

# 11<sup>th</sup> Annual CCSAW Research Symposium – 2018

May 9<sup>th</sup>, 2018 9:00am – 5:00pm (registration at 8:30am)

## Welcome

9:00 Dr. Malcolm Campbell, Vice-President (Research), University of Guelph

## Session #1: Poultry I (Chair: Dr. Stephanie Torrey, Animal Biosciences)

9:10 **The effect of calcium propionate in feeding strategies for broiler breeders on dietary palatability and conditioned place preference**

Aitor Arrazola\*, Tina Widowski, Michele Guerin, Stephanie Torrey

9:25 **Assessment of aversion and time to loss of consciousness to CO<sub>2</sub> gas exposure in turkeys**

Rathnayaka M.A.S. Bandara\*, Stephanie Torrey, Rebecca L. Parsons, Tina M. Widowski, Suzanne T. Millman

9:40 **Evaluation of different electroencephalographic and behavioural changes for on-farm euthanasia of turkeys**

Elein Hernandez\*, Fiona James, Stephanie Torrey, Tina Widowski, Karen Schwean-Lardner, Patricia V. Turner

## Poster Introductions (Chair: Dr. Derek Haley, Population Medicine)

9:55 1-min intros to posters

10:15 **POSTER SESSION & COFFEE BREAK – 60 min**

## Session #2: Poultry II (Chair: Dr. Alexandra Harlander, Animal Biosciences)

11:15 **A cross-sectional study of associations between management and feather damage in Canadian laying hens housed in furnished cages**

Caitlin Decina\*, Olaf Berke, Nienke van Staaveren, Christine F. Baes, Tina Widowski, Alexandra Harlander

11:30 **Too close for comfort? Effects of space allowance on the comfort behaviours of three strains of pullets in enriched cages**

Lorri Jensen\*, Tina Widowski

11:45 **Problem-solving in laying hens**

Isabelle Y. Kwon\*, Bishwo B. Pokharel, Ilka Boecker, Alexandra Harlander

12:00 **Startle reflex as a welfare indicator in laying hens**

Misha Ross\*, Georgia Mason, Alexandra Harlander

12:15 **LUNCH – 60 min**

## Session #3: CCSAW Research Grants (Chair: Dr. Karen Houle, Philosophy)

1:15 **Pharmacology and antinociception of a sustained-released butorphanol – poloxamer 407 formulation in Amazon parrots**

Delphine Laniesse, Hugues Beaufrère, David Sanchez-Migallon Guzman, Cornelia Mosely, Heather K. Knych, Dale A. Smith\*

1:35 **Does using internal state as a discriminative stimulus require conscious awareness?**

Georgia Mason\*, Dora Duka, Anile Seth, Mateo Leganes Fontenau, Christopher Brown, Kyriaki Nikolaou, Zoltan Dienes, Walter Sanchez Suarez

#### **Session #4: Mice, Dogs & Mink (Chair: Dr. Karen Houle, Philosophy)**

- 1:55 Female mice can distinguish between conspecifics raised with enrichment and those raised in standard 'shoebox' cages**  
Aimee Adcock\*, Emma Nip, Basma Nazal, Aileen MacLellan, Georgia Mason
- 2:10 Enriched mice are nice: long-term effects of environmental enrichment on agonism in female C57BL/6s, DBA/s, and BALB/cs**  
Emma Nip\*, Aimee Adcock, Basma Nazal, Georgia Mason
- 2:25 Effect of owner presence on dog responses to a routine physical exam in a veterinary setting**  
Anastasia Stellato\*, Tina Widowski, Cate Dewey, Lee Niel
- 2:40 Boredom-like states in mink are rapidly reduced by enrichment, and are unrelated to stereotypic behaviour**  
Andrea Polanco\*, Rebecca K. Meagher, Georgia Mason
- 2:55 COFFEE BREAK – 30 min**

#### **Session #5: Cattle (Chair: Dr. Charlotte Winder, Population Medicine)**

- 3:25 The effect of two stage weaning on the behavioural response of beef calves with the addition of creep feeding**  
Heather MacElwee\*, Derek B. Haley
- 3:40 Will dairy cows sort their diet in response to negative energy balance?**  
Sydney M. Moore\*, Trevor J. DeVries
- 3:55 Effect of solid feed location on feed consumption and growth of dairy calves**  
Sarah D. Parsons\*, Ken E. Leslie, Michael A. Steele, Trevor J. DeVries
- 4:10 Associations between the general physical condition and the selling price of culled dairy cows sold at three Ontario auction markets**  
Allison K.G. Moorman\*, Todd F. Duffield, Ann Godkin, David F. Kelton, Jeffrey Rau, Derek B. Haley
- 4:25 Receptivity of animal care feedback and advice: Dairy farmers' perspectives**  
Stephanie L. Croyle\*, Emily Belage, Deep K. Khosa, Stephen J. LeBlanc, Derek B. Haley, David F. Kelton

#### **Closing Remarks**

- 4:40 Questions Arising: Some Philosophical Reflections**  
Karen Houle
- 4:50 Student Awards & Acknowledgements**  
Derek Haley & Lee Niel
- 5:00 End**

## POSTER PRESENTATIONS

- 1. Assessing the welfare of fast-growing broilers reared in pens with or without enrichment**  
Zhenzhen Liu\*, Stephanie Torrey, Ruth C. Newberry, Tina Widowski
- 2. Assessing the welfare of turkeys euthanized with CO<sub>2</sub> under different fill rates**  
Logan Patterson\*, Tina Widowski, Karen Schwan-Lardner, Stephanie Torrey
- 3. Strain differences and effects of different densities during rearing on the musculoskeletal development of pullets**  
Danielle Fawcett\*, Teresa Casey-Trott, Lorri Jensen, Tina Widowski
- 4. Is there a relationship between feather pecking and response inhibition in laying hens?**  
Jennifer Heinsius\*, Nienke van Staaveren, Isabelle Kwon, Angeli Li, Joergen Kjaer, Alexandra Harlander
- 5. Gut motility in laying hens with different propensities for feather pecking behaviour**  
Julia Krumma\*, Nienke van Staaveren, Isabelle Kwon, Christine West, Paul Forsythe, Wolfgang Kunze, Alexandra Harlander
- 6. Behavioural and physiological consequences of stressors on laying hens**  
Claire Mindus\*, Nienke van Staaveren, Haylee Champagne, Wolfgang Kunze, Paul Forsythe, Joergen B. Kjaer, Alexandra Harlander
- 7. The use of tryptophan to decrease abnormal behaviour and positively affect growth in growing pigs**  
Maggie L. Henry\*, Robert M. Friendship, Anna K. Shoveller, Anita L. Tucker
- 8. Are “depression-like” responses really depression?**  
Aileen C. MacLellan\*, Georgia J. Mason
- 9. Determining human ability to recognize canine stress in familiar and unfamiliar dogs**  
Sydney D. Pearce\*, Lee Niel, Katrina Merckies
- 10. Expression of canine inherited behaviours in crossbreds versus purebreds**  
Victoria Shouldice\*, Lee Niel, James A. Serpell, and J.A.B. Robinson
- 11. Effect of type of gradual weaning program on intakes and growth of dairy calves fed a high level of milk**  
Sarah D. Parsons\*, Ken E. Leslie, Michael A. Steele, Trevor J. DeVries

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**Campbell Centre for the Study of Animal Welfare**



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**CO-ORGANIZERS:**

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**SESSION CHAIRS:**

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Tina Widowski (Poster)

Katrina Merkies (Poster)

Agnes Balzani (Oral)

Nienke Van Staaveren (Oral)

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## ABSTRACTS:

### Female mice can distinguish between conspecifics raised with enrichment and those raised in standard 'shoebox' cages

Aimee Adcock\*, Emma Nip, Basma Nazal, Aileen MacLellan, Georgia Mason

Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada

In *Drosophila* and mink, males raised with enrichment (EE) win more copulations with females than do males raised without, likely reflecting females' evolved tendencies to prefer low-stress mates. But in social species, do females similarly prefer E females over non-enriched (NE)? We investigated this using female mice. In our experiment and others, EE females are less aggressive, less stereotypic, and less likely to show depression-like changes (learned helplessness & anhedonia): effects that human research suggests reduce both social attractiveness and sociability. We therefore tested the hypotheses that NE-raised females are less attractive as companions than EE females, and also less interested in social contact. Forty C57BL/6 mice (hereafter 'focal mice') from EE or NE cages (N=20 each) were exposed to two unfamiliar females (one EE, one NE) from another strain (either DBA/2 or BALB/c), in a modified social preference apparatus (a square arena where stimulus mice are enclosed in opposite corners, and novel objects are enclosed in the other 2 corners). This was repeated for 10 min/day until focal mice were familiar with both stimulus mice (inferred from investigatory sniffing, which wanes with familiarity). Trios were then filmed for 30 minutes and a blind observer scored focal mouse proximity to each corner enclosure. Data were analysed using general linear mixed models. As predicted if EE mice are more social, they spent more time near other mice than did NE focal mice ( $F_{(1,30.44)}=13.75$ ,  $p=0.0008$ ). However, this effect interacted with stimulus mouse housing ( $F_{(1,32.59)}=6.74$ ,  $p=0.01$ ): EE focal mice unexpectedly tended to spend more time with NE stimulus mice than with EE stimulus mice ( $F_{(1,18)}=3.39$ ,  $p=0.08$ ); while as predicted if EE mice are more socially attractive, NE focal mice spent significantly more time with EE stimulus mice than NE stimulus mice ( $F_{(1,15)}=4.84$ ,  $p=0.04$ ). These data thus reveal for the first time that female mice can discriminate EE- from NE-raised conspecifics. However, our hypothesis that E-raised mice would be more attractive as companions was not fully supported, as this held only for NE focal mice. Furthermore, our hypothesis that EE mice are more sociable was supported, but in a nuanced way: they were only more social than NE focal mice when choosing to spend time near NE stimulus mice ( $F_{(1,31.45)}=17.64$ ,  $p=0.0002$ ). To determine what underlies these complex effects, and identify which cues focal mice were using, we are now investigating the role of stimulus mouse homecage behaviour. Analyses are underway, but time spent inactive but awake (IBA, a depressive-seeming behaviour that covaries with learned helplessness) seems to play a role. For NE focal mice, the group that avoided NE stimulus mice, the time spent near NE stimulus mice was negatively related to stimulus mouse IBA behaviour ( $F_{(1,12.99)}=6.15$ ,  $p=0.03$ ). This did not hold for EE focal mice. Research is ongoing, but together these findings provide novel evidence that mice can distinguish between NE and EE conspecifics. They also cautiously suggest that NE mice avoid other NE mice who show depression-like changes, indicating that their welfare may potentially be reduced if they are forced to live with individuals they would rather avoid.

