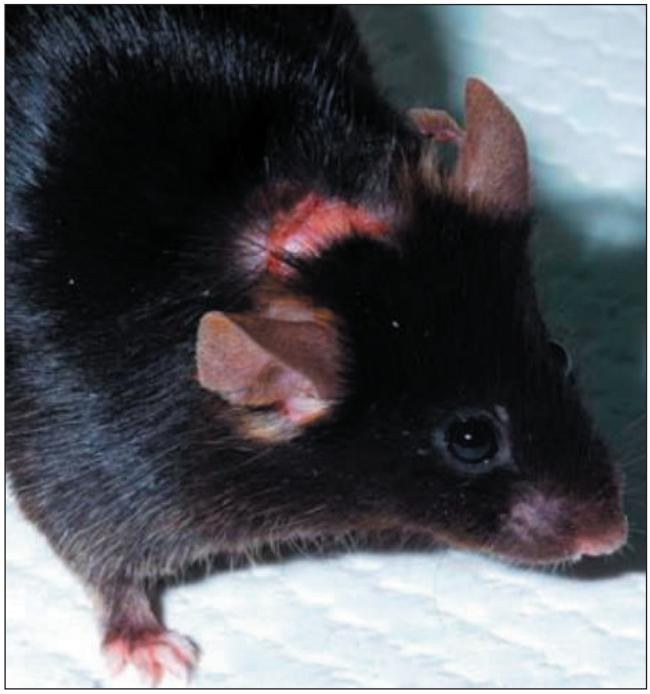

Figure 1. Mouse exhibiting dermatitis.

There are veterinary prescribed treatments available. Green clay is a non-invasive method and does not require veterinary approval nor does it have properties that may interfere with an experiment.

How it works

The clay is manufactured in a powder form (see figure 2).

Figure 2. Green clay as used at CRUK CI.

1. Using a petri dish, the powder should be mixed with sterile water until it resembles a thick consistency (cream).
2. Using a clean cotton bud, apply the clay to the lesion.
3. When not in use, it can be refrigerated for up to 7 days before it is discarded. The refrigerator allows 'cooling' properties.

We have found that the cage environment is modified changed by:

- Using a plastic tunnel instead of a cardboard tunnel.
- Sizzle Nest is provided instead of a Nestlet.
- Food is placed onto the floor.
- Woodchip bedding is changed to 3Rs bedding (like cat litter).

Refinement and findings

- Green clay can reduce the need for medicinal ointments or provision of analgesia.
- Mouse toenails can be trimmed in conjunction with the green clay to prevent further harm if the mice are scratching.
- Where possible reduced handling can prevent the possibility of contact dermatitis.
- It is most effective when lesions are found in early stages or just forming.
- It has been seen to improve animal welfare and their quality of life as a result.

Happy mice make great science!

Acknowledgements

Special thank you to all BRU staff at CRUK CI.

References

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Refining identification methods of *Gallus gallus domesticus*

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Abstract

Individual identification of birds used in scientific research at The Pirbright Institute has historically used invasive wing tags. Here we present the first results of an ongoing study which seeks to replace the wing banding with non-invasive leg bands in a variety of ages of an inbred chicken breed.

Introduction

Individual identification of birds is required when using poultry in infectious disease research. At The Pirbright Institute this had historically been achieved by placing a permanent wing tag through the webbing of an individual wing of day-old chicks. (See Figure 1)

In addition to the invasive nature of this type of identification (ID), other issues were noted including application in wrong places, rubbing and growth of feathers in older birds which cover the wing band. Here we describe the approach and initial results generated at The Pirbright Institute as part of a project attempting to replace the invasive wing band with a plastic leg band.

Method

Four different leg band designs were assessed against several criteria including:

- ease of application adjustment
- ID readability
- interference with chick mobility
- design functionality
- cost

Once a leg band design was selected, various sizes were evaluated on day old Rhode Island Red (RIR) chicks, available on a weekly basis. Every day after application, the leg bands on each bird were checked to determine:

– How many times they had to be loosened and/or changed.
– How often the band would fall off.

