

Proceedings of the 14th  
**ISAE North American  
Regional Meeting**



**April 29<sup>th</sup> & 30<sup>th</sup>, 2022**  
**University of California, Davis**  
**Davis, CA, USA**

Organizing and scientific committee:  
Richard Blatchford, Hanna Butler-Struben,  
Rachael Coon, Alycia Drwencke  
Kristina Horback (Chair), Claudia Lu  
Maja Makagon, Joy Mench  
Carly Moody, Allison Pullin and Cassandra Tucker

# Acknowledgments

**The organizing committee would like to thank the following persons for their help in organizing the conference and reviewing the abstracts:**

Department of Animal Science, volunteer reviewers (Joy Mench, Jeremy Marchant, Christopher Byrd, Carissa Wickens, Shawna Weimer, Leonie Jacobs, Grazyne Tresoldi, Julie Huzzey, Amy Robinson-Junker, Penny Lawlis, Derek Haley, Arlene Garcia, Lisa Gunter), moderators (Melissa Cantor, Anastasia Stellato, Grazy Tresoldi, Shawna Weimer), and judges (Carissa Wickens, Emma Grigg, Courtney Daigle, Julie Huzzey, Abbie Viscardi, Katherine Creutzinger, Maria Camila Ceballos, Joy Mench, Brianna Gaskill, Suzanne Millman, Melissa Cantor, Anastasia Stellato, Grazy Tresoldi, Shawna Weimer).

**Sponsorship for the conference has been generously provided by**



# Program Overview

## Friday, April 29<sup>th</sup>

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08:00 – 09:20	Registration Opens
09:30 – 11:30	Oral Presentation: <b>Human-Animal Interaction</b>
12:00 – 14:00	Lunch
14:00 – 15:00	Keynote Lecture: <b>Dr. Gail Patricelli</b>
15:00 – 16:00	Poster Presentations Group B & Coffee Break
16:00 – 16:30	Oral Presentation: <b>Feeding Behavior</b>
18:00 – 21:00	Banquet Dinner

## Saturday, April 30<sup>th</sup>

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09:00 – 09:30	Oral Presentation: <b>Social Behavior</b>
10:00 – 11:00	Poster Presentation A/B & Coffee Break
11:00 – 11:45	Oral Presentation: <b>Pain</b>
12:00 – 14:00	Lunch
14:00 – 14:45	Oral Presentation: <b>Housing and Management</b>
15:00 – 15:15	Coffee Break
15:15 – 16:00	Student Award Ceremony
16:00 – 18:00	Final Reception

## Sunday, May 1<sup>st</sup> Optional Excursion

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Matchbook Winery, Capay Valley (Zamora, CA)

\$30/person (includes bus ride and tasting)

# General Information

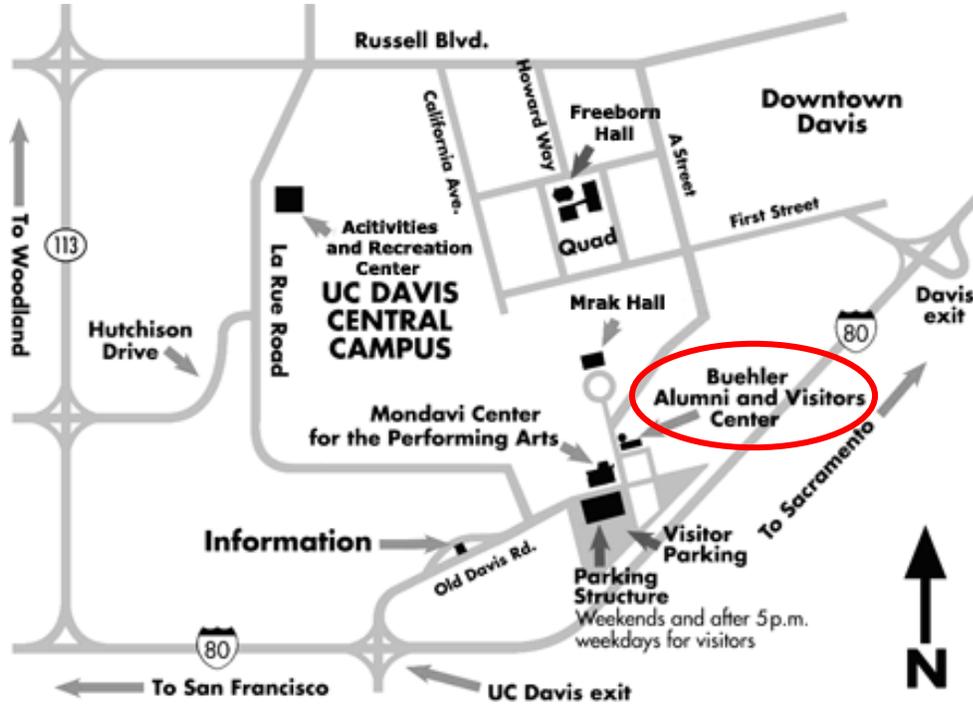
## Contact Information

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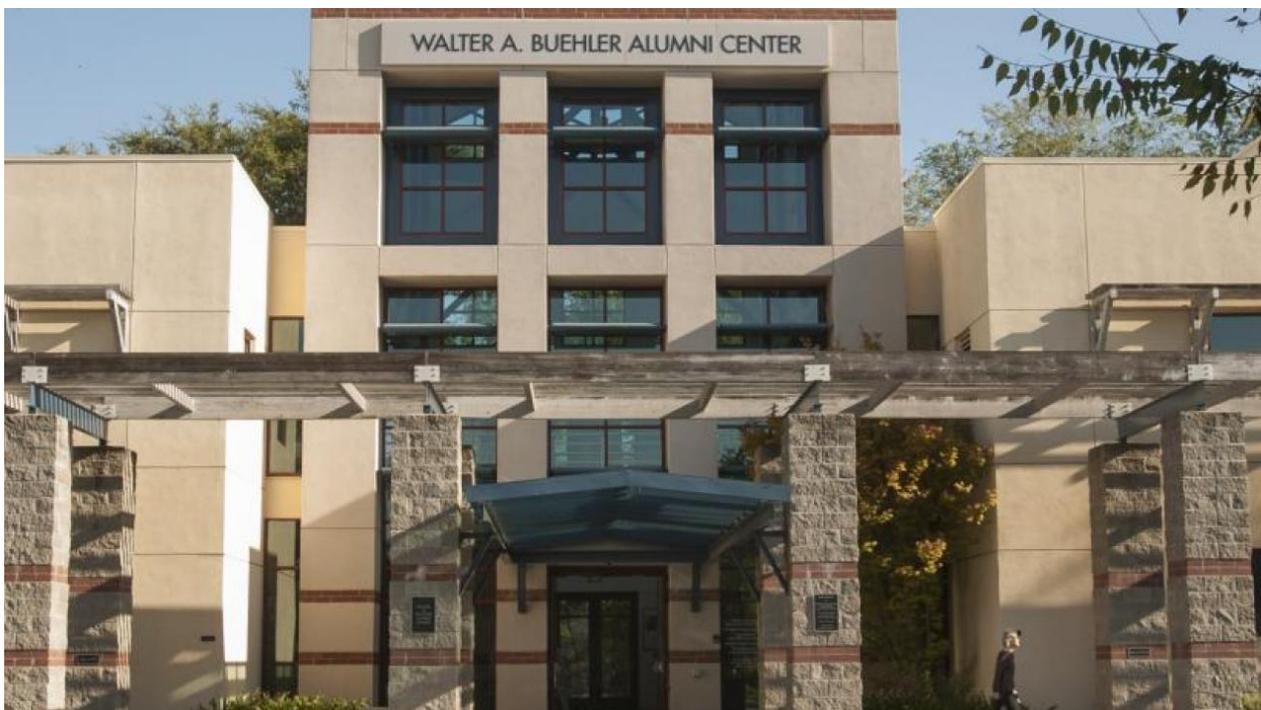
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# Map

Direction to Alumni Center:



Alumni Center (Front view):