## The effect of calcium propionate in feeding strategies for broiler breeders on dietary palatability and conditioned place preference

Aitor Arrazola<sup>1\*</sup>, Tina Widowski<sup>1</sup>, Michele Guerin<sup>2</sup> and Stephanie Torrey<sup>1</sup>

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Broiler breeder pullets are feed-restricted throughout rearing to avoid health-related problems attributed to fast body weight gain and to achieve a profitable laying performance. Feed restriction is a significant welfare concern due to chronic hunger and the development of alternative feeding strategies has focused on decreasing voluntary feed intake by the inclusion of appetite suppressants, such as calcium propionate (CaP). Dietary inclusion of CaP decreases feeding rate and it has been suggested that the decreased feeding motivation is due to lower palatability, gastrointestinal discomfort, or both. The objective of this experiment was to examine the effect of calcium propionate in alternative diets for broiler breeders on palatability and gastrointestinal discomfort. Three subsamples of Ross 308 broiler breeders were used: two for the palatability test (108 pullets at 2 pullets/cage on 3 weeks old, and 24 pullets in individual cages at 8 weeks old) and one for the condition place preference test (24 pullets at 4 pullets/pen). Palatability was assessed by measuring feeding rate with birds naïve to CaP, at weeks 3 (at 0% [control] or 1.44% CaP inclusion) and 8 (at 0% [control] or 3.19% CaP). Data were analysed by age using linear mixed regression models, with cage nested in the models and body weight as a covariate. Gastrointestinal discomfort was examined using a conditioned place preference test. On training days, pullets consumed two pills (160 mg of CaP/pill) followed by 20 g feed allotment. Training lasted for 90 min/pullet/day during 8 consecutive days at weeks 7 and 9, and pullets' choice was tested in a T-maze twice on two consecutive days at weeks 6, 8 and 10. Data were analysed using a linear mixed regression model, with pen nested in the model and age as a repeated measure. Pullets' preference was assessed using a binomial distribution. For the palatability assessment, pullets tested at week 3 at 1.44% CaP ate  $5.18 \pm 1.36$  g less in 10 min compared to pullets on the control diet ( $F_{1,45}=14.49$ ,  $P<0.001$ ). However, there was no significant effect of 3.19% CaP inclusion at week 8. For the gastrointestinal discomfort assessment, the choice for place conditioned with the consumption of the CaP pill linearly decreased over time ( $F_{1,18}=38.27$ ,  $P<0.001$ ) after repetitive conditioning place training. No pullets preferred the CaP pill while the preference for the placebo increased from week 6 to weeks 8 ( $+17.4 \pm 5.8\%$  of pullets [6/24];  $t_{18}=2.91$ ,  $P=0.01$ ) and 10 ( $+30.4 \pm 8.6\%$  of pullets [9/24];  $t_{18}=4.53$ ,  $P<0.001$ ). The inclusion of CaP at 1.44% reduced feeding motivation during early rearing at a low feed restriction level, and older pullets were less likely to choose the place conditioned with the consumption of CaP. These results suggest that inclusion of CaP at 1.44% for broiler breeder pullets can reduce palatability and induce a negative affective state, making its implications for positive welfare controversial.

## Assessment of aversion and time to loss of consciousness to CO<sub>2</sub> gas exposure in turkeys

Rathnayaka M.A.S. Bandara<sup>1\*</sup>, Stephanie Torrey<sup>1</sup>, Rebecca L. Parsons<sup>2</sup>, Tina M. Widowski<sup>1</sup>, Suzanne T. Millman<sup>2</sup>

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Carbon dioxide is widely used for poultry euthanasia. To claim CO<sub>2</sub> as a humane euthanasia agent, it is essential to identify CO<sub>2</sub> concentrations which are not aversive to the species and cause rapid loss of consciousness. The objectives of this study were to examine the aversiveness to four different CO<sub>2</sub> concentrations that were used for euthanasia in turkeys, by using approach-avoidance and conditioned place avoidance paradigms, and to find the time to loss of consciousness at each of the concentrations. A preference test was designed using two identical chambers separated by a sliding door and a curtain. The control chamber (CC) maintained ambient air conditions (1% CO<sub>2</sub>); the treatment chamber (TC) maintained predetermined CO<sub>2</sub> concentrations. Eleven turkeys (1.26±0.18kg; 4 wks old) were individually trained for 5 consecutive days to enter the TC by pushing through a curtain to access feed and enrichment, with ambient air in both the chambers. After 5 minutes in the CC, the sliding door was opened to provide access to the TC. Birds received 5 minutes access to the TC, after which they were removed and returned to the home pen. All birds learned to enter TC and were advanced to testing phase after two consecutive days of entering to TC. During the testing phase, the same procedures were used with the TC maintained at one of four CO<sub>2</sub> levels: 25%, 35%, 50%, or 70%. Tests were concluded after 5 minutes or when loss of neck tone (LONT) occurred during gas exposure and birds were removed from the chamber for recovery. Turkeys experienced each of the CO<sub>2</sub> treatments (in random order) on the gas day (G), preceded by one baseline day (B) and followed by one wash-out day (W) with ambient air condition. Time to loss of neck tone (LONT) and posture (LOP) were collected using live observations. Four birds never entered TC after the first gas experience. According to Fisher's exact test, there is a significant difference (P<0.0001) in the proportion of birds that entered TC on G for the different levels of CO<sub>2</sub>. All 8 turkeys tested entered TC at 25% CO<sub>2</sub>. Only three birds out of 8, entered TC for the 35% level and only one bird out of seven entered TC for the 50% CO<sub>2</sub>. Eight birds were tested for 70%, and none entered TC. Pairwise comparisons (2x2 Fisher's exact test with Bonferroni correction) showed that the proportion of birds that entered TC on G was significantly different between 25% and all other CO<sub>2</sub> levels (6 pairs = 25% vs 35%: P= 0.02, 25% vs 50%: P<0.001, 25% vs 70%: P<0.001, 35% vs 50%: P= 0.55, 35% vs 70%: P=0.07, and 50 vs 70%: P=0.46). LONT followed LOP in all birds that entered TC at all CO<sub>2</sub> levels (average latency ± SD; 25%: LOP= 18±7 s, LONT=49±14 s; 35%: LOP=15±2 s, LONT= 38±7 s; 50%; LOP= 8 s, LONT= 15 s). Conditioned place avoidance was observed in 2 birds following LOP at 25% CO<sub>2</sub>, and not for the other CO<sub>2</sub> concentrations. However, none of the turkeys tested actively avoid 25% CO<sub>2</sub>. These results suggest that the willingness of turkeys to gain access to the feed and enrichment is weigh more than avoiding the 25% CO<sub>2</sub>, and that exposure at this level causes LONT, indicative of loss of consciousness within less than one minute. The other CO<sub>2</sub> concentrations (35%, 50% and 70%) tested were aversive for the turkeys.

## **Receptivity of animal care feedback and advice; dairy farmers' perspectives**

Stephanie Croyle\*, Emilie Belage, Deep Khosa, Stephen LeBlanc, Derek B. Haley, David Kelton

Department of Population Medicine, University of Guelph, Guelph, ON, Canada

This study explored the perceptions of dairy farmers towards communication pertaining to receiving feedback and advice related to animal welfare issues on farm (i.e. lameness, disbudding practices). The objective of the study was to identify expectations in order facilitate communication for key advisee such as on farm consultants. Qualitative data were collected through focus group discussions. Five focus group discussions consisting of 36 farmers took place in distinct regions of Ontario, Canada. Participant ages ranged from 21 to 80 (median = 47). Representative herd size ranged from 25 to 550 (median = 75). Farm type included 'free-stall' (n=14) and 'tie-stall' (n=22). Rigor was incorporated by utilizing systematic thematic analysis; data were first examined live during the interviews, later by listening to the audio, and again examined through line by line coding of focus group transcriptions. Working codes were developed by highlighting segments and creating labels in the transcripts. Codes were connected or separated through a process of developing working themes and patterns in the data. Thematic analysis of the producer discussions suggested four major themes related to the receptivity of feedback: 1) an established relationship 2) perceived responsibility and expertise for animal care 3) communication approach and 4) providing 'practical' next steps. Providing insight into what makes farmers more open and responsive to animal care advice facilitates the implementation of feedback as well as the uptake of advice.



## **A cross-sectional study of associations between management and feather damage in Canadian laying hens housed in furnished cages**

Caitlin Decina<sup>1\*</sup>, Olaf Berke<sup>1</sup>, Nienke van Staaveren<sup>2</sup>, Christine F. Baes<sup>2</sup>, Tina Widowski<sup>2</sup>, Alexandra Harlander-Matauschek<sup>2</sup>

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Feather pecking, where birds peck, pull at, and often eat the feathers of their conspecifics, is a continuous welfare challenge in the housing of egg-laying hens. Canada is currently making the transition from conventional cages to alternative housing systems, such as furnished cages. However, feather damage (FD) due to feather pecking remains a welfare concern despite increased space allowance and better opportunity to perform natural behaviours. With the goal of improving hen welfare in practice, an explorative approach was taken to assess bird, housing, and management associations with FD in Canadian laying hens housed in alternative systems. A questionnaire, focused on housing and management practices, was designed and administered to 122 laying farms nation-wide in autumn of 2017 (response rate of 52.5%), providing information on a subset of 26 flocks kept in furnished cages. Additionally, a three-point feather cover scoring system (0: fully feathered, 1: broken/missing feathers with naked area smaller than a 2-dollar coin, 2: poorly feathered with a naked area larger than a 2-dollar coin) was developed for farmers to estimate the prevalence of FD present on-farm. Farmers visually assessed a sample of 50 birds per flock, providing a FD score for the back/rump area. Flock prevalence of FD was determined by the percentage of birds with a score greater than zero. After univariable testing of 54 variables, 24 related to FD prevalence in furnished cage flocks met the inclusion criteria for model building ( $P \leq 0.25$ , or retained for biological importance) and were applied to a linear regression model using a stepwise forward building approach in R. The 6 variables of hen age (weeks), feather colour, feed structure, frequency of feeder running, midnight feeding, and scratch area presence or substrate were included in the final model, presented as regression coefficients  $\pm$  SE, and accounted for 68% of the variation in FD between farms. Increasing age ( $+0.7\% \pm 0.23$ ), brown feather colour ( $+34.6\% \pm 9.04$ ), midnight feeding ( $+24.4\% \pm 9.20$ ), and no access to a scratch area ( $+17.7\% \pm 7.88$ ) were found to be associated with higher levels of FD. Frequency of feeder running ( $+2.4\% \pm 1.54$ ), mashed feed structure ( $+13.2\% \pm 7.70$ ), and presence of scratch substrate ( $-14.2\% \pm 9.08$ ) were not found to be associated with FD. However, there were high standard errors for these variables, likely due to the small study sample of 26 flocks. These results indicate that FD as a result of feather pecking is a multifactorial problem and support existing evidence that FD increases as birds age. These results also suggest that genetics, feeding patterns/management, and access to (or lack of) a scratch area of any type play a role in FD prevalence in furnished cages. Further longitudinal investigations into these associated factors are needed in the future to affirm their influence on FD and to assist farmers in improving hen welfare during the transition to alternative systems.