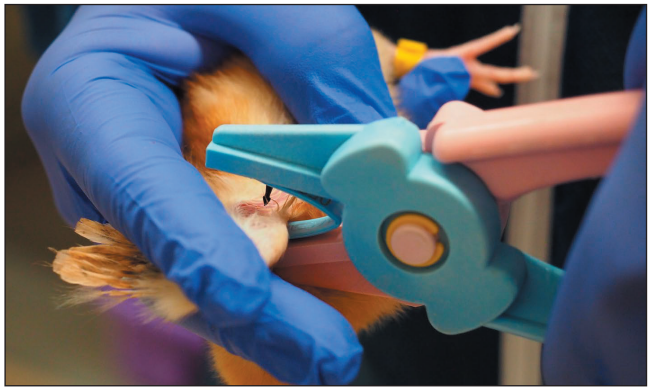


Figure 1. Placement of wing tag on day-old chick.

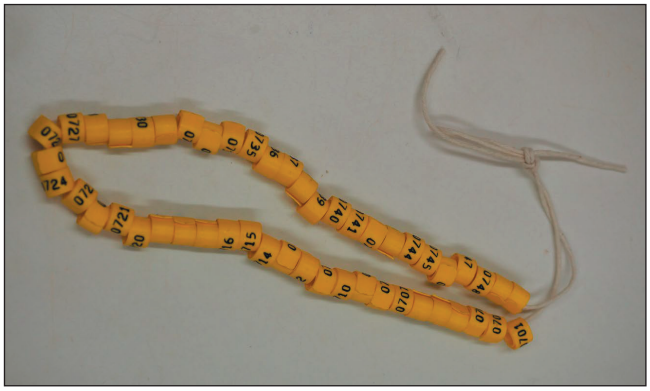
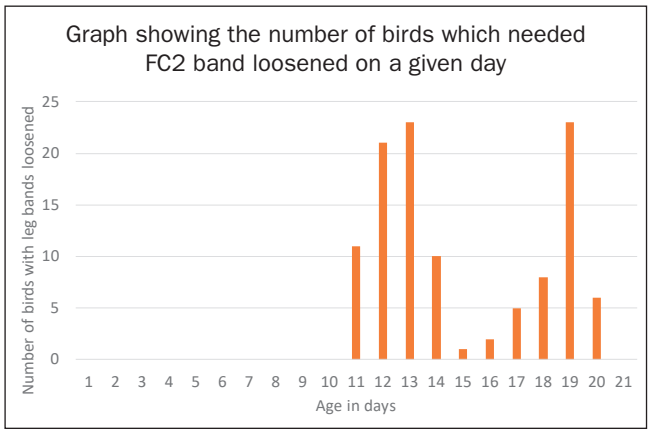


Figure 2. Example of chicken leg band.

Results

Every week for 6 months, the various sizes of leg bands were assessed on day old chicks to determine the optimal starting size. Preliminary results from the assessment of weekly hatched RIR chicks indicated that a single 6.4mm leg band (FC2) can be used on birds up to 3 weeks old before the leg band must be replaced (picture 2). It was observed however that loosening of the leg bands was required; with much of this happening between 2 – 3 weeks of age. In the 6 months thus far, no FC2 bands have fallen off chicks up to 3 weeks of age. It is important to reduce the frequency of leg band replacements to avoid transcription errors (see Graph 1).