### Venue and Surrounding Areas:

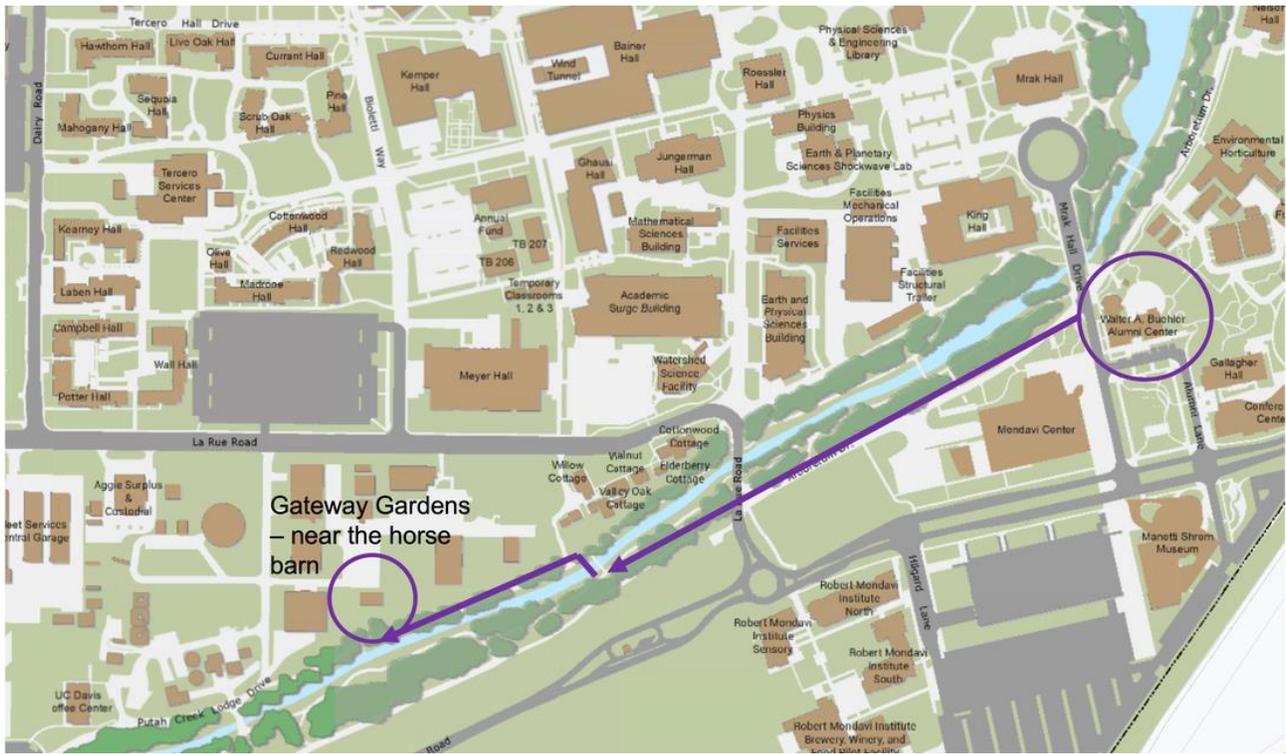


visitor parking



Alumni Center

### Direction to Gateway Garden from Alumni Center:



# Oral Presentations

Presenter name underlined

## Friday AM

## Human-Animal Interaction

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- 9:30 Evaluation of tube handling to improve the speed of post-shipment habituation in laboratory mice**  
Brianna Gaskill, Christina Boykin, Israel Zuniga, Kate Maynard, and Fabrizio Scorrano
- 9:45 Too much too soon: risk factors for fear behavior in kittens in foster care**  
Courtney Graham, David L. Pearl, and Lee Niel
- 11:00 Effects of caretaker interactions on the welfare of adult dogs in commercial breeding kennels**  
JoAnna Rogowski, Aitor Arrazola Durana, Traci Shreyer, Shanis Barnard, and Candace Croney
- 11:15 Associations between behavior modification program progression, cat stress core, and latency to emerge from hiding in shelter cats from hoarding environments**  
Bailey Egan, Karen van Haaften, and Alexandra Protopopova
- 11:30 Evaluating behavioral implications of calf handling and restraint methods used for processing pre-weaned beef calves**  
Lindsey Arkangel, Christy Goldhawk, Claire Windeyer, and Edmond Pajor

## Friday PM

## Feeding Behavior

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- 4:00 Hay provision affects 24h performance of normal and abnormal oral behaviors in milk-fed dairy calves.**  
Blair Downey and Cassandra Tucker

**4:15 Isolation box test and its relationship with daily patterns of activity and feeding behavior measured by precision technology.**

Megan Woodrum Setser, Heather Neave, and Joao H.C. Costa

**4:30 Can the supplementation of parental diets with omega-3 fatty acids increase the spatial memory of Shaver white and ISA brown chickens in a T-maze test?**

Rosemary Whittle, Elijah Kiarie, and Tina Widowski

**Saturday AM Social Behavior**

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**9:00 Effects of a disease challenge on social networks of group-housed dairy calves**

Katherine Burke and Emily K. Miller-Cushon

**9:15 Age and litter size influence puppy competitive behavior during nursing in the domestic dog.**

Quinn Rausch, Erica Gibson, Gordon Lo, and Lee Niel

**9:30 Are infrared thermography and automated feeding systems capable of identifying group-housed sow social hierarchies?**

Dominique Sommer, Jennifer M. Young, and Christopher J. Byrd

**Saturday AM Pain**

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**11:00 Wound healing following caustic paste disbudding in dairy calves**

Alycia Drwencke, Sarah Adcock, and Cassandra Tucker

**11:15 Effects of willow bark (Salix) on pain and stress in recently disbudded organic dairy calves.**

Madison Bacon, Marcia Endres, and Bradley Heins

**11:30 Evaluation of firocoxib, delivered transmammary to piglets, to alleviate pain associated with elective husbandry procedures**

Abbie Viscardi, Elizabeth A. Shirtcliff, Michael D. Kleinhenz, Emily Eppler, and Johann F. Coetzee

**11:45 Developing a Goat Grimace Scale to assess pain in goats**

Maria Lou, Michael D. Kleinhenz, Emily J. Reppert, Madeline G. Hall, Mikaela Weeder, Alyssa Leslie, Johann F. Coetzee, and Abbie V. Viscardi

**Saturday PM**

**Housing and Management**

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- 2:00 Laying hen welfare in furnished cages: Effect of sham dustbathing and rearing experience on motivation to dustbathe on peat**  
Kristen Panetta, Ana K. Rentsch, Ian J. Duncan, and Tina M. Widowski
- 2:15 Do housing-induced changes in brain activity cause stereotypic behaviours in mice?**  
Lindsey Kitchenham, Aileen MacLellan, Pietro Paletta, Ashutosh Patel, Elena Choleris, and Georgia Mason
- 2:30 Measuring the amount of vertical space needed for laying hens to flap their wings**  
Tesa Grebey, Junjie Han, Juan Steibel, and Janice Siegford
- 2:45 Impact of stocking density and exercise on the social and stereotypic behaviors of developing beef heifers**  
Courtney Daigle, Reinaldo F. Cooke, Logan Rhamel, Aiden E. Juge, and Kelsey S. Harvey

# **ABSTRACTS FOR POSTERS**

# Group A

## 1 Owner perspectives on canine handling techniques during a routine veterinary appointment.

Amber D. Carroll<sup>1\*</sup>, Alissa Cisneros<sup>1</sup>, Hannah Porter<sup>1</sup>, Carly Moody<sup>2</sup>, Anastasia C. Stellato<sup>1</sup>

<sup>1</sup>Department of Animal and Food Sciences, Texas Tech University, 2500 Broadway, Lubbock, TX, USA, 79409

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Veterinary care is a necessary part of animal care; however, it can be a source of stress for domestic dogs and their owners. If a dog owner is not satisfied with the veterinary experience, frequency of veterinary visits may reduce and impact a dog's health and welfare. Providing dog owners with the opportunity to offer their perspectives on aspects of the veterinary appointment may improve client satisfaction and increase owner willingness to bring their dog to the veterinary clinic. Therefore, our aim was to assess owner attitudes towards 13 handling techniques used on dogs during routine veterinary appointments. An online cross-sectional survey targeting current North American dog owners was used to examine the influence of participant's pet attachment and demographic information (age, sex, experience working in the veterinary field) on owner agreement towards the handling techniques. The Lexington Attachment to Pets Scale was used to indicate level of pet attachment. Likert-scale questions were designed to assess level of agreement with handling techniques when the participants' dog was calm, fearful, or aggressive. Overall, participants (n= 1,176) disagreed with higher restraint techniques (e.g., full-body restraint, muzzle hold) and tools (e.g., dog mask), and agreed with lower restraint techniques (e.g., minimal restraint) regardless of dog demeanor. Logistic regression models revealed that dog owners were more likely to agree with the use of full-body restraint on fearful dogs if they had veterinary experience ( $p < 0.001$ ), or were male ( $P = 0.015$ ), and less likely to agree if they were older than 45 years of age ( $P = 0.04$ ). Further, dog owners were more likely to agree with the use of minimal restraint on fearful dogs if they had a higher pet attachment score ( $p < 0.001$ ), or if they did not have veterinary experience ( $p < 0.001$ ). Owner agreement with techniques involving lower levels of restraint and disagreement with methods involving higher degrees of restraint, is in line with current dog handling recommendations. Overall, using low stress handling methods may help improve owner satisfaction, as well as dog welfare at the veterinary clinic.

## 3 Utilization of enrichment objects by growing pigs and the impact on lesion scores.

Kristofer Smith<sup>1</sup>, Meghann K. Pierdon<sup>2</sup>

<sup>1</sup> University of Pennsylvania School of Veterinary Medicine; <sup>2</sup>VMD ACAW

**Statement of problem:** Public concern for the welfare of agricultural animals has brought attention to the environment in which pigs are grown and pigs' ability to perform

normal behaviors. The goal of enrichment is to allow the pigs to perform species specific behaviors and prevent destructive behavior including aggression toward pen mates.

**Objective:** The goal of this study is to investigate whether growing pigs demonstrate a preference for various readily available enrichment objects and to see if those same objects impact lesions.

**Materials and Methods:** The study was performed in a wean-to-finish facility. 129 pens were randomly allotted to 1 of 8 enrichment objects. Pens held on average  $27.1 \pm 2.9$  pigs. Enrichment was placed on a single day 42-56 days after pigs were delivered. Objects included AllBite™ blocks, Bite-Rites, chains, branched chains, apple Porcichews®, vanilla Porcichews®, ropes, EasyFix® Luna, and nothing. The objects were replaced or repaired as needed. Scan sampling was performed two days per week, 4 times per day, for 7 weeks, starting at 0800. A single observer performed direct observation counting the number of pigs interacting with each object. Once per week, one pen of each object was randomly chosen for lesion scoring. Lesion scoring was performed by enumerating all scratches and abrasions on the body, head, and ears of 25% of the pigs in each pen.

**Statistics:** Statistics were done using Stata IC15.1. All data were non-normal. A Kruskal-Wallis test, with a post-hoc Dunn Test, was used to evaluate the effect of object and day on the proportion of pigs interacting with the object as well as the effect of the object type on lesions. A Bonferroni correction was used to account for multiple comparisons.

**Results:** There was a significant effect of the object on the proportion of pigs interacting with the object ( $P < 0.001$ ) with rope being the most visited and chains the least. There was a significant effect of day on the proportion of pigs interacting with the object ( $P < 0.001$ ) with the proportion on day 1 ( $8.2\% \pm 9.2\%$ ) significantly higher than all other days ( $P < 0.001$ ). Pigs were still visiting the objects on day 50 ( $2.5\% \pm 3.0\%$ ). There was a significant decrease in lesions with chains ( $16.4 \pm 7.8$ ), rope ( $15.3 \pm 6.5$ ), EasyFix® Luna ( $15.6 \pm 5.9$ ), and All Bite™ blocks ( $14.5 \pm 6.8$ ) having fewer lesions than pens with no enrichment ( $20.4 \pm 5.7$ ) ( $P < 0.05$ ).

**Discussion:** Rope, which is readily available, was the most visited object compared to the other objects in the study. Provision of rope decreased the number of lesions as did several of the other objects suggesting that pigs redirected some aggressive interactions toward the enrichment objects. Though there was a decrease in visits to the objects over time, there were still a proportion of pigs visiting the objects when the study was terminated. The data suggest such readily available objects are utilized by pigs in this life stage. Further work is needed to determine whether such object could prevent specific vice behaviors such as tail and flank biting, as opposed to total lesions on body.