## **Strain differences and effects of different densities during rearing on the musculoskeletal development of pullets**

Danielle Fawcett\*, Teresa Casey-Trott, Lorri Jensen and Tina Widowski

Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada

Osteoporosis is a major concern in the laying hen industry and poses a serious welfare concern due to the pain associated with subsequent bone fractures, especially the keel bone. Most previous research has focused on improving skeletal health during the laying phase. However, a more proactive approach is to focus on the rearing phase of the birds. This phase is often overlooked because the production cycle of hens does not begin until around 18 weeks of age. However, this phase represents a critical period for musculoskeletal growth, particularly structural bone which ceases development at the onset of lay. Therefore, it is critical to lay down a solid framework of structural bone during the rearing phase to minimize the risk of osteoporosis and bone fractures. Rearing pullets with perches or in an aviary system has been shown to have life-long effects on bone strength which may be attributed to opportunities to exercise and perform load-bearing activities, such as running, jumping, perching, wing-flapping and flight. However, only a limited number of previous studies have targeted the pullet stage. The objectives of our study were to determine if strain or stocking density affected musculoskeletal development of pullets reared in a cage system, furnished with perches and a raised platform. We hypothesized that 1) pullets reared at a lower density would have superior musculoskeletal qualities compared to pullets reared at a higher density, due to more space and opportunities to move and exercise and 2) that larger strains of birds would be more affected by density than smaller strains. This study was conducted at the Arkell Poultry Research Station, where 48 cages were distributed between 2 rooms. A platform and 3 length-wise perches were provided in each cage. This study was run as a 4 x 3 factorial, with 4 densities (38.2 in<sup>2</sup>/bird, 41.9 in<sup>2</sup>/bird, 46.3 in<sup>2</sup>/bird and 51.9 in<sup>2</sup>/bird), 3 strains of pullets (LSL-lite, Dekalb, and Lohmann Brown) and 4 replicates per strain per density. At 16 weeks of age, 8 birds per treatment-strain combination were randomly selected and euthanized for collection of muscle and bone tissue. The bicep brachii, pectoralis major, pectoralis minor and a collection of leg muscles were extracted and weighed. The humerus, radius, femur and tibia were extracted to test bone breaking strength. The keel bone was extracted to measure cartilage percentage and area. Data collection is on-going and preliminary results will be presented. Our results will provide insight on the musculoskeletal development between strain and how development is impacted by the rearing environment. Results of this study can also be used to help implement space allowances for different strains of pullets that may benefit their life-long skeletal health. Additionally, basic insight on differences in keel bone development may be acquired from this study to add to our understanding on the development and occurrence of keel bone damage in adults.

## Is there a relationship between feather pecking and response inhibition in laying hens?

Jennifer Heinsius<sup>1\*</sup>, Nienke van Staaveren<sup>1</sup>, Isabelle Kwon<sup>1</sup>, Angeli Li<sup>1</sup>, Joergen Kjaer<sup>2</sup>, Alexandra Harlander<sup>1</sup>

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Behavioural inhibitory control mechanisms provide the substrate by which inappropriate responses are suppressed, so that slower cognitive processes can guide behavior. Individuals with inhibitory control disorders exhibit repetitive behaviours where they have a deficit in the mechanism that enables them to inhibit acting. Behavioural control disorders (such as obsessive-compulsive spectrum disorders (OCD)) are a multi-faceted concept where impulsivity is a core feature, comprising impulsive action and impulsive choice. Some research suggests that feather pecking (FP) in laying hens, repetitive pecking (motor action) to the feather cover of other birds, may represent an avian analog to OCD. Whether this is true for laying hens selected for FP and whether FP contains features of impulsivity is unknown. Therefore, our objective was to use laying hens from a line selectively bred for high (H) feather pecking as well as an unselected control line (C) to measure impulsive action in a Go/No-Go task. This assessed the ability of hens to peck or withhold pecking appropriately, depending on the visual cues present in the operant chamber. We hypothesised that the level of established measurements such as pre-cue responding and incorrect withholding during a No-Go cue would be higher, and efficiency ratio (ability of the hen to peck and not peck appropriately) would be lower in H hens. Twenty 64 weeks-old White Leghorn laying hens were divided into four groups of laying hens, each based on the presence of high (H) and low (L) FP phenotypes (P) and high (H) and control (C) FP genotypes (G) [G/P: C/L n=5; C/H n=5; H/H n=6; H/L n=4]. The operant chamber measured 37 cm x 60 cm x 60 cm and had one lighted pecking key 40 cm above the floor of the chamber. Each hen learned to peck at a key in the chamber upon seeing a lighted pecking key indicating a Go cue (green light (n=10) or red light (n=10)) or to withhold pecks at a No-Go cue (the alternate light from the Go cue color) paired with an aversive sound. The hens went through five phases of learning the task by reinforcing successful pecks and successful withholds with a luxury food reward. Criteria to move on to the next phase was a 75% success rate in the last two consecutive sessions (average of 9 NoGo cues and 7 Go cues per session). The sixth phase was considered the experimental phase to assess group differences of pre-cue responses, efficiency ratio, and incorrect withholding using a mixed-model ANOVA with genotype and phenotype as factors. Analysis of data is ongoing. The results will show if breeding for high FP may also select for birds that have impaired response inhibition and/or if impulsivity is associated with FP behaviour.

## The use of tryptophan to decrease abnormal behaviour and positively affect growth in growing pigs

Maggie L Henry\*<sup>1</sup>, Robert M Friendship<sup>1</sup>, Anna K Shoveller<sup>2</sup>, Anita L Tucker<sup>1</sup>

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In growing pigs, aggression and aberrant behaviour such as tail- and ear-biting can result in decreased growth, diminished health and welfare, increased morbidity and mortality, increased labour on the part of the producer and a significant economic loss to the producer and pork industry. Tryptophan (TRP), an essential amino acid in the diet, is known to diminish aggression in humans and rats through the serotonergic system, however, this has not been examined in pigs. The objectives of this study were to determine the effect of varying inclusion rates of dietary TRP on aggression, aberrant behaviour and growth in grower pigs. This study examined the effects of 3 feed treatments on a total of 90 grower pigs (over 2 trials) divided equally across the 3 diet treatments. Sex, weight and litter origin were balanced across pens in each trial. There were 5 pigs/pen and 3 pens/treatment. This feeding trial lasted a total of 29 days/trial, with feed and water being fed *ad libitum*. A single diet was formulated based on providing all nutrients at or above their estimated NRC requirements, and providing TRP at 100% of its standard ileal digestible (SID) requirement. Amino acid mixtures were then added to the base diet to provide: 1) Control diet (TRP at 100% SID requirement), 2) TRP at 175% of its SID requirement, and 3) TRP at 250% of its SID requirement. From day 1 to 8 all pens received the control diet. On day 8, pens that were assigned to the 2 high-TRP diets began receiving their specific feed, while control pens continued receiving the control diet. All pigs had tail, ear and scratch score lesions recorded, as well as their weight taken, on day 1, 8, 15, 22 and 29. All feed was weighed and feed intake was measured for the duration of the trials on a pen level. All pigs had continuous behaviour recordings done for 12 hours (06:00-18:00) 3 days/week. An ethogram was used to examine 13 mutually exclusive behaviours and to evaluate the severity and duration of aggressive interactions taking place, including tail- and ear-biting. Plasma and serum samples were taken from 3 pigs/pen (N=27/trial) at four different time points (day 8, 15, 22 and 29). The TRP and serotonin levels will be measured using ELISA. Tail lesions were not found during the first trial, while the odds of having ear-lesions were higher on day 1 vs. day 29 (OR=19.8;  $p<0.0001$ ) across all diets; scratch score lesions were present in low numbers across all pens during the first trial. Data continues to be analyzed as it is gathered, however, it appears that TRP in excess of National Research Council requirements, does not have a positive effect on decreasing aggression or decreasing aberrant behaviour, and does not impact growth in growing pigs.

## Evaluation of different electroencephalographic and behavioural changes for on-farm euthanasia of turkeys

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Cervical dislocation (CD), either manual or mechanical, is a conditionally acceptable method of euthanasia in poultry with several potential welfare issues including prolonged time to loss of sensibility, operator training, and body weight limitations. Development of appropriate on-farm euthanasia methods is required for all poultry species. Electroencephalography (EEG) is considered the most objective method for assessing time to insensibility and brain death for mammalian and avian species. Brain death is associated with electrocerebral inactivity observed as an isoelectric (flat) line in the EEG. Behavioural changes associated with time to brain death have not been described in poultry. The aim of this study was to evaluate EEG and behavioural responses of turkeys euthanized with intravenous (I.V.) barbiturate overdose (considered the gold standard technique for euthanasia) or mechanical or manual CD. Female turkeys (8 weeks old, mean body weight 2.5kg) were randomly assigned to treatment groups and euthanized with I.V. pentobarbital sodium (1mL/4.5kg) (N=6), manual CD (N=10) or the Koechner Euthanasia Device (KED, mechanical CD, size medium) (N=12). EEGs were recorded in lightly anesthetized birds (1.5% isoflurane/100%O<sub>2</sub>) using Lifelines Ambulatory EEG system with four 25G needle electrodes inserted subcutaneously to the head for baseline activity prior to application of the euthanasia method. EEGs and bird behaviour were recorded continuously for a maximum of 5 minutes following euthanasia method application. Data were analyzed by using Proc Glimmix procedure (SAS ver.9.4).

Birds euthanized with pentobarbital presented with isoelectric EEG signals at 18±46.4s after drug administration, with feather erection occurring at 57.9±43.4s and loss of eye reflexes and auscultated cardiac function within 1 minute. For the KED, isoelectric signals occurred in only 6 birds at 268±50.8s with a mean onset of clonic convulsions at 135±24.3s. Loss of eye reflexes and cardiac arrest were only observed in 3 and 1 bird, respectively, before the 5-minute endpoint in the KED group. For manual CD, the average time to isoelectric signal was 238.63±40.2s with a mean time to onset of clonic convulsions at 77.6±31.3s. Cardiac arrest was only noted in 5 birds before the 5-minute endpoint. Loss of eye reflexes occurred approximately at 177s in 7 birds. Barbiturate overdose is an effective euthanasia method for poult, but has on-farm limitations. Mechanical and manual CD presented prolonged time to brain death compared to barbiturate overdose ( $P=0.002$  &  $P=0.002$ , respectively) and the use of KED resulted in highly inconsistent onset of brain death. Alternative euthanasia methods should be considered for this age group of turkeys.