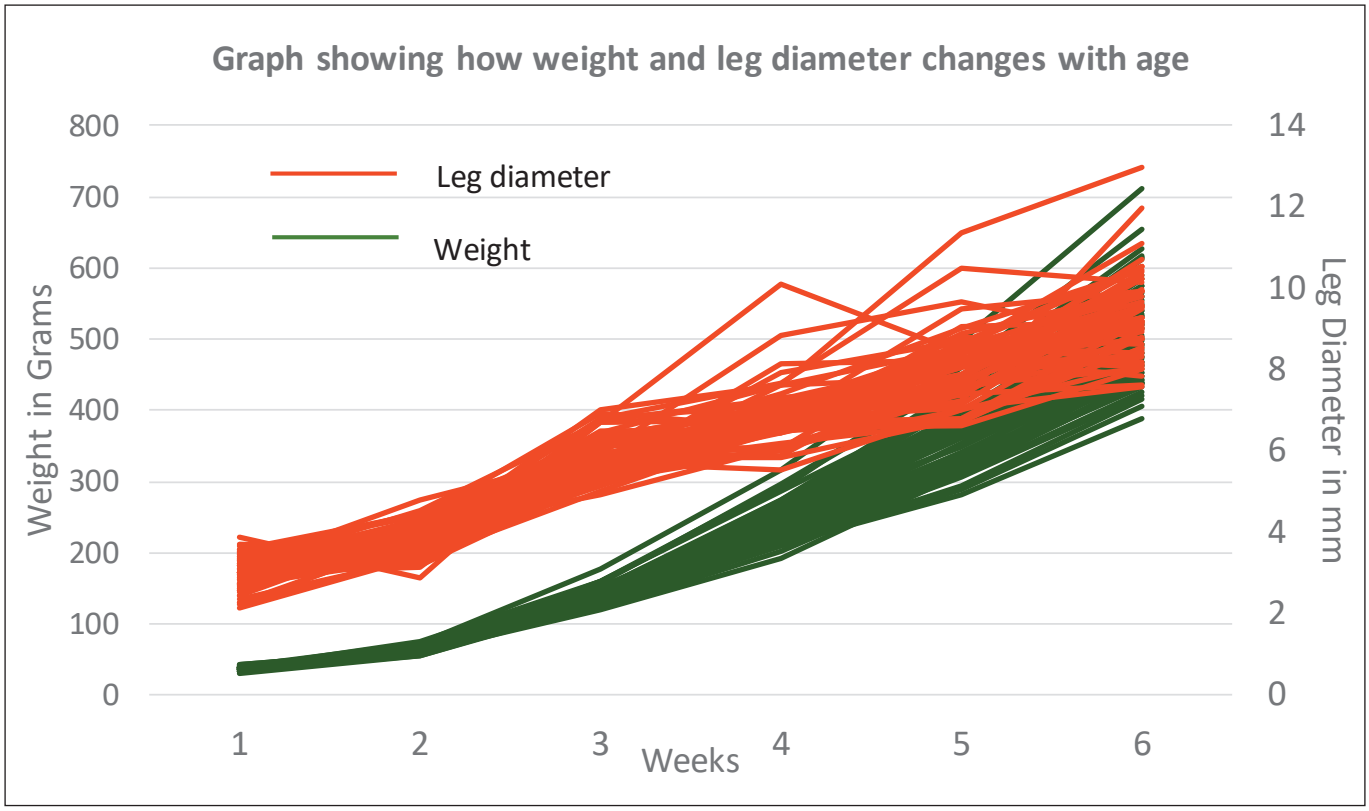
Graph 1. Number of birds requiring leg bands to be loosened and when.

Due to the chicks' rapid growth rate, it was confirmed that daily checks of the leg band tightness were essential to avoid discomfort to the chicks (See Figure 3).

Using this information, a method was developed which detailed the criteria for assessing leg band tightness to standardise the data collected.

The other significant finding was that a single 11mm leg band (FC5) was identified as the largest band which did not fall off from 2 weeks of age. The duration this size can remain on the bird beyond 2 weeks before needing to be replaced will be assessed during study specific hatches which frequently extend beyond 5 weeks of age.

Figure 3. Showing check having leg band checked.



Graph 2. Changes in weight and leg diameter.

Future work

Data will continue to be collected from weekly and study specific hatches. This will be statistically analysed to determine the optimal leg band size and age at which replacement is necessary for a given chicken breed for differing study lengths. The failure rate of a given size of wing band for a particular breed/age range of chicken will also be determined from this data. Importantly, any difference in effect of wing and leg banding on weight gain will also be examined as in Dennis *et al* 2008,[3] with ours being based on plastic leg bands in contrast to their data using metal.

Correlations of leg band replacement events with weight gain and leg diameter (see Graph 2) will also be determined to elucidate which of these two parameters is the most significant. This could be used to predict leg band replacement times for breeds previously unrecorded.

Variability between operators and within an individual operator will also be assessed to determine whether the individual influences the failure rate and frequency of loosening.

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