## **6 Measuring motivation for alfalfa hay in feedlot cattle using voluntary interaction with an aversive stimulus.**

*Rachael Coon<sup>1\*</sup>, Cassandra B. Tucker<sup>1</sup>*

<sup>1</sup>Center for Animal Welfare, Department of Animal Science, University of California, Davis, CA, USA

\*Presenting author: [recoon@ucdavis.edu](mailto:recoon@ucdavis.edu)

Finishing cattle are fed high-grain diets that may result in sub-acute ruminal acidosis, but it is unclear if these animals want more forage or not. Previous work has asked this question with weighted gates and found ceiling effects of physical strength. Other options, like operant tasks, are difficult to interpret in terms of what they mean to the animal and often require individual housing to execute. To address limitations, we asked cattle if they would voluntarily engage with electric current, a universal adverse stimulus that can be presented in a graded manner, to access forage when fed a finishing diet. We predicted that animals would engage at a higher current when accessing alfalfa hay than animals accessing an additional offering of their primary diet or nothing at all. Twenty-four steers were each assigned exclusive access to 2 automated feed bins. One bin contained their primary diet, TMR fed ad-libitum (115% of previous day's intake), and the other bin contained 1 of 3 treatments (n=8 each): 1) 0.2 kg alfalfa hay (AF), 2) 0.6 kg of additional TMR, measured to match the alfalfa by volume or 3) Empty (E). The primary diet was fed 2x/d while the treatments were delivered only during the morning feeding. To access a treatment, the steer voluntarily pushed his muzzle against an electrified barrier which rose out of his way. The animals were exposed to treatments for 24 h while the barrier was in place but not electrified. The current then increased exponentially every 48 h (0, 156, 312, 625, 1250, 2500, 5000  $\mu$ A) until the animal ceased accessing the bin. To provide context for these values, 156  $\mu$ A is not perceptible to humans, an electric prod is reported to deliver 2,500 to 3,000  $\mu$ A and levels above 3000  $\mu$ A are painful for humans. Visits to the bin were recorded continuously 24 h/d. Five animals (1 AF and 4 E) were removed for failing to visit after the initial 24 h exposure. The proportion of steers successfully accessing treatments at each current level was analyzed using survival analyses (logrank). No animals visited at 2500  $\mu$ A, thus 5000  $\mu$ A was not tested; 50% visited at 1250  $\mu$ A and 72% at 625  $\mu$ A. The probability of advancing to the next current level was higher for AF and TMR animals than E animals ( $p < 0.01$ ), but not different between AF and TMR animals ( $p = 0.78$ ). A pairwise Wilcoxon test found that, compared to E animals, AF and TMR animals both accessed the treatments at higher currents (mean ( $\pm$ SE) maximums: 195 $\pm$ 39, 1042 $\pm$ 122, and 976 $\pm$ 137 $\mu$ A for E, AF, and TMR, respectively;  $p = 0.01$ ), but did not differ from one another ( $p = 0.76$ ). This work demonstrates that contrafreeloading is far more important to cattle than previously understood. Future work needs to untangle if relatively barren housing conditions contribute to this motivation. This research also demonstrates that alfalfa can be a valued resource for cattle, much more so than an empty bin, but it does not indicate that this forage type, above and beyond being an additional food item, matters for them. While high-concentrate diets may cause health problems for finishing cattle, what they want, in terms of opportunities to consume forage, may be satisfied by this form of management.

Ethical note: We recognize the potential ethical concerns of using electric current and, at each step of the process, took this very seriously. This experiment was designed to allow cattle to touch the electrified barrier *entirely voluntarily*. Cattle did not have to

touch the electrified barrier to access any essential nutrients, as they were freely fed to 115% of their intake from the previous day from their 2<sup>nd</sup> bin (with no barrier). We deliberately chose a starting current that was as low as possible (below our ability to feel it) and below the upper limit of what humans report as painful (3000  $\mu$ A and above). The maximum of 5000  $\mu$ A was used by animals in pilot testing, but in this experiment, the upper limit was 2500  $\mu$ A and no animals engaged with it at this point. This work was approved by the UC Davis IACUC and adheres to the ISAE ethical guidance about use of aversive stimuli.

## **9 Response to novel feed in dairy calves is affected by prior hay provision and presentation style.**

*Morrow<sup>1,2</sup>, Chelsea M., Downey<sup>1</sup>, Blair C., and Tucker<sup>1</sup>, Cassandra B.*

*1Center for Animal Welfare, Department of Animal Science, University of California, Davis, CA, USA; 2School of Veterinary Medicine, University of California, Davis 95616, USA*

Pasture-raised cattle begin to process forage within days of birth. Dairy calves in the US, however, are often raised in restricted environments without access to forage until weaning. Introducing novel forage at a time when calves must quickly adapt to processing a solid-based diet may lead to challenges. We set out to assess how early feed experience, particularly the type and presentation of forage, affected initial response to a novel forage mix at weaning. Twenty-seven Holstein heifer calves were housed individually on sand bedding and fed a diet of starter grain and milk replacer (5.7 - 8.4 L/d step-up) via a bottle (C) or given additional access to mountaingrass hay presented in 2 formats: in a bucket (B), or in a PVC pipe feeder (P), which was designed to encourage different feeding movements to extract hay from holes with the tongue. Treatments were applied from birth through 50 d of age, when step-down weaning began. All calves had 3 buckets (l to r: grain, water, hay or empty) and pipe feeder (empty or with hay) in the same location in their respective pens, regardless of treatment. On d 50 before milk was withheld in the morning to start weaning, each calf was blocked inside the hutch with an opaque board for less than 1 min. TMR (total mixed ration; novel grain + alfalfa) was put in the 3<sup>rd</sup> bucket that previously contained hay (B) or was empty (C, P). The calf was released and video-recorded for 30 min. Intake was calculated, while videos were scored for latency to eat TMR, proportion of time spent eating TMR, and the number of startle and retreat responses. B calves approached and began eating TMR in  $4.6 \pm 1$  s (mean  $\pm$  SE), faster than both P ( $38.4 \pm 14$  s; generalized linear model,  $P = 0.005$ ) and C ( $60.2 \pm 12$  s,  $P = 0.003$ ) calves. Startles and retreats differed across groups (chi-square,  $P = 0.017$ ), with C calves reacting more than B (20 vs. 2 combined occurrences) and P calves (10 occurrences). While TMR intake was similar across groups (C:  $40 \pm 10$  g, B:  $42 \pm 15$  g, P:  $39 \pm 8$  g; generalized linear model,  $P = 0.978$ ), B calves spent less time eating TMR than C calves (proportion of time:  $0.26 \pm 0.10$  vs.  $0.57 \pm 0.10$ ; beta regression,  $P = 0.002$ ), while P calves tended to spend less time eating than C calves ( $0.38 \pm 0.10$ ;  $P = 0.07$ ), suggesting previous experience with hay improves processing ability when presented with a novel forage. Overall, calves with previous experience with hay in the bucket demonstrated no apparent caution to approaching or processing novel TMR. Calves

with previous experience with hay, but not in the bucket, were similarly adept at processing novel TMR but demonstrated apparent transient neophobia to the novel placement of TMR (previously empty bucket). Control calves faced heightened challenges in both regards: transient neophobia to both forage and presentation location, and slower processing times of TMR, attributable to lack of experience in the preweaning period. Therefore, response to novel feed in dairy calves is affected by both early life experience, such as opportunities to process forage, and the presentation of the feed itself.

### **13 Development and application of a scoring system for septum injuries in beef calves with and without a nose flap.**

Ashlynn A. Kirk<sup>1\*</sup>, Cassandra B. Tucker<sup>1</sup>

<sup>1</sup>Center for Animal Welfare, Department of Animal Science, University of California, Davis, CA, USA

\*Presenting author: [aakirk@ucdavis.edu](mailto:aakirk@ucdavis.edu)

The weaning period is a stressful time for beef calves because they must quickly gain nutritional and social independence from their dam. Previous work has found that gradual methods of weaning, such as when the calf is fitted with a nose flap to prevent suckling, reduce the behavioral and physiological indicators of stress. Nose flaps are held in place by the nasal septum and are worn for 4 to 7 days. The majority of literature regarding nose flaps examine effectiveness of weaning at d 7 after insertion, but there is little mentioned about injuries caused. In the present study, we aimed to identify if one type of plastic nose flap that was administered for 7 days caused nasal injuries and to create a reliable scoring system to characterize these injuries. Eighty-two (n=82) Angus and Angus-Hereford crossbred beef calves were assigned to 'Flap' or 'No Flap' treatments in a between-animal design. Data were collected by taking images of each nostril at d 0 (before; final n=164 photos), d 7 (on day of removal; final n=158 photos), and d 13 (final n=80 photos; 'Flap only' animals) relative to insertion. Wounds were scored for the presence/absence of 3 characteristics: damage, impression, and blood (each characteristic scored as 0 or 1). Evidence of damage was described as when the tissue in the site where the flap rested was a different color than surrounding nostril; natural variation in nostril color (e.g. spots) were not included as wound-related difference. Impression was defined as when the edges of the wound were clearly raised or sunken, distinct from surrounding tissue. Presence of blood was defined as bright red liquid present in the nostril area, either in or around the site where the flap rested. One trained observer scored a subset of photos (n=64 from d 7 and n=80 from d 13) twice, in a consistent manner for all 3 characteristics (damage, impression and blood, 95%, 91% and 97% agreement between first and second evaluations, respectively). Once trained, the observer then scored the full dataset. No nostrils in the 'No Flap' treatment that had damage, impression, or blood present. All nostrils from animals that kept their flap in for 7 days had damage and impressions and 76% of nostrils had blood present immediately after nose flap removal (p<0.001; Chi-square 2x2 contingency of presence/absent and Flap/No Flap for each wound characteristic). Wounds were scored 1 week later (d 13) and 100% of nostrils still had visible damage, 45% of nostrils had an impression where the nose flap was placed and 14.2% of nostrils had blood present.

We created a reliable method to score nasal septum injuries, which were present in all calves fitted with a nose flap for 7 days. These injuries were still present 1 week after nose flap removal.