## **Questions Arising: Some Philosophical Reflections**

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At the end of the day's session, having listened to all the presentations, I will share some deeper philosophical questions that emerged *from the work* being done by CCSAW students and faculty, questions that hopefully spur more reflection and aid in the shaping of further research questions in animal welfare.

## **Too close for comfort? Effects of stocking density on the comfort behaviours of three strains of pullets in enriched rearing cages.**

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In 2017, Canada was the first country to set welfare standards for pullets, and one of the first provisions addressed was stocking density. However, there was very little existing research on the effects of stocking density on pullets, none of which addressed behavior or welfare. Comfort behaviours such as wing flaps, leg stretches, feather raises, and body shakes are natural behaviours seen in various avian species and these behaviours often require considerable area for performance. Thus, greater performance of these behaviours may indicate a more suitable stocking density for caged pullets. This study focused on the effects of stocking density (SD) on the number of comfort behaviours performed by pullets in rearing cage system (Combi Pullet, Farmer Automatic: 240x80x84 cm<sup>3</sup>) furnished with three 238 cm perches and a raised platform running the length of the cage. Floor space allowance (cm<sup>2</sup>/bird, including platform) and number of birds per cage were as follows: 246.5 cm<sup>2</sup>, 91 birds; 270.3 cm<sup>2</sup>, 83 birds; 298.7 cm<sup>2</sup>, 75 birds; and 334.8 cm<sup>2</sup>, 67 birds. Two white-feathered strains (LSL-lite and Dekalb) and one brown-feathered strain (Lohmann Brown) were used, and there were 3-4 replicate cages per strain and SD (n=45). Cages were located in two rooms and were balanced for strain and SD. Researchers performed live observations of the pullets in their home cages at 14 weeks of age for 20 min per cage, three times per day (after lights on, midday, and prior to lights off). Focal animals were selected by choosing the bird closest to certain points in the cage at the start of an observation. Five start locations were on the cage floor, two on perches, and one on the raised platform. During a 20 min observation, a focal bird was selected according to a pre-assigned start location and observed continuously for 10 min. This was repeated for a second focal from the same cage with another starting location. Occurrences of wing flaps, leg stretches, feather raises, and body shakes were recorded. We hypothesized that greater space restriction would result in fewer comfort behaviours performed. Therefore, the largest strain (Lohmann Brown) housed at the highest SD would perform the fewest comfort behaviours and white strains at the lowest SD would perform the most. A generalized linear mixed model was used to evaluate the effects of strain, SD, time of day, and start location on the sum of all comfort behaviours performed. Room was included as a random effect. Overall, the pullets performed an average of  $0.94 \pm 0.089$  comfort behaviours per 10 min observation. Feather raises occurred most frequently ( $0.39 \pm 0.047$ ) and wing-flaps were the least frequent ( $0.14 \pm 0.030$ ). SD and time of day did not affect comfort behaviours ( $P=0.851$  and  $P=0.235$ , respectively). Lohmann Browns performed the fewest comfort behaviours and LSLs performed the most (strain;  $P=0.049$ ). However, there was no interaction between strain and SD ( $P=0.537$ ). Start location was significant ( $P<.001$ ) with birds performing three times more comfort behaviours on the perches and raised platform than on the cage floor. This suggests that pullets may seek out the necessary space to perform comfort behaviours on perches or platforms, away from the commotion of the floor. Thus, the provision of vertical spaces away from feeders or other busy resources not only provides for perching needs but may also give more birds the opportunity to perform comfort behaviours.

## Gut motility in laying hens with different propensities for feather pecking behaviour

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An increased number of behavioural disorders are seen in the majority of human patients with gut motility disorders. In laying hens, feather pecking is one of the most important behavioural and animal welfare problems. Feather pecking (FP) in laying hens is a redirected behaviour from foraging and feed pecking to the feather cover of other birds, often leading to injuries and cannibalism. Previous work has demonstrated an association between FP and feather eating. Moreover, FP birds accelerate gut feed passage time by ingesting feathers (insoluble fiber) and a genetic difference in gut feed passage time between birds selected for high (H) and low (L) FP propensities has been found. However, it is unknown whether feed passage time differences are achieved by gut peristalsis to propel digestive contents or by feathers per se. In an effort to start addressing this question, we hypothesized that these differences in feed passage time might be caused by differences in ceca smooth muscle contraction, as the ceca plays an important role in insoluble fibre digestion and triggering peristalsis. As no study has analyzed gut motility in an ex-vivo model in FP laying hens in detail, we compared ceca motility in a perfusion organ bath in H birds and unselected control (C) birds. Twenty-five birds at 64 weeks of age were selected on the basis of genotype and phenotype (11 C and 14 H). Birds were euthanized via cervical dislocation and cecal segments were excised out and placed in the well of a tissue bath perfusion system filled with an oxygenated buffer solution. Silicone tubing was used to cannulate the cranial and caudal ends of the tissue to allow for flow of oxygenated buffer solution through the lumen of the tissue. Contractions of cecal segments were recorded using a JVC camcorder and recordings were analyzed using specialized ImageJ software with a specific plug-in (DMaple©) in order to generate spatiotemporal maps. These maps depict contractility of a tissue over time and enable the measurement of multiple parameters, including frequency, velocity, and amplitude. These measurements have been validated by numerous other studies measuring intestinal motility in other species. Frequency was defined as the number of contractions per second (1/period). Velocity was defined as the slope of a contraction (distance/time). Amplitude was defined as the difference in gut diameters before and during peak contractions. The practical portion of this experiment has been completed. Analyses will soon be finalized and will make use of the GLIMMIX procedure in SAS version 9.4. While this study will not be able to assess the effect of feathers on feed passage time, results have the potential to set a baseline for further investigations into the effect of gut smooth muscle contractions on behavioural welfare problems in laying hens and other species.



## Problem-solving in laying hens

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The ability to change behaviour by developing new responses to novel stimuli, or alter existing responses to familiar stimuli, is beneficial to the welfare of animals living in complex environments. In the case of laying hens, finding food in the complex environment of a multi-tier system is facilitated if birds are flexible with respect to their associative learning and motor skills. Strain differences in fearfulness and extinction learning between brown- and white-feathered hens that may affect their ability to successfully find food in these systems are reported. This may partly be due to differences in problem-solving skills. The present experiment was designed to test problem-solving skills in brown- and white-feathered birds by evaluating cognitive abilities using a novel motor task and a reversal learning task. We hypothesized that different strains would require a different number of sessions required to successfully learn either task. Seventy-three hens of two strains – brown ( $n = 35$ ) and white ( $n = 38$ ) – housed in a multi-tier system were presented with a two-choice colour discrimination task. Food rewards were placed into one of two holes within a wooden block, which could be concealed by circular lids. Shaping was conducted in two steps in which birds did not need to manipulate the lid to access the reward: 1) the reward was placed inside one hole and the lid was positioned next to the hole, and 2) the lid was positioned covering half of the hole. Once birds had associated the lid of their assigned colour with a reward, they were presented with the novel task of completely dislodging the lid to access the hidden reward. Birds were considered to have learned the task when they correctly performed the task five out of six times in two consecutive sessions. The same procedure was used to test reversal learning, in which reward contingencies were reversed. Differences between strains were assessed using generalized linear mixed models (PROC GLIMMIX). We found that 57 out of 73 birds were successfully able to solve the novel task of dislodging the correctly coloured lid to access the concealed reward over a period of four weeks. Similarly, 44 out of those 57 were able to successfully reverse the previously learned association. The mean number of sessions (1 session = 6 trials) required to learn to access the reward was  $1.6 \pm 1.29$  (when the reward was not covered),  $2.8 \pm 1.31$  (when the reward was half-covered),  $4.7 \pm 2.83$  (during the novel task of completely dislodging the lid) and  $12.8 \pm 2.94$  (during the reversal learning task). No differences were found between strains ( $P > 0.05$ ) with respect to the mean number of sessions required to achieve the learning criteria throughout each task. In conclusion, white- and brown-feathered birds did not differ in their ability to solve a novel motor task and reverse a previously learned stimulus-reward association. This suggests that both strains could be able to navigate complex, multi-tier systems. However, further work is needed to evaluate other types of cognitive and motor skills in hens, and the possible influence they can have on their welfare within these multi-tier systems.

## Pharmacology and antinociception of a sustained-released butorphanol – poloxamer 407 formulation in Amazon parrots

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Butorphanol is an opioid drug used for pain management in Psittacidae. It has a short duration of action (2-3h) when administered IM or IV. In order to decrease frequency of handling and injections, there is a need for a long-acting opioid analgesic in avian medicine. Poloxamer 407 (P407) is a hydrogel commonly used to prepare sustained-release drugs. P407 is liquid when cold and becomes a gel at body temperature. The objective of this study was to evaluate the rheological properties and in vitro diffusion of a formulation of butorphanol in a P407 base (But-P407), as well as its pharmacokinetic parameters and nociceptive effects in Amazon parrots. But-P407 25% (8.3 mg/mL) had in vitro characteristics that made it a good candidate for a sustained-release analgesic medication: it was a gel at avian body temperature, could be sterilized by microfiltration, and its diffusion through a dialysis membrane was slower than that of butorphanol tartrate. Butorphanol was well absorbed from But-P407 25% in Hispaniolan Amazon parrots (*Amazona ventralis*) (12.5 mg/kg SC), and its pharmacokinetic profile was compatible with a sustained-release drug. Plasma concentrations stayed above the suspected therapeutic threshold for 3-8h. However, no analgesic effect of this formulation could be detected in orange-winged Amazon parrots (*Amazona amazonica*) (12.5 mg/kg SC) delivered a thermal noxious stimulus. These results could reflect poor analgesic effect of butorphanol, confounding of the poloxamer base, or a lack of sensitivity of the thermal analgesiometric model in this species. Further studies are warranted to provide recommendations for the clinical use of But-P407 25% in birds.