## **16 Gait traits and digital dermatitis in beef cattle**

Anice Thomas<sup>1\*</sup>, Karin Orsel<sup>1</sup> and Edmond Pajor<sup>1</sup>

<sup>1</sup>Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, Canada

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Digital dermatitis (DD) is a major cause of infectious lameness in cattle and, significantly impacts cattle productivity and welfare. The aim of this study was to determine the association between DD disease stages, locomotion, and gait traits (arched back, asymmetric gait, head bobbing, tracking up, reluctance to bear weight) and determine which traits are most associated with DD in beef cattle. Cattle ( $n = 310$ ) from 13 pens across three commercial feedlots were enrolled. Locomotion and gait scores were assigned to cattle as they walked four strides down a dirt alleyway. Next, cattle were restrained in a chute, each hind foot lifted, and DD disease stage; no lesion, active (ulcerative) or chronic lesion determined. In total 104 cattle had DD; 69 presented with active lesions and 35 with chronic lesions. Cumulative odds ordinal and binomial logistic regression models were developed to determine the association between DD disease stage, locomotion score (LS), and gait traits. Test characteristics, sensitivity (Se) and specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) were calculated to determine which traits were most associated with DD. There was a significant association between DD disease stage and cattle being lame ( $P < 0.001$ ). Odds ratios (OR) for cattle with DD being lame or moderately to severely lame was 8.6 ( $P < 0.001$ ) and 10.8 ( $P < 0.001$ ) times more than cattle without lesions. Cattle with active lesions had the greatest odds of being lame (OR 10.2;  $P < 0.0001$ ) and moderately to severely lame (OR 16.0;  $P < 0.001$ ) compared to those without lesions. There was a significant association between DD disease stage and all five gait traits ( $P < 0.001$ ). Asymmetric gait (AG), and reluctance to bear weight (WB) had the greatest OR for classifying cattle with DD as having altered gait (OR 5.5;  $P < 0.001$  and OR 5.8;  $P < 0.001$  respectively) or having severely altered gait (OR 8.2;  $P < 0.001$  and OR 17.9;  $P < 0.001$  respectively), compared to cattle without lesions. The OR for cattle with active lesions having altered and severely altered gait was greatest for WB which was 6.0 ( $P < 0.001$ ) and 25.2 ( $P < 0.001$ ) times greater than cattle without lesions. The OR for cattle with chronic lesions having altered gait was greatest for AG and was 6.5 ( $P < 0.001$ ) times more than cattle without lesions. All gait traits had low Se for detecting cattle with DD and varied greatly from 6.7% to 34.6% with LS (Se 34.6%; PPV 76.6%) and AG (Se 25.0%; PPV 74.3%) being most associated. For all traits Sp was high ranging from 94.1% (NPV 72.1%) for LS to 98.4% (NPV 68.9%) for WB. In conclusion, locomotion score is still the best tool to detect cattle with DD and gait traits WB and AG had the strongest association with cattle that had DD. Priority should be given to these traits when assessing cattle and when developing detection guidelines.

## 22 Too much too soon? Risk factors for fear behaviour in kittens in foster care.

*Courtney Graham, David L. Pearl, Lee Niel*

*Department of Population Medicine, Ontario Veterinary College, University of Guelph, Canada*

Many companion kittens spend their sensitive period for socialization (~2 to 9 weeks of age) in foster care, and the quality of these early life experiences can impact behavioural development. This study aimed to improve early kitten care by using an online Qualtrics survey to investigate risk factors for fear behaviour in foster kittens prior to adoption (7 to 9 weeks of age) based on early management practices, foster parent personality traits, and evaluations of kitten behaviour. Worldwide recruitment resulted in 74 foster parent responses for a total of 241 kittens. Our main behavioural outcome was whether kittens showed fear behaviours during interactions with unfamiliar people, which included displays of avoidance, freezing, crouching, ears back, piloerection, and aggression. We also analyzed the impacts of being reported as fearful at intake into foster care, high vs. low amounts of gentling (i.e., general handling, mimicking handling during a vet visit, exposure to people), high vs. low amounts of stimulus exposure (e.g., to interactive toys, scratching material, etc.), and foster parent personality traits. Of the 241 kittens analyzed, 69 were reported fearful at intake, 109 received high gentling, and 95 received high stimulus exposure. Using a mixed logistic regression model, with foster parent as a random effect to account for foster home clustering, we found that kittens who were reported fearful at intake into foster care were more likely to display fear behaviours towards people than kittens who were not reported fearful at intake (OR=43.43 [95% CI=9.06, 208.26];  $p<0.001$ ). Kittens who received a high amount of stimulus exposure were more likely to display fear towards people compared to those who received a low amount of stimulus exposure (OR=15.90 [2.84, 89.06];  $p=0.002$ ), regardless of fear level at intake. Interestingly, we did not find a reduction in displays of fear behaviour towards people with increased gentling ( $p=0.17$ ), which was predicted based on previous literature. We also found that 48% of the variance in displays of fear behaviour toward people was at the foster-home level, suggesting this outcome was impacted by either litter effects or the foster parent's personality or approaches to socialization. Related to foster personality, kittens whose foster parent had a high score for emotional stability were more likely to display fear towards people compared to those who had a low score for emotional stability (OR=8.86 [1.66, 47.33];  $p=0.011$ ). However, this finding might reflect a bias towards emotionally stable foster parents being more willing to foster fearful kittens. Overall, these findings suggest that high amounts of stimulus exposure and gentling are not necessarily beneficial during early socialization. High gentling was ineffective at reducing fear levels in the current study, and high stimulus exposures appear to have exceeded kitten limits for optimal behavioural development, potentially due to flooding. Our previous research has shown that clear and concise training in identifying kitten behaviour improves human accuracy of rating fear in kittens—the current study reiterates the importance of accurately identifying when kittens are fearful and adapting socialization practices appropriately.

## 24 The association between feeding behaviors and disease in automatically fed group-housed preweaned dairy calves in the Upper Midwest.

Rielle K. Perttu\*, Mateus Peiter\*, Tiago Bresolin#, Joao R. R. Dórea#, Marcia I. Endres\*

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The majority of preweaned dairy calves are housed individually on US dairy farms. Housing calves individually can minimize the risk of disease; however, it can have detrimental effects on the calf's affective state. Automated milk feeders (AMF) make it easier for producers to feed calves in group settings. These feeders offer a more flexible labor schedule and greater behavioral freedom for the calves. Since housing calves in groups potentially leads to increased morbidity and mortality, feeding behavior changes recorded by AMF software could potentially be used as an indicator of disease. Therefore, the objective of this study was to investigate the association between feeding behaviors and disease in automatically fed group-housed preweaned dairy calves in the Upper Midwest. The study farm was visited on a weekly basis from May 2018 to May 2019 when calves were visually scored for health and AMF software data were collected. Calf health scores included calf attitude, ear position, ocular discharge, nasal discharge, hide dirtiness, and cough score. A generalized additive mixed model (GAMM) approach was used to identify associations between feeding behavior and disease. The final GAMM ( $n = 599$  calves) included the fixed effects of rewarded visits (with milk being offered), unrewarded visits (without milk), total milk intake (mL per day), average milk intake per visit (mL/day), drinking speed (mL per min), interval between visits (min), visit duration (min), and calf age (day). Total intake per day, drinking speed, interval between visits, age, and rewarded visits were associated with dairy calf health status ( $P < 0.05$ ). Interactions between total intake per day and number of rewarded visits, interval between visits and number of rewarded visits, and drinking speed and total intake per day were also significant (Figure 1). We plan to use these variables to develop a predictive model that flags a sick calf days before a sickness event. Our results suggest that calf growers could use the behavioral traits collected by AMF as a potential indicator of calf disease. In addition, GAMM showed to be a simple and flexible approach to model calf health status, as this approach can deal with non-normal data distribution of the response variable, capture nonlinear relationships between explanatory and response variables, and accommodate random effects.

Figure 1. Predicted health status of dairy calves on the odds ratio scale for the significant interaction number of rewarded visits and total intake (A), number of rewarded visits and interval between visits (B), and total intake and drinking speed (C).

## 27 Spatial skills of 3-week-old laying hen chicks are affected by flock, housing, and genetic strain.

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Early experience is important for development of spatial skills in laying hens. Rearing aviaries vary in their space and complexity, mainly in the brooding compartments where chicks are confined for the first few weeks of life. We asked how environmental complexity during early life (i.e., in brooding compartments) and genetic strain (brown vs white feathered) affect spatial skills of 3-week-old chicks. In 4 consecutive flocks, we split 3000 chicks each (brown- and white- feathered) into 4 commercially available rearing systems; conventional pullet cages (CC, barren cage), a rearing aviary of low complexity (A1, cage with 2 perches), intermediate complexity (A2, cage with 3 perches and an elevated platform), and high complexity (A3, large open floor with 6 perches and a swinging elevated platform). Of these chicks, spatial learning was assessed in 972 using a T-maze where a social reward (a mirror) was located in the one arm, while the other was empty. A test included 4 minutes of habituation, followed by training (2 forced visits into both arms of the maze), then chicks were given 5 tries to navigate the maze and locate the reward, each lasting until a choice was made or 90 sec passed. Only chicks that explored the maze (reached the decision fork) were included in the statistical analysis (n=449). We analyzed the number of correct and incorrect choices, and indecisions by fitting a generalized mixed effect model with Poisson distribution, and latency to choose by fitting a linear mixed effect model. Fixed effects were flock, strain, and housing; random effect was the housing section nested in housing treatment; and p-values were significant at < 0.05 for main effects and < 0.1 for interactions where main effects were significant. A flock by strain by housing interaction explained the number of correct choices ( $\chi^2=16.35$ ,  $df=9$ ,  $p=0.06$ ) though post-hoc analysis showed inconsistent effects for each of the factors. Number of wrong choices was not affected by either flock, strain, or housing. The number of trials where no choice was made was explained by a housing by flock interaction ( $\chi^2=15.89$ ,  $df=9$ ,  $p=0.07$ ) with flock 1 choosing more often than flock 4 ( $p<0.05$ ) and in flock 3, A2 and A3 being more often undecided than A1 ( $p<0.05$ ). Latency was explained by flock ( $\chi^2=42.18$ ,  $df=3$ ,  $p<0.0001$ ) and a strain trend ( $\chi^2=3.29$ ,  $df=1$ ,  $p=0.07$ ). Flock 1 had a shorter latency to choose than all other flocks, and the latency of white chicks tended to be shorter than that of browns.

At 3 weeks of age, chicks were showing flock and strain differences, and housing effects were inconsistent. The flock effects suggest that maternal effects (e.g., maternal age) or day-of-hatch experience affect chicks at this age as much, if not more, than their subsequent early life environment. This study improves understanding of development of spatial skills in laying hens, specifically how and when the environment contributes. It

further adds to the steadily growing body of evidence showing behavioral differences between white and brown feathered laying hens.

## **29 Development of Ultrasound Technology as a Non-Invasive Tool to Assess Reproductive Condition in Red Abalone (*Haliotis rufescens*).**

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Abalone (*Haliotis* spp.) are edible marine gastropods with a reproductive status that can be difficult to determine using traditional visual assessments and often rely on lethal histological analyses for conclusive results. Because species of abalone are endangered, lethal examinations are prohibited. Since abalone lack necessary clotting factors and are haemophiliacs, there exists considerable risk of mortal injury during the handling process. Ultrasound technology presents a unique tool to non-invasively monitor gonadal maturation, thereby increasing animal welfare by reducing handling stress for abalone conservation and food production aquaculture facilities. This study explored the usefulness of ultrasonography as an equivalent means of establishing the reproductive state of abalone compared to lethal histological evaluations. Here, cultured red abalone (*H. rufescens*) were used to test these methods as a surrogate for endangered black abalone (*H. cracherodii*). To examine these methods, sexually mature (n=12) red abalone were randomly selected from a cohort (n=200) produced at an abalone farm for comparative histological and ultrasonography assessments. Non-invasive ultrasonography successfully differentiated between reproductive and digestive tissues, and allowed for the characterization of abalone reproductive tissue on a categorical scale of one to five, with an ultrasound gonad index score of one being the lowest (gonad tissue absent or very thin) and an index score of five being the greatest (gonad tissue has increased thickness, compressing the digestive gland) for red abalone. To assess seasonal changes in gonadal development, a separate cohort of red abalone (n=62) produced at the UC Davis Bodega Marine Laboratory were monitored for seven weeks and then induced to spawn. Ultrasonography was able to detect changes in gonadal maturation over the spawning cycle without removing animals from their tanks, reducing associated handling stress. In addition, ultrasound imaging also successfully captured changes in gonad thickness before and after spawning.

## **34 Comparing different types of reward for assessing behavioural flexibility of weaned dairy heifers in a T-maze.**

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Many animal behavioural tests require animals to seek a reward. This pilot study aimed to determine the most effective reward for post-weaned heifers during behavioural testing in a T-maze, used to assess cognitive flexibility. The test consisted of a T-shaped maze with an entrance aisle and two 'arms', using cattle gates for structure and corrugated plastic to prevent visual contact to the outside of the maze and to the arms of the maze from the entrance aisle. Holstein dairy heifers ( $n = 15$ ) were randomly assigned to one of three reward types; food (FR;  $n = 5$ ), social contact (SR;  $n = 5$ ), and escape (ER;  $n = 5$ ). The food reward was a grain-based concentrate mixed with chopped hay placed in a feed bucket in the rewarded arm. The social contact reward was a non-study heifer from the home pasture enclosed in a small pen in the rewarded arm, with only an open bar gate in between the study heifer and reward heifer to allow for tactile, visual, and auditory contact. The escape reward consisted of the exit door in one arm of the maze being left open so that heifers were able to 'escape' the maze into the holding pen and have visual contact with their home pen. Heifers were tested at 9 weeks of age, one-week post-weaning and following movement to a group pen on pasture. Heifers each received a maximum of 6 testing sessions per day for a maximum of 5 days or until successfully reaching a learning criterion of direct movement to the rewarded arm of the maze for three consecutive sessions. In each session, heifers entered the base of the T, were observed for 3 min, and then exited through the rewarded arm of the maze. Once heifers had met the learning criterion during the initial stage, the rewarded side was reversed, and the number of sessions taken to meet the same learning criterion was recorded. The reward type did not affect the pass rate for initial learning ( $P = 0.24$ ; pass rates FR = 80 %, SR = 80 %, ER = 100 %), or for reversal learning ( $P = 0.16$ ; pass rates FR = 40 %, SR = 60 %, ER = 60 %). In addition, the reward type did not affect the number of sessions required to pass initial learning ( $P = 0.24$ ; FR =  $13 \pm 2$ , SR =  $9 \pm 2$ , ER =  $9 \pm 2$  sessions; mean  $\pm$  SE) or reversal learning ( $P = 0.68$ ; FR =  $16 \pm 4$ , SR =  $15 \pm 4$ , ER =  $12 \pm 3$  sessions; mean  $\pm$  SE). Overall, the high pass rates for all types of reward during the initial learning stage suggest that all three reward types may be considered effectively rewarding for future behavioural testing in weaned heifers. Further, while pass rate was lower during the reversal learning stage as consistent with previous findings, this preliminary data set suggested no significant effect of reward on outcomes in this test of behavioural flexibility.

### **38 Can housing system and detection method affect estrus detection in Holstein dairy heifers?**

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Precision dairy technologies rely on changes in activity levels to detect estrus in dairy cows. Yet, it is known that the animal housing system can affect the overall activity of dairy animals. Thus, the objective of this study was to evaluate the effects of three dairy

cattle housing systems on estrus detection in dairy heifers. Further, we aimed to evaluate the performance of three estrus detection methods in dairy heifers. We evaluated the performance of three estrus detecting methods (visual detection, estrus detection patch, and behavior monitoring tag) in dairy heifers across three housing systems (bedded-pack, free-stall, and pasture). Thirty-six Holstein heifers ( $14.2 \pm 2.2$  months old;  $297.7 \pm 64.6$  kg) were enrolled in this study from March to June 2019. Heifers were fitted with a behavior monitoring tag (CowManager SensOor, Agis Automatisering, Harmelen, the Netherlands) and randomly assigned to a housing system in a 3x3 Latin Square. Each period lasted 29 days before cows were moved to a different housing system. Heifers had 2 weeks for habituation, then their estrus was synchronized using a 7-day protocol. The protocol started (D0) with a CIDR insertion plus GnRH (100 mcg). Seven days later (D+7), the CIDR was removed, PGF2 $\alpha$  (25mg) was administered, and heifers received an estrus detection patch (Estroject, Rockway, Inc.; Spring Valley, WS). Visual estrus observations were conducted twice daily for 60 minutes 12h apart. Uterine ultrasonography was performed on days -7, +2, and +7. True estrus was defined if ovulation was confirmed via uterine ultrasound. Estrus detection standards were established for each of the methods tested in the study. For visual observation, estrus detection was considered a true event if the researchers observed standing heat. For the estrus detection patch, estrus detection was true if more than 50% of the patch was activated. For the behavior monitoring tag, estrus detection was considered true if the system's software generated an automatic estrus detection alert. We then calculated sensitivity (proportion of heifers in true estrus detected), specificity (proportion of heifers not in true estrus undetected), and accuracy (proportion of heifers that were correctly identified as in estrus or not) for each of the estrus detection methods. A logistic model (PROC LOGISTIC; SAS 9.4, SAS Institute Inc. Cary, NC) was used to determine the effects of housing system and estrus detection method on true estrus detection. A receiver operating characteristic curve was used to evaluate the estrus detecting capabilities of each estrus detection method by calculating an area under the curve (AUC). Overall, the ovulation rate was 82.4%. True estrus detection was not affected by housing method ( $P = 0.41$ ). However, true estrus detection was affected by the estrus detection method used ( $P < 0.01$ ). Sensitivity, specificity, accuracy, and AUC were: 52.4%, 100%, 61.0%, and 0.53 for visual; 87.7%, 91.7%, 87.0, and 0.55 for the patch; 90.8%, 91.7%, 90.7%, and 0.60 for the behavior monitoring tag. In summary, estrus detection in dairy heifers was affected by method, but no effects of housing were observed in any of the analysis.

#### **40 Effects of a probiotic capsule containing *Megasphaera elsdenii* on the feeding behavior of crossbred Angus X Holstein calves.**

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*Megasphaera elsdenii* probiotics have been shown to aid animals in risk of subacute ruminal acidosis. Thus, the objective of this study was to evaluate the feeding behavior of calves fed a probiotic capsule (*Megasphaera elsdenii* NCIMB 41125; Lactipro FLX Calf, MS Biotec, Wamego, KS). The study was conducted at the University of Kentucky Large Animal unit between August 2020 and April 2021. Thirty-one Holstein x Angus

calves ( $45.3 \pm 7.1$  kg;  $8.2 \pm 2.0$  days old) were enrolled in a 76-day randomized trial in two blocks. Calves were assigned one of three treatments: placebo (ME0), probiotic administration on day 15 (ME15), or on days 15 and 39 (ME15+39). Calves were individually housed and had unlimited access to water and calf starter. Calves were fed 7L of milk replacer daily divided in two meals until day 41 (pre-weaning period). On day 42, milk was reduced in half (3.5L/day; weaning period) and then calves were weaned on day 56 (post-weaning period). Calf starter orts were weighed daily and calf starter intake was calculated by disappearance. A camera (Moultrie M-40i, Birmingham, AL) recorded the calf starter and water buckets in 1-minute intervals on d 13, 32, 53, and 67. Images were examined by a single observer and classified using an ethogram. Behaviors exhibited in the images were classified as: eating, drinking, non-nutritive oral behaviors, or others. Eating and drinking behaviors were defined as the calf having its muzzle inside the calf starter or water bucket. Non-nutritive oral behaviors were classified as the calf licking the pen walls or buckets. Other behaviors were standing idle, laying down, or nonvisible. The effects of the probiotic capsule were determined using mixed linear models. The model included treatment, enrollment weight, enrollment age, immune status, if the calf was ever treated with antibiotics, study period, and the interaction between treatment and period. Study period was specified as a repeated measure and calf as subject, using a compound-symmetry structure. Block was considered a random factor. Calves receiving the probiotic had greater daily calf starter dry matter intake (ME0 =  $1.17 \pm 0.13$ , ME15 =  $1.62 \pm 0.13$ , ME15+39 =  $1.68 \pm 0.11$  kg/d;  $P = 0.01$ ). There was a significant treatment by period interaction for calf starter intake where calves receiving the probiotic had greater intakes during the weaning ( $P = 0.01$ ) and post-weaning ( $P < 0.01$ ) periods. No differences between the ME15 and ME15+39 treatments were observed ( $P = 0.72$ ). Calves receiving the probiotic spent more time drinking water (ME0 =  $2.46 \pm 1.20$ , ME15 =  $6.32 \pm 1.19$ , ME15+39 =  $6.73 \pm 1.08$  min/d;  $P = 0.03$ ). However, time spent eating (ME0 =  $38.09 \pm 4.75$ , ME15 =  $37.51 \pm 4.68$ , ME15+39 =  $40.86 \pm 4.45$  min/d;  $P = 0.75$ ) or performing oral behaviors (ME0 =  $32.96 \pm 4.53$ , ME15 =  $31.74 \pm 4.75$ , ME15+39 =  $21.31 \pm 3.82$  min/d;  $P = 0.14$ ) did not differ between treatments. No treatment by period interactions were seen for eating ( $P > 0.45$ ). However, we observed a significant treatment by period interaction for drinking ( $P = 0.03$ ). Overall, the probiotic increased calf starter intake and the time the animals spent drinking water but had no effect on the time spent eating or performing oral behaviors.