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## Assessing the welfare of fast-growing broilers reared in pens with or without enrichment

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Over the past 50 years, broiler chickens have been under intense genetic selection for economically important traits like high breast meat yield and high feed conversion efficiency. However, this high productivity comes at a cost, with rapid growth and emphasis on breast over leg development being discordant with skeletal and cardiovascular health. Consequently, rapidly growing broilers are at risk of developing welfare problems such as limited mobility, contact dermatitis, and heart insufficiency. Previous research indicates that broilers make some use of environmental enrichments such as platforms, and suggests that activity stimulated by enrichment may help to improve leg strength. However, the extent to which welfare can be improved by providing broilers with an enriched environment remains unclear. Our objective was to assess the health, behaviour, and welfare of fast-growing broilers reared in pens with or without enrichment. We hypothesized that broilers living in an enriched environment would be more active, have better leg strength and healthier footpads, and would be more motivated to perform play behaviour than those reared in a more barren environment. We also hypothesized that body weight would be negatively related to leg strength and activity levels, and there would be an interaction between body weight and environmental enrichment. We housed 456 Ross 708 broiler chickens from 1 day of age in 12 floor pens (38 birds/pen; each pen with 19 females and 19 males) until 43 days of age (6 weeks). Each pen (1.68 x 2.29 m<sup>2</sup>) was equipped with one tube feeder, a line of nipple drinkers and wood shavings as a litter substrate. Half of the pens had enrichment (raised platform, swinging scale, pecking stone and suet feeder filled with wood shavings) while the other half lacked enrichment. Individual body weights and litter moisture were measured on a biweekly basis. Beginning at 3 weeks of age, two males per pen (1 heaviest, 1 lightest) were outfitted with Actical<sup>®</sup> automated activity monitors to quantify activity levels. These same birds, plus two additional males per pen (1 heaviest, 1 lightest) received a latency-to-lie test in week 5 to assess leg strength and determine any association between leg strength and activity level in the home pen. We selected male birds because they are generally much bigger and heavier than females which make them more susceptible to leg disorders, thus having worse welfare. All pens were video-recorded for 5 hours between 10am to 3pm every Friday from week 3 to determine time budgets of the focal birds performing some specific behaviour such as eating, drinking, sitting, standing, walking, dust bathing, and preening, etc. Behavioural tests performed at the pen level included an obstacle test in week 5 (to assess lameness), and two tests intended to stimulate play behaviour (opening free space and offering objects suitable for worm running) to determine any effects of providing enrichments on mobility and motivation to play. The prevalence and severity of foot pad lesions was determined at 42 days. Data are currently under analysis. The results of the project will lead to a better understanding of the effects of enrichment and body weight on the health, behaviour, and welfare of fast-growing broiler chickens. This knowledge will provide a basis for recommendations on the use of enrichment for broiler chickens.

## The effect of two stage weaning on the behavioural response of beef calves with the addition of creep feeding

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Artificially weaning beef cattle is stressful on the dam and offspring, largely because their social bond is suddenly and unexpectedly severed. Ethologists have validated weaning methods that reduce the behavioural signs of distress, and animal scientists had previously validated the practice of creep feeding calves to ease their weaning transition and maintain their weight gain by providing supplemental feed that is only accessible to calves. Before our study these two beneficial practices had never been integrated in attempt to wean beef cattle in a way that protects their well-being while maximizing productivity. We hypothesized that the reduced behavioural responses of beef calves weaned in two stages would result in them spending more time eating creep-feed, compared with creep-fed calves weaned by the traditional method of abrupt separation. The study involved 24 Angus-cross cow-calf pairs observed over a 12-day period. Treatments were applied over three phases, in equal numbers, as follows: abrupt weaning of cow and calf (CONTROL1); nose flaps applied to calves for 5 days prior to separation (TWOSTAGE); and a second group abruptly weaned the same day as the TWOSTAGE pairs were physically separated (CONTROL2). Treatments were evenly divided between two pens. Behavioural observations were recorded live daily from 0830 to 1630 h. Using instantaneous sampling every 10 min, the observer recorded individual calf location (in the creep area or not) and activity: walking, lying, standing idle, eating, ruminating, nursing, and drinking water. Immediately following each instantaneous sample, vocalizations by individual calves were recorded for 2 min. When the calves were weaned, the dams were relocated to an area on-site within auditory range, but without visual and physical contact with their calves. Using SAS, data was analyzed with general linear mixed models (GLIMMIX). Preliminary results indicate a significant interaction between treatment, day, and where calves spent their time within the pen ( $P < 0.001$ ). Significant differences were found between time spent in the creep-feed area between CONTROL1 and TWOSTAGE calves, and between TWOSTAGE and CONTROL2 calves ( $P < 0.05$ ), and no difference was observed between CONTROL1 and CONTROL2 calves ( $P > 0.05$ ). On average, TWOSTAGE calves spent 47.77% of their time in the creep-feed area, while CONTROL1 and CONTROL2 spent 32.87% and 25.92%, respectively. Treatment had a significant effect on eating ( $P < 0.01$ ), with TWOSTAGE calves spending 33.54% of their time eating, while CONTROL1 and CONTROL2 calves spent 25.38% and 26.15% of their time eating, respectively. CONTROL1 calves also vocalized more than TWOSTAGE calves ( $P < 0.01$ ). The data suggests weaning stress was reduced in TWOSTAGE calves and on-going analyses are aimed at determining whether there is an interaction between treatment and use of the creep feeder, which would have implications for producers aiming to maximize productivity.

## Are “depression-like” responses really depression?

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Laboratory mice are typically housed in barren, shoebox-sized cages. These are restrictive, reducing welfare and often induce repetitive, unvarying, apparently functionless stereotypic behaviour (SB). However, not all mice in such cages perform SB. Recent research has shown that instead, some spend increased time awake but standing still. This is consistent with the reduced activity common in clinically depressed human patients. Furthermore, time spent inactive but awake (IBA) predicts increased immobility in forced swim tests (FST), a common measure of ‘learned helplessness’ in rodent models of depression. Highly stereotypic individuals instead show little or no such learned helplessness. Because of these findings, this specific form of inactivity has been said to be “depression-like”. However, although the FST is commonly used in depression-related research on rodents, this result alone is not sufficient to conclude that these IBA mice experience depression. The aim of this study is therefore to test this hypothesis. Depression is a heterogeneous disorder that cannot be diagnosed through measurement of one single trait, and it has at least two subtypes, ‘melancholic’ and ‘atypical’. Thus, a multifaceted approach is needed to assess whether IBA behaviour is a manifestation of depression, and if so, what subtype. This study will first attempt to replicate the finding that IBA behaviour predicts increased immobility during the FST, and then assess mice for six additional indicators consistent with depression: poor self-care, hypo- or hyper-cortisolaemia, anhedonia or loss of pleasure (decreased sucrose preference), elevated serum proinflammatory cytokine levels (e.g.  $\text{TNF-}\alpha$ ,  $\text{IL-1}\beta$ ), significantly increased or decreased body mass indices, and reduced hippocampal volumes. Subjects will be 72 females (24 C57BL/6, 24 DBA, and 24 BALB/c) that have been randomly assigned to standard non-enriched (NE), or larger environmentally enriched (EE) cages and reared to adulthood. It has already been established that more time is spent IBA in the standard barren cages than in the large enriched ones for these subjects. If the depression hypothesis is correct, IBA mice will spend more time immobile in the FST, have poorer coat condition, demonstrate anhedonia, and have smaller hippocampi. If their depression is of the atypical sub-type, they will also exhibit lower levels of faecal corticosterone, elevated serum proinflammatory cytokines, and greater body mass indices. If these hypotheses are accepted and standard-housed IBA mice do show symptoms of atypical depression, this would indicate that the chronic stress of barren laboratory cages creates states consistent with those experienced in clinical depression.

## Does using internal state as a discriminative stimulus require conscious awareness?

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In humans, emotions and other 'affective' states involve feelings: subjective states that we are consciously aware of. Which other species have such states is unknown (e.g. it is debated whether injured fish feel states of pain, or merely show unfelt nociceptive responses). Finding tasks that humans can only do when aware of feelings (using verbal self-report as the gold standard) could be a way to identify which other beings have similar states. One potential task is the use of internal states as 'discriminative stimuli': stimuli that determine which of two alternate operants will yield a reward. For example, humans dosed with an unknown drink (drug or placebo) can often perform the operant task of picking the label that identifies it (winning cash rewards if correct); and successful performance in this 'pick the label' task usually only occurs if the drug causes changes in self-reported feelings. However, such results are merely correlational: they do not show that drug-induced feelings are required to succeed in the task. Here, we therefore experimentally investigated whether changes in feelings play a causal role in abilities to discriminate a drug-induced state. Our drug was alcohol, chosen because it typically increases positive emotions: feelings that we could also manipulate in other ways, via music chosen to increase or decrease positive emotions. If subjects use their feelings to guide selection in the 'pick the label' task (as they choose between alcohol and placebo labels to identify what they have just drunk), then changing emotions with music could alter their tendencies to label the unknown drink as alcohol; for example, inducing positive emotions with music should increase their tendencies to pick the 'alcohol' label in the cash-rewarded choice task. 42 subjects were first trained to discriminate alcohol from placebo, with 32 successfully learning this task. Self-reported changes in state (across a wide range of feelings) were also recorded. 13 subjects emerged as reporting emotional reactions to the alcohol, although unexpectedly often negative ones. These were then exposed to a new set of unlabeled drinks, this time in the presence of music by Albinoni or Mozart: respectively sad or happy. Subjects exposed to emotional music concordant with how they felt on alcohol ( $n = 8$ ; thus the Mozart if upbeat on alcohol, and the Albinoni if negative on alcohol) were significantly more likely to pick the 'alcohol' label in the choice task, compared to subjects exposed to music whose emotional effects opposed how they reacted to alcohol ( $n = 5$ ) ( $t = 2.53$ ,  $p = 0.0297$ ). This was despite all having been given placebo. Thus altered feelings did cause changes in operant behaviour, in this task where the presence or absence of alcohol was a discriminative stimulus. The strengths and weaknesses of using such tasks to identify conscious awareness in animals, and the types of additional research still needed on humans, will be discussed.