#### **45 Commingling Beef Calves: Is There an Impact from Familiarity With Penmates on Performance and Activity in Beef Calves Transitioning to a Feedyard System?**

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Beef calves entering feedyards experience many stressors including weaning, transportation, social mixing, and castration. We are interested in whether familiar (F) calves might confer stress resilience through social buffering relative to calves unfamiliar (U) with their pen-mates. The F calves were defined as those from the same source farm and U calves were sourced from different farms. We hypothesized that F calves would experience less stress than U calves during mixing (D0), castration

(D+14), and wound healing (D+28) phases, resulting in enhanced performance. Calves (n=102) from 23 source farms were randomly assigned to F or U treatments and placed into 17 pens, each comprised of 6 calves (3F, 3U). Calves were fitted with IceQube (IceRobotics Ltd) accelerometers. Calves were processed weekly for 4 consecutive weeks; outcomes included average daily gain (ADG), chute exit speed, activity, and castration wound healing score. Blood samples were analyzed for plasma cortisol and a CBC panel was performed on D28. Mortality, morbidity, and feed intake data were collected from farm records; home pen video was digitally recorded for behavioral analysis of stress and social responses. Preliminary analysis showed no effect of treatment on overall calf activity that included standing duration, number of steps or number of lying bouts ( $p>0.12$ ). F calves spent more time lying than U calves ( $p=0.03$ ). Trial day affected all outcomes ( $p<0.01$ ). F and U calves did not differ in weight ( $p=0.67$ ), neutrophil count ( $p>0.47$ ), or lymphocyte count ( $p>0.47$ ). However, weekly feed intake was lower in F calves ( $p=0.02$ ), and F calves showed a higher post-castration ADG than U calves at D21 ( $p<0.02$ ). In conclusion, we found some evidence to support our hypothesis of enhanced performance amongst F calves, perhaps due to stress resilience from social buffering.

#### **47 Social distancing: The association between owners' close, daily interactions with pet rabbits & thoughts about giving them up.**

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Rabbits are common companion animals with millions of pet rabbit estimated worldwide. Despite their popularity with some owners, many rabbits are relinquished and in need of rehoming in North America and beyond. Limited research assesses pet rabbits' social and environmental requirements, and factors important for strengthening the human-rabbit bond, and owner care and commitment. Our aim was to explore the association between self-reported, daily time spent interacting in close contact with pet rabbits (e.g., playing, socializing, sitting with, petting) and owners having thought about giving them up. Existing data (N=2893) from an online, snowball sampled survey were analyzed. Descriptive statistics were used to characterize owner and rabbit demographics (owner gender, age, country, income, housing type, education, number of rabbits-most recently acquired if >1; and rabbit age, sex, neuter status, litter training status) and close contact time (measured categorically). Logistic regression (univariable and multivariable with backwards stepwise elimination) was used to explore associations between independent variables (demographics and close contact time) and the yes versus no outcome of thoughts of giving the rabbit up. Participants were mostly female (91%), median age 32 years (range 18-79), lived in the USA (63%) or Canada (21%) vs. elsewhere, had \$20-39K annual household income (mode category), lived in detached houses (52%), graduated college/university or had some graduate/professional school (63%), and had 1 rabbit (44%). Of 2860 owners reporting daily close contact time, 358 (13%) reported <30 min, 954 (33%) reported 30 min-1 hr, 1065 (37%) reported 1-3 hr, and 473 (17%) reported >3 hr. Rabbits were median age 2.8 years, male (53%),

neutered/spayed (68%), and partially (urine or feces only, 9%) or fully litter trained (85%). Of owners, 325 (11%) reported thinking about no longer owning their rabbit. The odds of thinking about no longer owning a pet rabbit were all significantly greater – and different from each other – if owners reported spending <30 min, 30 min-1hr, or 1-3 hr per day in close contact compared to those that reported spending >3 hr per day in close contact (all  $P < 0.05$ ). None of the owner and rabbit demographic variables were retained in the final model (all  $P > 0.05$ ). Participants that spend more time closely interacting with their rabbits than those who do not may be less likely to think about giving them up for a number of reasons (e.g., they notice more interesting behaviours). Owners thinking about not keeping their rabbits may not have been interested in spending time with them due to undesirable rabbit behaviours (e.g., aggression) or a lack of desirable rabbit behaviours (e.g., rabbit willingly sitting in lap, friendly attentiveness to owner). Since causation cannot be inferred from cross-sectional data and since thinking about versus actually no longer owning rabbits (e.g., relinquishing them, setting them loose outside) are different, further research is needed to explore the reasons why owners enjoy and commit to pet rabbits.

#### **49 Assessing the Influence of Food Treats on Dog Stress Responses During Low Stress Handling: A Preliminary Study.**

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Veterinary care can be a stressful experience for many dogs. The use of food treats for distraction and counterconditioning during potentially-stressful interactions is a frequently-recommended low-stress handling technique. We compared canine stress levels when food treats were used in this way during routine handling of dogs by unfamiliar handlers vs. when treats were not used. Stress behaviors exhibited by 38 dogs during routine handling in a veterinary exam room were recorded, using digital video; behavioral data were coded using event-logging software (BORIS). A canine 'stress index' (SI; rate of stress behaviors observed/min) was calculated for each handling session. Sessions were then coded as: 0=no treats offered or 1=treats offered (whether or not accepted by the dog). Comparisons of the SI of dogs between the categories were made, using a two-sample t-test. Although SI values were consistently higher in sessions where treats were not offered (mean: 39.2, range: 0-115.9; n=39) than when treats were offered (mean: 29.8, range: 0-67.2; n=22), the results were not statistically significant ( $t=1.229$ ,  $df=59$ ,  $p=0.22$ ). This lack of significance may reflect a real lack of effect of food treats on stress levels, or may be due to the convenience nature of our sample: these data were collected as part of a larger study assessing dog responses to handling by unfamiliar humans, and handlers were free to decide for themselves whether or not to give treats. Frequently, treats were offered by handlers when dogs appeared to be somewhat stressed, and conversely not deemed necessary

with relaxed dogs, which likely complicated our analyses. Additionally, handlers who perceive the dog as stressed may transmit their nervousness to the dogs via body language or chemosignals. Despite this, it is interesting that the dogs' SI levels were still generally lower in sessions where treats were offered. Given the many variables at play, understanding the veterinary experience from the dog's perspective is challenging, and we suggest further systematic research on the use of treats to reduce stress and increase welfare of dogs during handling is warranted.

## **51 Changes in the standing behavior of Jersey and Holstein dairy cows during the period around calving.**

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Despite Jersey cows being the second most common dairy cow breed in the United States, very little work has focused on describing their behavior, particularly during the period before and after calving. Previous work with Holstein cows has shown that changes in standing behavior around calving may serve as an early indicator of disease. The first step towards determining if this could be true in Jersey cows is to begin by better understanding how Jersey behavior changes around calving in a healthy population of animals. The objective of this research was to compare changes in standing behavior before and after calving between healthy Jersey and Holstein dairy cows. A total of 31 dairy cows ( $n = 15$  Jersey and  $n = 16$  Holstein) were monitored from 15 d before to 15 d after calving. Daily standing time, number of standing bouts and average standing bout duration were measured using the HOB0 Pendant G Data Logger (Onset Computer Corporation, Bourne, MA) which recorded leg orientation every minute. These behaviors were summarized into 5 periods for data analysis: wk -2 (d -15 to -9, relative to calving), wk -1 (d -8 to d-2), calving period (d-1 to d+1), wk +1 (d+2 to d +8) and wk +2 (d +9 to d+15). Behavior was analyzed using a mixed model which included the effects of breed, parity, and period, including the 2-way and 3-way interactions of these fixed effects. In the model, cow was considered a random effect, and period a repeated measure. Average daily standing time differed by parity ( $P = 0.02$ ), breed ( $P = 0.02$ ) and period ( $P < 0.001$ ) and there was also a breed x period interaction ( $P = 0.04$ ). Cows that were beginning their 3<sup>rd</sup> lactation or higher spent less time standing than 1<sup>st</sup> or 2<sup>nd</sup> lactation cows ( $767.3 \pm 16.4$  min/d vs  $833.8 \pm 20.8$  min/d, respectively). Standing time was higher after calving compared to before calving ( $714.6 \pm 19.1$  min/d vs  $850.8 \pm 19.1$  min/d, respectively). The significant breed x period interaction revealed that differences in standing time between breeds only occurred during the calving period; Jersey cows stood on average 2.25 h/d more than Holstein cows during this period (d-1 to d +1;  $939.9 \pm 29.4$  min/d vs  $804.0 \pm 25.6$  min/d, respectively). There was an effect of period on the average number of standing bouts ( $P < 0.001$ ); standing bouts were highest during the calving period ( $10.7 \pm 0.5$  bouts/d) compared to the two periods before and after calving (average of the two periods being  $9.3 \pm 0.5$  bouts/d and  $8.9 \pm 0.5$  bouts/d, respectively). Breed had no effect on the number of standing bouts ( $P > 0.77$ ), and 1<sup>st</sup> or 2<sup>nd</sup> lactation cows tended to have more standing bouts compared to the older cows ( $9.6 \pm 0.4$  bouts/d vs  $8.8 \pm 0.3$  bouts/d,  $P = 0.10$ ). Jersey cows tended to have longer average standing bout durations compared to

Holstein cows ( $109.7 \pm 8.2$  min/bout vs  $91.4 \pm 7.5$  min/bout, respectively,  $P = 0.11$ ). There were no effects of parity ( $P = 0.99$ ) or period ( $P = 0.24$ ) on standing bout duration. Changes in standing behavior during the period around calving differ between Jersey and Holstein cows; this may suggest that the relationship between standing behavior and disease in Jersey cows may also be different.