## Behavioural and physiological consequences of stressors on laying hens

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Laying hens are often subjected to adverse early life events causing stress such as constant mixing and limited access to environmental enrichment when birds are housed in large flocks. These events predispose animals to perform not only feather pecking (FP) (repetitive pecks at the plumage of conspecifics) but behaviors towards conspecifics such as threats or aggressions causing poorer body condition and welfare. Under stress, there is an alteration of tryptophan (TRP) metabolism which affects the immune system and the bacterial community in the gut. However, it is unclear whether chronic, unpredictable stressors induce both those behavioural phenotypes and physiological alterations in laying hens which could affect hens' welfare and egg productivity. Therefore, the aim of the study was to investigate how a combination of social and non-social stressors during adolescence affect laying hens' reactions towards conspecifics, their physiological response and egg production. Eighty-six White Leghorn laying hens (19 week of age) were housed in enriched floor pens and randomly assigned to 6 stressed and 6 non-stressed groups of 7 birds (baseline period). At 23 weeks of age, we examined the behavioural and physiological consequences of combinations of social (mixing with unfamiliar birds, physical individually manual restrain for 5 min, physical collective restrain in a transport crate in a crouched position for 1 hour) and non-social (introduction to a novel environment, missing nest boxes or perches) stressors on hens. Stressors were repeated 4 to 5 times each in an unpredictable way over 3 weeks (treatment period). During both the baseline and treatment period, individual behavioural data (FP, threat and aggression), body condition (feather cover, injuries; 22, 26 wks) blood TRP metabolites (22, 26 wks) as well as excreta microbiota (22, 26 wks) were recorded/collected. Egg were collected and recorded daily per pen. A generalized linear mixed model (Proc Glimmix) will be used to analyze the data (SAS V9.4, SAS Institute, Cary NC). Variance of variables will be partitioned into the fixed effect stress using the most appropriate distribution. We expect behaviours frequencies of FP, threat and aggression and related feather cover and injury scores to be increased by the stress treatment. Stressed birds will show distinct TRP blood and excreta metabolites levels. Results of this study have the potential to show whether combined social and non-social stressors might trigger specific behaviour and physiological changes causing poorer body condition (feather loss and injuries) and reduced egg production of the birds and therefore impair the hens' welfare.

## Will dairy cows sort their diet in response to negative energy balance?

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Dairy cattle often succumb to periods of negative energy balance (NEB) in early lactation, as they are unable to consume sufficient dry matter intake to meet their energy requirements for milk production. As a result, cows begin to mobilize body fat as a source of energy, putting the animal at risk for ketosis, a condition that leads to a greater risk of other metabolic and infectious diseases, thus having a negative impact on cow welfare. It is important, therefore, to identify ways in which dairy cows may mitigate the impacts of NEB. The objective of this study was to determine how feed sorting behavior of dairy cows is altered in response to NEB. Holstein cows ( $n=30$ ; days in milk= $59\pm 5$ ) were followed for 2 wk where they were fed (on a dry matter basis) a lactating diet ( $NE_L=1.66$  Mcal/kg; 68% forage) and produced  $44.6\pm 1.2$  kg/d of milk. To induce negative energy balance, cows were then exposed for 3 wk to a total mixed ration (TMR) formulated for a 12% reduction in energy available for milk ( $NE_L=1.56$  Mcal/kg; 73% forage). Blood samples were taken 1x/wk during the baseline period and every 4 d while on the treatment diet and analyzed for non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB). TMR samples (fresh and orts) were collected every 3 d and separated into: long ( $>19$ mm), medium ( $<19, >8$ mm), short ( $<8, >4$ mm), and fine ( $<4$ mm) particles. Feed sorting was calculated as: actual intake of each particle fraction expressed as a % of predicted intake. Data were analysed in repeated measures, mixed-effect linear regression models. During the baseline period cow NEFA and BHB averaged  $0.29\pm 0.04$  mmol/L and  $0.71\pm 0.08$  mmol/L, respectively. During the experimental period, NEFA and BHB increased ( $P<0.001$ ) to  $0.32$  mmol/L and  $1.00$  mmol/L, on average, with a peak of NEFA ( $0.59\pm 0.06$  mmol/L) and BHB ( $1.12\pm 0.11$  mmol/L) occurring 4 d after dietary change. During the baseline period, cows sorted against long particles ( $95.3\pm 1.4\%$ ), did not sort medium particles ( $99.8\pm 0.2\%$ ), and sorted for short ( $101.1\pm 0.8\%$ ) and fine ( $101.8\pm 0.6\%$ ) particles. During the experimental period, cows increased ( $P=0.008$ ) sorting against long particles ( $92.2\pm 1.3\%$ ), continued to not sort medium particles ( $100.0\pm 0.1\%$ ), increased ( $P=0.002$ ) their sorting for short particles ( $102.1\pm 0.8\%$ ), and continued to sort for fine ( $102.5\pm 0.6\%$ ) particles. In the baseline period there was no association ( $P>0.6$ ) of feed sorting and blood NEFA levels. In the experimental period, higher NEFA levels were associated with greater sorting against long particles (% long sorting =  $-15.0 \times \text{NEFA (mmol/L)} + 97.0$ ;  $R^2=0.20$ ;  $P=0.01$ ). Further, in that period higher NEFA levels were associated with greater sorting for short particles (% short sorting =  $4.8 \times \text{NEFA (mmol/L)} + 100.5$ ;  $R^2=0.18$ ;  $P=0.02$ ). The results indicate cows alter their feed sorting behavior, to consume greater energy, in response to experience of a period of negative energy balance. This suggests that allowing high-producing dairy cows the ability to selectively consume the higher energy portions of the diet, may limit the effects of NEB, and promote greater health and welfare.



## **Associations between the general physical condition and the selling price of culled dairy cows sold at three Ontario auction markets**

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About one third of Ontario's dairy cows are culled annually from the herd and are commonly sold at auction markets to enter the food chain. The most common reasons for culling are fertility problems, low milk production, or udder problems. Poor health condition or injuries can also contribute to the decision to cull, either directly, or indirectly, by negatively impacting fertility or production. Disease or injury can also impact the cow's ability to handle the stress of transportation and subsequently may reduce their welfare. The purpose of this study was to determine the general physical condition of culled dairy cows sold at Ontario auction markets, and to determine how this related to the sale price. We hypothesized that culled cows sold at auction markets in suboptimal condition, according to the proAction® Animal Care animal-based evaluation methods (2017), would sell for less compared to cows that were in good condition. Culled dairy cows were observed and evaluated during weekly sales at three licensed Ontario livestock auction markets. Data were collected on 4460 dairy cows sold over 16 weeks (June to August inclusive). As cows passed through the sales ring the primary observer recorded each cow's hock injury score, body condition score (BCS), gait score, and whether they had a tail, according to the standards of the proAction Animal Care program. Secondary observers recorded each cow's bodyweight from the ring scale, their breed, and the sale price paid. Statistical analysis software (SAS 9.4 Institute Inc., Cary, NC, USA) was used to analyze the data. The frequency of each variable was calculated, and a mixed model was used to calculate the least squares means, which determined the relationship of the price paid and each variable measured. Body weight and week variables were held constant in the analysis. Results showed that 27.2% of these culled cows had a hock injury with swelling greater than 2.5 cm and/or a lesion present, 40.7% had a BCS $\leq$ 2, 72.7% had an abnormal gait, and 12.5% had docked tails. Cows with a BCS $\leq$ 2 sold for \$0.20 less per kg (SE=0.0087 \$/kg) compared to those with a BCS $>$ 2, which equated to an average loss of \$117 per cow ( $P < 0.001$ ). Cows with an abnormal gait sold for \$0.04 less per kg (SE=0.0080 \$/kg) compared to culled cows with a normal gait, which equated to an overall average loss of \$32.45 per cow ( $P < 0.001$ ). Except for location, 2-way interactions between all explanatory variables were tested. There was only an interaction between BCS and gait score seen. The most common issues identified in this study were low BCS and lameness. Both translated into a lower price paid for the animal compared to those that were in good condition. The authors hope that these results will encourage producers to refrain from transporting cows that are in suboptimal condition, and may not be able to withstand the stress of transport. These results indicate that further research is required to determine ways to prevent the transport and sale of cows in poor general condition to protect their welfare.

## Enriched mice are nice: long-term effects of environmental enrichment on agonism in female C57BL/6s, DBA/2s, and BALB/cs

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Laboratory mice often live in non-enriched (NE) shoebox-sized cages. Adding environmental enrichment (EE: preferred, complex stimuli) usually enhances welfare, but in male mice can cause resource-defence agonism. In contrast, past work found that large EE cages *reduce* agonism in female mice, with a potential link to reduced stereotypic behaviour (SB). Female agonism includes aggression (mounting, rough grooming, chasing & pinning) and milder negative interactions: displacement (pushing) & genital sniffing. Using 3 strains - C57BL/6 (C57s), DBA/2 (DBAs) and BALB/c (BALBs), we sought to replicate findings that EE females are 'nicer'. We assessed whether this is a side-effect of lower interaction rates that are likely to occur in larger cages; and whether EE agonism is qualitatively milder (e.g. genital sniffing) and less aggressive. We then re-checked if agonism relates to SB, plus inactive-but-awake behaviour (IBA), an abnormal, potentially depression-like response. 165 females were weaned at 3-4 weeks into mixed-strain trios in 33 NE cages, and 22 2x2x1 ft. cages with various enrichments. We scan-sampled in-cage behaviour from 9am to 1pm for 5-6 days, in four periods (2, 4, 7 and 9 months post-weaning). Data were analysed using repeated measure general linear mixed models (unless otherwise stated). Similar to past work, EE mice were less agonistic than NE mice (2.3% vs. 5.7%;  $F_{1,158}=338.24$ ,  $P<0.0001$ ). There were fewer social interactions in EE cages than NE cages (C57:  $F_{1,52}=102.66$ ; DBA:  $F_{1,53}=59.38$ ; BALB:  $F_{1,53}=166.58$ ;  $P<0.0001$  for all), but EE mice performed less agonism even after correcting for this ( $F_{1,158}=137.02$ ,  $P<0.0001$ ). Qualitatively, NE C57s were more aggressive relative to all agonism than EE C57s ( $F_{1,46}=4.28$ ,  $P=0.04$ ), while the EE C57s performed more genital sniffing (Mann Whitney U:  $Z=-2.59$ ,  $P=0.009$ ). In contrast, EE BALBs performed more aggressive agonism than NE BALBs ( $F_{1,52}=36.95$ ,  $P<0.0001$ ). Stereotypic NE BALBs were more agonistic, but *only* in month 9 – a possible false positive; whereas stereotypic NE DBAs both displayed ( $F_{1,90}=14.34$ ,  $P=0.0003$ ) and received ( $F_{1,90}=8.51$ ,  $P=0.005$ ) less agonism, perhaps because they stereotyped so often (31% of all observations) that they evaded social interactions. NE DBAs ( $F_{1,90}=4.22$ ,  $P=0.04$ ) and EE BALBs ( $F_{1,59}=5.55$ ,  $P=0.02$ ) who performed more IBA were more agonistic. The repeated measure term also revealed that agonism levels declined over time in NE mice ( $F_{1,255.8}=12.61$ ,  $P=0.0005$ ), though never reaching the low levels of EE mice - which were stable throughout the study ( $F_{1,104.4}=0.35$ ,  $P=0.56$ ). Overall, providing EE thus improves welfare by reducing agonism in female mice. In C57s, EE also reduced how aggressive the agonism was. Future work should see if depression-like effects promote aggression in NE C57s, and also test a hypothesis inspired by our time course patterns: that EE mice may form stable hierarchies faster than NE mice.