### **53 Preference for natural versus synthetic odors as olfactory enrichment for captive jaguars (*Panthera onca*).**

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To increase the performance of species-appropriate behaviors, and decrease the potential performance of stereotypic behaviors, captive wildlife are given appropriate environmental enrichment as sensory stimulation. Because felids rely on olfaction to locate food, mates, and as a communication tool (e.g., pheromones), captive felids are often given enrichment which has a strong odor component. Unfortunately, there is little information on the specific chemical compound that is best to use as olfactory enrichment for each zoo-housed large cat. In this study, nine adult jaguars (6 F, 3 M) were assessed for preference to interact with a wooden object that is either sprayed with a “natural” odor (elk urine) or a synthetic order (perfume). The scented objects were placed in the animal enclosures one at a time, on alternating weeks, for 4 weeks. Each animal was observed for six 60 min observations each week (12 hrs/jaguar total), with animal order randomized per day. Live focal follow recordings for all-occurrence of behavioral events (spray mark, dirt roll, scratch, and, eight types of interact with object) and 5-minute scan samples of behavioral states (walk, rest, drink/eat, pace) were conducted at each hour session. Non-parametric Mann-Whitney U Tests were conducted to assess the impact of odor type on the performance of specific behavior events and the proportion of observation time animals were in specific behavioral states. Overall, the jaguars interacted with the enrichment object significantly ( $z = -2.6$ ,  $p < 0.01$ ) more often when it was sprayed with a synthetic perfume odor ( $13.1 \pm 6.3$  interactions/hr) as compared to the elk urine spray ( $4.8 \pm 3.5$  interactions/hr). No other behavioral differences were found between the treatments. Identifying the odor preference for captive wild felids can help caretakers provide an appropriately stimulating environment. While our study population is limited to nine individuals living at the same zoological institution, we suggest that animal care staff for captive jaguars use synthetic, non-toxic perfume or cologne odors when attempting to increase the olfactory stimulation in the enclosure.

### **55 Behavioural Characterization of Dairy Cows using 3-Axis Accelerometer Ear Tags to Detect Estrus.**

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Estrus behaviours are used to identify the most accurate time to artificially inseminate (AI) dairy cows. Newer technologies such as accelerometers can provide behavioural parameters that can be used to detect estrus with minimal labour input. The objectives of this study were to characterize behavioural parameters that can detect estrus using accelerometer (CowManager Sensor™ Agis Automatisering) ear tags compared to progesterone (P<sub>4</sub>) concentrations in milk (via Herd Navigator System™ DeLaval International: HN) and to identify factors influencing behaviour responses during estrus in a commercial dairy herd. Forty-six lactating Holstein cows were monitored using accelerometer ear tags and milk P<sub>4</sub> concentrations during early lactation (20 to 90 d after the calving) to characterize behaviour durations (%/60 min in 24 h periods) during the most critical time to AI dairy cows (i.e. cost-efficient time period). Non-active (NA: Laying or standing inactive or resting), Rumination (Rum: Laying or standing ruminating), Eating (Eat: muzzle on feed), Active (Act: head movements while standing), and High-active (HA: walking with head movements) behaviours were measured. Independent variables in the model (Proc Glimmix SAS 9.4) were infradian rhythm (estrous cycle), circadian rhythm (sample time: in 24 h periods), age groups (1-2 lactation [Young] and 3-6 lactation [Mature]), ambient temperature (°C), and estrus alert quality (an index based on AI success according to the interval between previous luteal phase and the drop in P<sub>4</sub> [%]) were tested to identify factors affecting behavioural responses during the estrus period. Estrus alerts were flagged (d 0) by HN if P<sub>4</sub> concentrations fell below 5 ng/mL for 2 consecutive days after being above 5 ng/mL. Changes in behaviour responses ( $P < 0.05$ ) were observed (LS Means  $\pm$  SEM) in Act ( $5.01 \pm 0.14$  %/60 min) and HA ( $8.70 \pm 0.25$  %/60 min) at (d 1) compared to the luteal phase (Act:  $4.46 \pm 0.13$  %/60 min and HA:  $6.40 \pm 0.22$  %/60 min). However, as expected, behaviour parameters differed ( $P < 0.01$ ) within a circadian cycle (increase of Eat, Act, and HA at early morning & late evening compared to increases of Rum and NA at mid-day). In addition, Younger cows exhibited greater ( $P < 0.01$ ) activity (Rum:  $23.45 \pm 0.14$  %/60 min, Eat:  $10.77 \pm 0.11$  %/60 min and HA:  $7.39 \pm 0.06$  %/60 min) compared to Mature cows (Rum:  $23.00 \pm 0.13$  %/60 min, Eat:  $10.30 \pm 0.11$  %/60 min and HA:  $5.50 \pm 0.05$  %/60 min) except for Act (Young:  $4.12 \pm 0.03$  %/60 min; Mature:  $4.77 \pm 0.03$  %/60 min;  $P < 0.01$ ). Ambient temperature range during the study was 7.6 to 29.4° C, but no effect of temperature was observed on behaviour responses during estrus ( $R^2 < 0.03$ ) and non-estrus periods ( $R^2 < 0.01$ ). Estrus alert quality differed among behaviour responses ( $P < 0.05$ ); Act and HA were higher in estrus quality of 74% (Act:  $9.46 \pm 0.38$  %/60 min; HA:  $31 \pm 0.09$  %/60 min) compared to the highest estrus quality 83% (Act:  $6.83 \pm 0.07$  %/60 min; HA:  $28.65 \pm 0.02$  %/60 min) and the lowest estrus quality 67% (Act:  $4.63 \pm 0.10$  %/60 min; HA:  $4.56 \pm 0.14$  %/60 min). Increases in walking (HA) and head movement (Act) activity as measured using CowManager system had the highest correspondence 1 d after the drop of P<sub>4</sub> of Herd Navigator system and the factors that affected behaviour response were time of day and parity.

# Group B

## 2 Modulating captive mammalian social behavior: a scoping review on olfactory treatments.

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Many species communicate via olfaction; therefore, odor signals could improve captive animal welfare by reducing aggression and promoting socio-positive behavior. However, to fully gauge the potential benefits of odor manipulations, existing literature must be evaluated and weighed by quality. Accordingly, a systematic search and scoping review was conducted to summarize prevalent methods, treatment outcomes, and modulating factors in existing literature on how intraspecies odors impact social behavior. Articles from a systematic search of three databases were included 1) if they were published in a peer reviewed journal, 2) used a terrestrial mammalian species, and 3) contained original data evaluating how odor signals from the subject species directly affected non-reproductive social behavior. Two researchers screened all articles, one extracted data, and both assessed reporting quality using the SYRCLE risk of bias tool. This tool measures whether studies report sources of bias such as randomized treatments and housing, researcher blinding, and proper analyses. Sixty-three articles were included based on this criteria. Eighty percent of subjects were rodents, while 71% of subjects were sexually mature males. Urine based treatments were used 60% of the time and primarily aggression was measured (60%). In contrast, socio-positive behaviors were measured only 2% of the time. Overall, urine treatments had a variable effect on aggression. Concerningly, most articles showed unclear or high risk of bias: most did not report enough information to confirm randomization, blinding, or proper analyses. Data from this review highlights a need for additional research on how odor signals from sources other than urine affect behavior and how socio-positive behaviors are influenced. Further, it emphasizes the need for more transparent reporting: the current body of literature hinders each experiment's quality assessment based on omitted methodology.

## 4 Effects of complex feeding enrichment on the behavior of captive Malayan sun bears.

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In captivity, a common indicator of poor welfare is the presence of stereotypic behaviors. Even though the underlying motivations behind the development of these behaviors may be difficult to identify, a growing field of research is investigating how

enrichment objects in an animal's environment can be manipulated in order to reduce the performance of stereotypic behaviors. In this study, we test whether providing complex feeding enrichment decreases the duration of stereotypic behavior and increases enrichment use for three adult female sun bears (*Helarctos malayanus*) at Oakland Zoo in California. Taking the species-typical behaviors of sun bears into consideration, we designed enrichment devices that would encourage the bears to use their long claws and tongues. We compared the effects of two different devices: honey-log and PVC cross-shaped feeder. Both devices were presented to the bears at three levels of complexity, with each level containing a different food item to maintain novelty of the devices. The honey-log had holes drilled around the circumference, and in level 1 it was freestanding and holes were open. In level 2, wooden dowels were inserted into the holes, so bears had to remove dowels to access food. In level 3, the log was attached to a structure in the exhibit (i.e., no longer free-standing). The cross-shaped feeder was constructed by the interlacing of PVC pipes. The bears accessed food through holes drilled into the middle of food-filled pipes that were revealed when pipes were moved along opposing pipes. In level 1, the device had a total of four pipes and with each successive level, one additional pipe was added. For data collection, we used video cameras to record pacing and head-rolling stereotypies using all-occurrence sampling, and we conducted in-person observations to measure interaction with the devices using one-zero sampling. After three weeks of baseline data collection, we introduced the complex enrichment devices three times a week per level over six weeks. Statistical analyses were conducted using linear mixed models, in which bear identity was a random effect with a REML method to yield estimates of the variance components. Significant differences were assessed using Least Square Means which controls for the variation between individual bears. When compared to baseline, the complex enrichment devices decreased the duration of stereotypies ( $F_{2,4642} = 23.68$ ,  $p < 0.001$ ), with no significant difference between the two devices. Across the six weeks, the duration of stereotypies was significantly less on the complex enrichment days compared to the days when complex enrichment was absent ( $F_{7,4740} = 11.14$ ,  $p < 0.001$ ). Increasing the complexity of the devices had a significant effect on the duration of stereotypies ( $F_{6,4637} = 11.36$ ,  $p < 0.001$ ). As the complexity of the honey-log increased, the duration of stereotypies decreased. As the complexity of the cross-shaped feeder increased, the duration of stereotypies increased. Increasing enrichment complexity also had an effect on enrichment use ( $F_{5,43} = 2.62$ ,  $p = 0.038$ ). As the complexity of the honey-log increased, enrichment use decreased. As the complexity of the cross-shaped feeder increased, enrichment use increased. Even though increasing enrichment complexity produced contrasting trends in the duration of stereotypy and enrichment use, our results indicate that introducing complex enrichment into zoo husbandry routines may help decrease stereotypic behaviors by creating more opportunities for animals to exhibit species-typical behaviors. This study offers promise in using the approach of increasing enrichment complexity for targeting stereotypies and enhancing animal welfare practices.