## Effect of solid feed location on feed consumption and growth of dairy calves

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When feeding high quantities of milk to dairy calves, encouraging sufficient solid feed intake during weaning can be a challenge. Insufficient intake increases the risk of health challenges, causes hunger, decreases growth after weaning and delays rumen development, consequently reducing welfare. The objective of this study was to investigate how the location of solid feed offered to dairy calves affects their intakes and growth during the milk-feeding (d 1-42), weaning (d 43-56), and post-weaning (d 57-70) periods. Sixty Holstein heifer calves were housed in individual pens (152 × 167 cm) and randomly assigned to 1 of 2 treatments: solid feed adjacent to the milk source or solid feed opposite from the milk source within their pen. All calves were offered 12.5L/d of milk replacer (in 2.5 L meals, 5x/d) by an automated rail milk feeder (AMF) until d 43, when they were gradually weaned off milk by d 57. All calves had access to ad libitum water from birth and starter ration of concentrate (95%) mixed with chopped (2.54 cm) wheat straw (5%) from d 5. At d 57, calves had access to only water and starter ration and were monitored to d 70. Feed and water intakes were measured daily and milk intakes were recorded automatically by the AMF. Calf Body weight was measured 2x/wk. Data were summarized by period and analyzed using generalized linear mixed models with repeated measures. Feed adjacent to the milk source tended to result in greater Dry matter intake (0.067 vs 0.056 kg/d; SE=0.005; P=0.09) during the milk-feeding phase. Feed consumption did not differ between treatments during the weaning (adjacent=0.81 kg/d, opposite=0.75 kg/d; SE=0.056; P=0.46) and post-weaning (adjacent=2.99 kg/d, opposite=2.87 kg/d; SE=0.093; P=0.42) periods. Adjacent treatment calves had greater water intakes during the milk-feeding (0.75 vs 0.55L/d; SE=0.49; P=0.005) and post-weaning (10.11 vs 9.30L/d; SE= 0.27; P=0.04) periods, and tended to consume more water during weaning (2.55 vs 2.12L/d; SE=0.16; P=0.06). Greater intakes for the adjacent treatment calves may have contributed to a tendency for greater average daily gain (1.11 vs 1.05 kg/d; SE=0.023; P=0.08) during the milk-feeding period. Growth was similar between treatments for the weaning (0.83 vs 0.83 kg/d; SE=0.04; P=0.96) and post-weaning periods (1.22 vs 1.14 kg/d; SE=0.06; P=0.28). The results indicate that placement of solid feed close to the milk source encourages calves to increase their feed intake and water consumption during the milk-feeding stage, which may contribute to more consistent growth during that period. This may improve rumen development and health, thus contributing to greater calf welfare.

## Effect of type of gradual weaning program on intakes and growth of dairy calves fed a high level of milk

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Gradual weaning programs are known to result in better growth, behaviour and welfare of dairy calves compared to abrupt weaning programs. Gradual weaning programs encourage greater feed intake, which improves rumen development, reduces behavioural signs of hunger and decreases health challenges, thus improving welfare. However, the optimal gradual weaning program is not yet known. The objective of this study was to investigate how two gradual weaning programs affect intake and growth of dairy calves during the milk-feeding (d 1-42), weaning (d 43-56), and post-weaning (d 57-70) periods. Sixty Holstein heifer calves were housed in individual pens (152x167 cm) and randomly assigned to 1 of 2 treatments: continuous gradual weaning program (CG) or multi-step gradual weaning program (MSG). Calves were offered 12.5L/d of milk replacer (in 2.5 L meals, 5x/d) by an automated rail milk feeder (AMF) until d 43, when weaning commenced according to their assigned treatment. Calves had access to ad libitum water from birth and starter ration of mixed concentrate (95%) and chopped (2.54 cm) wheat straw (5%) from d 5. Calves on CG program are weaned from 12.5L/d to 2.0L/d in small, equal increments until d 57. Calves on MSG program were offered 10L/d for 3 d, 8L/d for 4 d, 6L/d for 3 d, and 3L/d for 4 d until d 57. At d 57, all calves did not receive milk and were monitored until d 70. Feed and water intakes were measured daily and milk intakes were recorded automatically by the AMF. Calf body weight was measured 2x/wk. Data were summarized by period and analyzed using generalized linear mixed models with repeated measures. Milk intake was similar during the milk-feeding period (CG=9.1L/d, MSG=8.9L/d; SE=0.21; P=0.63). During weaning, milk intake varied by day depending on treatment (treatmentxday = P<0.01), but on average CG calves consumed more milk than MSG (6.7 vs 6.1 L/d; SE=0.12; P<0.01). Feed intake did not differ between treatments in the milk-feeding (CG=0.063 kg/d, MSG=0.060 kg/d; SE=0.0047; P=0.66), weaning (CG=0.75 kg/d, MSG=0.80 kg/d; SE=0.056; P=0.54), or post-weaning periods (CG=2.97 kg/d, MSG=2.91 kg/d; SE=0.093; P=0.68). Growth rates did not vary by treatment (P=0.8) during the milk-feeding (1.08±0.03 kg/d) and post-weaning (1.18±0.06 kg/d) periods. Growth was subject to a treatmentxwk interaction (SE=0.051, P=0.006) during weaning, resulting in CG calves growing at 1.08 kg/d during wk 7 and 0.59 kg/d in wk 8, versus MSG calves growing at 0.94 kg/d in wk 7 and 0.70 kg/d in wk 8. The results indicate that type of gradual weaning program may impact nutrient consumption and growth during the weaning period. This may result in improved nutrient digestibility, health, and growth, thus improving calf welfare.

## **Assessing the welfare of turkeys euthanized with CO<sub>2</sub> under different fill rates**

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Due to aesthetic concerns with physical methods of euthanasia such as blunt force trauma, there has been increasing use of gaseous agents for poultry euthanasia. Carbon dioxide (CO<sub>2</sub>) is the most commonly used gaseous agent for euthanasia on-farm, and commercially manufactured units that deliver controlled concentrations of CO<sub>2</sub> for euthanasia of small animals are available for purchase. Research on mammalian species has shown that gas fill rates and chamber stocking density influence animals' response to CO<sub>2</sub> prior to loss of sensibility. In piglets, for example, increasing group size resulted in an increased number of kicks and strikes experienced by group-mates, but also shorter latency to death as reduced air volume from increased stocking density resulted in higher CO<sub>2</sub> concentrations. This is a concern for turkey welfare because birds are likely to perform convulsive wing flapping during gas euthanasia, which can cause pain and distress to other conscious animals if there is significant asynchrony in time to loss of sensibility. Further research is still needed to determine the full welfare implications of gas euthanasia for turkeys of all ages and weights, and determine the optimum conditions for minimizing welfare-related concerns. Our objective is to compare distress related behaviour of turkeys at four ages and four chamber fill rates. It is hypothesized that birds will display less head shaking and gasping behaviour with faster fill rates. In this project, cull turkey poults at 1 day, 3 days, 7 days, and 3 weeks of age will be used. Poults will be euthanized in pairs using a Euthanex box at one of four gas fill rates (3, 6, 9, or 12 L/min), or immersion in a pre-filled chamber with a sample size of n=12 per treatment. Behaviours indicative of distress (vocalizations, gasping, defecation, wing flapping) and reflexes indicative of loss of sensibility (neck tone, jaw tone, rhythmic breathing, loss of posture) will be recorded by live observations and video cameras once birds enter the chamber. Birds will be exposed to CO<sub>2</sub> for a full minute after last movement is recorded. Data will be analyzed with an analysis of variance, with fill rate and age as fixed effects and poult body weight as a covariate. The results of this project will provide a better understanding of CO<sub>2</sub> euthanasia methodologies and their impact on the welfare of turkeys. This will allow for science-based policies for on-farm euthanasia of turkeys that improve animal welfare.

## Determining human ability to recognize canine stress in familiar and unfamiliar dogs

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Dogs can experience stress through novel environments, loud noises, and other stress-inducing stimuli. Owners are in the optimal position to alleviate stress, but need to be able to recognize signs of stress before they can take action to reduce it. Previous research showed that dog owners readily identified moderate to extreme stress behaviours in dogs, but performed less reliably when the behaviours were subtle, such as lip licking. This project aimed to determine human ability to recognise canine stress. It was hypothesized that dog owners would not accurately identify canine stress. Dog owners (n=29) registered at the Dogs in The Park (DITP) training facility in Guelph, ON, participated in this study. Classes focussed on obedience behaviours and skills that could elicit a variety of stress levels. Dog owners answered demographic questions about themselves and their dogs, including how confident they felt in their ability to determine stress in dogs. During 1hr classes, researchers asked DITP clients to rate on a scale of 1-5 (1=zero stress, 5=extreme stress) their dog's stress level during a 1min timeframe while participating in training activities. Clients were also asked to rate an unfamiliar dog's stress level after a 1min observation period. Researchers and DITP instructors assigned a rating for each rating provided by clients. During the 1min observations for familiar and unfamiliar dogs, researchers counted stress behaviours (lip licking, body shaking, body scratching, self-chewing, yawning, vocalizations) on an all-occurrence basis, and ears back behaviour on 10sec intervals. A GLIMMIX procedure determined differences between client, instructor and researcher ratings. Spearman correlations examined differences between researcher ratings and observed behaviours. Regression analyses examined the effect of client and dog demographics on observed behaviours. Results indicated that dog owners and DITP instructors rated canine stress significantly lower than the researcher ( $p < 0.04$ ). Additionally, owners rated their own dog's stress significantly higher than stranger dogs ( $p = 0.04$ ). Vocalizations and ear positioning (neutral and backward) occurrences correlated with the researcher's canine stress rating ( $p < 0.0008$ ) and these behaviours decreased with increasing number of classes attended by the dog in the previous week ( $p < 0.066$ ). Yawning decreased with increasing dog age ( $p = 0.079$ ). These results indicate that owners do not recognize stress in their dogs comparable to researchers, but owners are more attuned to their own dog than a stranger dog. Stress behaviours observed in dogs were tempered by age and familiarity with the facility. Determining human ability to identify canine stress highlights the need to improve owner recognition of stress behaviours, with emphasis on unfamiliar dogs. This is important to keep human interactions with unfamiliar dogs safe, thus improving dog welfare, as well as improve owner and dog interactions.

## **Boredom-like states in mink are rapidly reduced by enrichment, and are unrelated to stereotypic behaviour**

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Captive animals often live in barren housing that may trigger stereotypic behaviour (SB). Barren housing is also suggested to cause “boredom”: a negative state triggered by monotony that increases motivations to obtain stimulation. Farmed mink are excellent models for testing the hypotheses that: 1) monotony causes boredom (as it does in humans), and 2) boredom causes SB (since SB is often reduced by diverse enrichments, suggesting that SB could reflect motivations to obtain stimulation). We previously did so in two independent populations (Meagher & Mason, PLoS One. 7:e49180, 2012; Meagher et al., Appl. Anim. Behav. Sci. 197:112, 2017), by exposing mink to diverse stimuli (ranging from rewarding to aversive) when housed in monotonous (non-enriched [NE]) or stimulating (enriched [E]) conditions. We inferred minks’ interest in these stimuli from latencies to contact, and time spent oriented towards and in contact with them. As predicted if NE housing causes boredom, interest in all stimuli was significantly higher in NE than E mink (using orientation and contact values). However, SB did not positively predict interest in these stimuli (the first study finding the opposite for contact duration; and the second, no link at all). Using a new, third sample of young adult males ( $n=32$ ) from an experimental farm, we re-tested this hypothesis, and tested a new hypothesis: that stimulating environments rapidly reverse boredom. Minks’ interest in stimuli was tested after seven months in typical commercial, minimally-enriched (ME) cages (containing 1 wiffle ball and 1 shelf, to meet Canadian Codes of Practice), and two days after half the mink were moved to extra-enriched (EE) housing (larger cages with more physical enrichments and shelves). Mixed models tested for housing effects on boredom (mink ID set as a random effect), while Spearman correlations tested whether SB and boredom covaried. Compared to ME mink, contact times were increased ( $F=15.70$ ,  $P=0.0004$ ) and orientation times tended to ( $F=3.03$ ,  $P=0.09$ ), although latencies were not, in EE mink. SB was reduced in EE mink too ( $Z=2.78$ ,  $P=0.006$ ). But again, SB did not predict more interest in stimuli in ME conditions (save for a negative trend with latency to contact:  $\rho=-0.32$ ,  $P=0.08$ ); and after the move to EE housing, nor did reductions in interest in the stimuli covary with reductions in SB. Finally, given the contradictory patterns across studies, we pooled data from all three to re-analyse, with greater power, links between SB and boredom-like elevated exploration (in GLMs with ‘study’ as a blocking factor, and SB and housing as predictors). Overall, there were no significant associations between interest in stimuli and SB. Thus, boredom-like states (i.e., generalised motivations for stimulation) do not seem to cause SB in mink. Our new study also supports the hypothesis that commercial cages induce boredom-like states which can be rapidly reduced by enrichment.

## Startle reflex as a welfare indicator in laying hens

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Startle reflexes are rapid, involuntary responses to sudden and intense stimuli (e.g. sudden sounds). In mammals, startle reflexes indicate affective states (increasing in magnitude in negative states, and decreasing in positive ones), but whether these findings generalize to any avian species is unknown. We therefore investigated the potential of the startle reflex as an indicator of affective state in laying hens. We hypothesized that adult laying hens in relatively positive affective states would show diminished startle magnitudes. We manipulated affective state in two ways: first, by training hens to associate the startle test apparatus with reward; and second, in a larger study, by allowing hens long-term access to preferred housing conditions. All data were analyzed using GLMs unless otherwise noted. In Experiment One, 24 ISA Brown laying hens were individually placed in a dark startle chamber and exposed to a xenon light flash, during which their baseline startle magnitudes were measured using a force plate. Sixteen hens then received 20 sessions of reward conditioning in the startle chamber (meal worms and sweet corn), while the remaining eight were used as controls. To assess the effectiveness of this reward conditioning, latencies to enter the startle chamber were measured by placing the hens on a 1m long runway. The startle test procedure was then repeated for all hens. Conditioned hens' approach latencies were shorter than control hens' ( $F_{1,23}=42.02$ ,  $P<0.001$ ), confirming that they formed a positive association with the startle chamber. However, contrary to our prediction, reward conditioning did not reduce hens' startle magnitudes compared to controls ( $11.08$  Newtons [N]  $\pm$   $11.29$  vs.  $10.57$  N  $\pm$   $11.80$  [LSMs  $\pm$  SEs];  $F_{1,17}=0.15$ ,  $P=0.7$ ). In Experiment Two, 96 ISA Brown laying hens were randomly divided into 24 groups of four. Twelve control groups were housed in  $1.5\text{m}^2$  pens with a nest box, a perch, bedded with spruce shavings. Twelve enriched groups were kept in larger ( $10\text{m}^2$ ) more spatially complex enclosures containing a priori preferred resources including: foraging enrichments, dust bathing substrates, a heat lamp, and perches of various heights and diameters. Enriched hens also had free access to a proxy control pen, the use of which was assessed using instantaneous scan sampling. After five weeks in their respective housing conditions, hens were individually startled using the same procedures as Experiment One. Groups of enriched hens spent little time in their proxy control pens even after correcting for its smaller floor area (one-sample Wilcoxon:  $Z_{1,11}=-39.0$ ,  $P<0.001$ ), confirming that the resources were preferred and thus produced more positive affect. As predicted, the startle magnitudes of enriched hens were significantly lower than control hens ( $1.79$  N  $\pm$   $1.56$  vs.  $19.65$  N  $\pm$   $1.55$ ;  $F_{1,23,53}=104.00$ ,  $P<0.001$ ). Experiment Two's results thus support the validity of the startle reflex as a hen welfare indicator: the first ever demonstration that previous findings for mammals also hold for birds. However, the lack of a similar pattern in Experiment One indicates that more research is needed to assess and improve this indicator's sensitivity.



## Expression of canine inherited behaviours in crossbreds versus purebreds

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Purebred dog temperament and behaviour are well studied and understood but the same cannot be said for crossbred dogs. As crossbreds gain popularity, how they express inherited behaviour traits in comparison to their purebred constituent breeds is of interest. Owners may acquire one of these crossbreds unaware of potential behaviour challenges, which may lead to these animals being surrendered or euthanized. In this study, we investigated behaviours exhibited by crossbred dogs in comparison to the expression of those behaviours by their constituent breeds. We focused on the popular Goldendoodle and Labradoodle crossbreds and compared them to their corresponding constituent breeds: Standard and Miniature Poodle, Golden Retriever or Labrador Retriever. The data for this study were provided by 2,013 volunteer dog owners who filled out the C-BARQ online survey created by Hsu and Serpell in 2003. The survey results were used to analyze fourteen different representative behavioural trait scores: trainability, stranger aggression, owner directed aggression, dog directed aggression, dog rivalry, dog directed fear, stranger directed fear, non-social fear, touch sensitivity, separation related problems, excitability, attachment seeking behaviours, energy, and chasing. Data were analysed separately for each crossbreed using general linear mixed models (GLMM in SAS V9.4) that included the effects of breed, sex, where acquired, country of residence, and owner as a random effect. As expected from a first-generation crossbred (F1), the crossbreds in our study tended to fall between the two constituent parent breeds with some interesting exceptions. Our results suggest that the F1 Labradoodle differed significantly from one of the pure constituent breeds in dog rivalry ( $P=0.0012$ ), non-social fear ( $P=0.0310$ ), and energy ( $P=0.0342$ ). In contrast, the F1 Goldendoodle crossbreds expressed behaviour that varied from the Golden Retriever for excitability ( $P=0.0206$ ). We also found statistically significant differences ( $P<0.05$ ) between the behaviour trait scores of the purebred retrievers despite their very similar work origins and current service use. These results can help advise future dog owners on the appropriate choice in a companion animal for their family and help reduce the risk of these crossbreds being surrendered to animal shelters.

## Effect of owner presence on dog responses to a routine physical exam in a veterinary setting

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Many companion dogs show signs of fear and have impaired welfare during veterinary visits. Dogs are often removed from their owners for aspects of exams or procedures, but the effect of this removal has not previously been examined and anecdotal reports suggest that it can be both beneficial and detrimental to dog welfare. Therefore, we assessed the effect of owner presence on behavioral and physiological indicators of fear during a routine physical exam in a companion animal veterinary clinic. Owned dogs and their owners were recruited locally and the dogs received a standardized physical examination with (n=16) or without (n=16) their owner present. Dog behavior was recorded and responses were assessed by a blinded observer during each exam phase (head exam, lymph node palpation, abdominal palpation, temperature, heart rate, and respiratory rate). For behavioral measures, mixed poisson and logistic regression models assessed the effects of owner presence, exam phase, sex, and age, with dog as a random effect. For physiological measures, linear regression models were used. When owners were present, dogs had a lower frequency of certain fear indicators. In particular, when the owner was present versus absent, they had a reduced rate [95% CI] of vocalizations (e.g., whining, barking) (present, 0.11 vocalizations/min [0.03-0.39] vs absent, 1.84 vocalizations/min [1.2-2.8];  $F_{(1,29)}=19.84$ ,  $P<0.0001$ ), reduced mean temperature (present, 37.2°C [36.7-37.7] vs absent, 38°C [37.5-38.5];  $F_{(1,27)}=6.13$ ,  $P<0.0198$ ), and reduced heart rate (present, 82.4 beats per minute [74.2-90.6] vs absent, 98.6 beats per minute [89.7-107.5];  $F_{(1,27)}=7.58$ ,  $P<0.0104$ ). Rate of lip licking showed an interaction with age ( $F_{(1,28)}=6.39$ ,  $P<0.0174$ ), and was lower with the owner present in older dogs. Interestingly, dogs yawned more when their owner was present (0.24 yawns/min [0.08-0.69] vs absent (0.05 yawns/min [0.01-0.21];  $F_{(1,30)}=4.25$ ,  $P<0.048$ ). Owner presence did not significantly influence posture reductions, avoidance, escape attempts, or respiratory rate. However, exam phase also influenced fear indicators, including the frequency of posture reductions ( $F_{(5,150)}=3.49$ ,  $P<0.0052$ ), avoidance ( $F_{(4,152)}=8.71$ ,  $P<0.0001$ ), escape attempts ( $F_{(5,154)}=5.68$ ,  $P<0.0001$ ), and lip licking ( $F_{(5,155)}=5.7$ ,  $P<0.0001$ ). Overall, fear indicators appeared to be higher during the head exam, lymph palpation, and body palpation phases of the exam in comparison to assessments of temperature, heart rate, and respiratory rate. These results suggest that fear levels are reduced in dogs when their owners are present during an examination and that owners should be encouraged to remain with their dog whenever possible during veterinary visits to improve dog welfare within this setting.