## **7 Variability of abnormal repetitive behaviors in dairy cattle.**

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Dairy cattle perform oral abnormal repetitive behaviors (ARBs), including tongue rolling (TR) and non-nutritive oral manipulation (NNOM). Anecdotally, breeds like Jerseys (J) are thought to perform these behaviors more than Holsteins (H). Little is understood about the variability of these behaviors within and across days, particularly when assessed on farm. It is also unclear if the performance of these ARBs relate to one another. We set out to evaluate ARB performance of individuals within groups, within and across days, and across breeds (H vs J). TR and NNOM were evaluated in growing heifers (n=97 H n=22 J) and lactating cattle (n=68 H, n=15 J). Cattle were observed in 7 distinct groups, grouped by age as per farm protocol (ranging from 15-58 individuals/group) for daylight hours (6:00 to 20:00) of 2 d. The chosen days for a given group were separated by no more than 48 h. Each group was observed using behavior sampling, where performance of TR and NNOM was recorded for each individual (present or absent) in 30-min intervals for 24 intervals/d. Individuals were identified by the number on their ear tag. Both TR and NNOM occurred in this population: on average, 15±3, and 25±7% of animals per group performed each behavior (mean±SE over 2 d), respectively. Using the group as the experimental unit, Pearson correlations were performed: TR and NNOM performance were positively correlated across days ( $r \geq 0.96$ ;  $p < 0.01$ ). Also, the total performance of TR was positively correlated ( $r \geq 0.88$ ;  $p < 0.01$ ) with total performance of NNOM. The circadian patterns across 14 h were highly variable (min to max over 2 d, TR: 0 to 63%; NNOM: 0 to 88% animals per group/30 min). Furthermore, using the individuals as the experimental unit and t-tests, J showed more ARBs compared to H (J: 27±2 and 29±3%, H: 9±1 and 19±1% intervals with TR and NNOM performance, respectively, mean±SE over 2 d,  $p < 0.01$ ). These results demonstrate that J are more likely to perform ARBs than H and that there is overall consistency of these behaviors across days and forms (TR; NNOM), despite high variability within days.

Keywords: abnormal behavior, dairy, breed, variability

## 11 Neonatal Piglet Interaction with Environmental Enrichment Ropes and Impacts on Mortality and Litter Weight Gain.

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In the United States swine industry, preweaning mortality represents the highest mortality rate of any production phase, with nearly half these deaths attributed to being crushed by the sow. If piglets could be enticed away from the sow when not suckling,

then their livability could increase. Therefore, the aim of this study was to determine if neonatal piglets would interact with environmental enrichment ropes placed away from the sow, and if this enrichment would impact litter weight gain or mortality. A total of 84 crossbred piglets from nine sows (parities 1 - 5) were housed in farrowing stalls. Litters were provided with environmental enrichment cotton ropes hung at piglet eye level above a heat mat on Day 2 after farrowing. Litters were assigned to one of three rope treatments: 1) sunflower oil (OIL), 2) milky cheese (MC), and 3) semiochemical (SC). Piglet rope investigations (defined as purposeful snout contact with a rope with the mouth open or closed) and number of piglets that died when enrichment ropes were present were collected. Litter average weight gain, calculated as the difference between litter average bodyweight on Day 1 after farrowing and at weaning, was collected. Results will be presented descriptively as percentages and means ( $\pm$  SD). Regardless of treatment, 75% (63/84) of piglets interacted with the ropes at least once. More piglets assigned the OIL treatment interacted with the ropes compared to piglets assigned the MC or SC treatments (OIL = 100% [28/28 piglets], MC = 75% [18/24 piglets], SC = 53% [17/32 piglets]). Prewaning litter average weight gain was greatest for MC piglets, and smallest for SC piglets (OIL =  $9.2 \pm 4.1$  kg, MC =  $11.4 \pm 0.9$  kg, SC =  $7.6 \pm 1.4$  kg). Piglet mortality during rope presence was not different between treatments (OIL = 7% [2/28 piglets], MC = 8% [2/24 piglets], SC = 9% [3/32 piglets]). Overall, this study determined that piglets interacted with enrichment ropes away from the sow. This is encouraging, because at this early life stage a piglet's main motivators are sow-focused (suckling, warmth, and protection). Further research on larger number of litters is needed to examine enrichment ability to improve suckling performance and livability.

## **15 The Impact of Providing Hiding Places to Farmed Animals: A Scoping Review.**

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Farmed animals are typically housed in barren environments; however, growing concerns about animal welfare have sparked research into ways of allowing animals to perform species-specific natural behaviours. In the natural environment, many animals will perform hiding behaviours, which are not usually possible in a farm setting. The aim of this scoping review is to describe the existing literature exploring the provision of hiding places to farmed animals, including 1) identifying the farmed species that are most used in research investigating the provision of hides, 2) describing the context in which hides are provided to farmed animals, and 3) describing the impact that hides have on animals, including animal behaviour and other indicators of animal welfare, health, or production. A comprehensive search was designed and implemented in three online databases (CAB Abstracts, Web of Science, and PubMed) with a target population of farmed animals and an intervention of hide provision. From this search, 4,631 citations were procured and screened. Of these, 147 articles were confirmed to meet the search criteria and were included in the review. A total of 14 animal types were

found to be represented in the literature. Most frequently represented animal types included chickens (50% of the papers), cattle (9% of the papers), foxes (8% of the papers), and fish and crustaceans (all species pooled, 7% of the papers). Hides were used in four general contexts: 1) at parturition or oviposition (54% of the papers), 2) for general enrichment (44% of the papers), 3) for neonatal animals (3% of the papers), or 4) for sick or injured animals (1% of the papers). A total of 212 outcomes relevant to our objectives were found in the 147 papers. To assess the impact of hide provision on animals, 7 categories of outcome variables were identified: 1) hide use, motivation, and/or preference (47%), 2) behavioural indicators of affective state (17%), 3) health, injuries, and/or production (16%), 4) agonistic behaviour (8%), 5) abnormal repetitive behaviour (7%), 6) physiological indicators of stress (5%), and 7) affiliative behaviour (1%). Outcomes were then labeled as having positive, negative, or neutral impact on the animals. Hiding places resulted in 157 positive outcomes (74%), 14 negative outcomes (7%), and 41 neutral outcomes (19%). In papers that reported negative or neutral outcomes in response to hide provision, some may have resulted from other housing factors included in the study, such as crowding and competition over the hiding spaces. Researchers have provided hides for a variety of species and contexts; more research is encouraged for under-represented species, neonatal animals (species-dependent) and sick or injured animals. Providing hiding spaces appears to be beneficial to farmed animals in many circumstances; however, it is also critical to address fundamental housing concerns that may be detrimental to animal welfare.

## **20 Impact of stationary brush quantity on brush use in group-housed dairy heifers.**

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Grooming is an important behavior for cattle and can be facilitated with brushes. To date, no studies have evaluated how many brushes should be provided in group housing. Our objectives were to evaluate the effect of the number of stationary brushes on brush use patterns and competition in weaned dairy heifers naïve to brushes. Sixty-three Holstein heifers (95±5.7 d old) were housed in groups of 8 (with the exception of 1 group of 7) with 2 or 4 deck scrub brushes (n=4 groups/treatment). From video, we continuously recorded brush oral manipulation, grooming, and displacements during 0-6, 18-24, 120-126 and 138-144 h of exposure to brushes. Bout characteristics were calculated for individual heifers, using bout criteria calculated from data pooled by exposure stage (more novel: 0-6 and 18-24 h of exposure; less novel: 120-126 and 138-144 h of exposure), based on descriptive similarities within those respective 24-h periods. Linear mixed models were used to evaluate the effect of brush quantity and hours of exposure on brush use (including bout characteristics) and competitive events with a random effect of heifer group to account for repeated measures; no interactions were found. Latency to use any brush after entering the pen was 4.0±8.4 min (mean±SD; individual range: 7 s to 58.4 min). All heifers used a brush at least once (individual range: 3 s to 37.5 min of total brush use per 6-h period). Brush quantity did not impact the duration of oral manipulation (4 vs. 2 brushes: 1.9±0.2 vs. 1.5±0.2 min/6

h, mean $\pm$ SE;  $P=0.20$ ), grooming (5.8 $\pm$ 0.7 vs. 4.0 $\pm$ 0.7 min/6 h;  $P=0.11$ ), or total brush use (7.7 $\pm$ 0.8 vs. 5.4 $\pm$ 0.8 min/6 h;  $P=0.10$ ). Heifers provided with more brushes performed longer bouts (4 vs. 2 brushes: 35.1 $\pm$ 1.9 vs. 27.5 $\pm$ 1.8 s/bout;  $P=0.03$ ), but no difference was observed for bout frequency (17.4 $\pm$ 1.8 vs. 14.6 $\pm$ 1.8 bouts/6 h;  $P=0.31$ ). Total brush use was greatest at both 0-6 and 138-144 h of exposure (7.1 $\pm$ 0.7 and 8.5 $\pm$ 0.8 min/6 h, respectively, mean $\pm$ SE;  $P\leq 0.01$ ) compared to 18-24 and 120-126 h of exposure (5.1 $\pm$ 0.7 and 5.5 $\pm$ 0.8 min/6 h, respectively). Bouts were more frequent at 0-6 h than 120-126 h (18.8 $\pm$ 1.6 vs. 12.2 $\pm$ 1.7 bouts;  $P=0.01$ ), but otherwise similar across 6-h periods (18-24 and 138-144 h of exposure: 17.1 $\pm$ 1.6 and 15.8 $\pm$ 1.7 bouts;  $P\geq 0.051$ ). Bout durations were shorter when brushes were more novel (0-6 and 18-24 vs. 120-126 and 138-144 h of exposure: 22.5 $\pm$ 2.5 and 19.9 $\pm$ 2.5 vs. 41.2 $\pm$ 2.7 and 41.6 $\pm$ 2.7 s/bout;  $P<0.001$ ), but were similar within 24-h periods ( $P\geq 0.88$ ). Competition was not impacted by brush quantity (4 vs. 2 brushes: 6.2 $\pm$ 1.1 vs. 5.7 $\pm$ 1.0 events/6 h;  $P=0.74$ ). More displacements occurred during the initial and final 6-h periods compared to 120-126 h of exposure (8.6 $\pm$ 1.0 and 7.1 $\pm$ 1.1 vs. 3.1 $\pm$ 1.1 events;  $P=0.04$ ); an intermediate number occurred at 18-24 h of exposure (4.1 $\pm$ 1.0;  $P\geq 0.35$ ). In conclusion, naïve heifers used brushes soon after exposure, and use varied over time. Although total brush use and competition were not affected by brush quantity, heifers with access to more brushes used them for longer bouts. Further research is needed on brush stocking density to inform management strategies.

Key words: grooming, oral behavior, competition, behavioral bouts

### **23 Do conventional laboratory cages impact rodent health? A systematic review and meta-analysis.**

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Over 120 million mice and rats are used in research each year in biomedical research. Conventional housing (CH) consists of small shoebox-sized cages that thwart highly motivated natural behaviours such as running, hiding, nesting and burrowing. As a consequence, these cages increase abnormal (e.g. stereotypic) behaviours and aggression between females, and potentially impair thermoregulation and abilities to regulate bodyfat. But are these cages also chronically stressful? In humans, chronic stress can increase disease morbidity and reduce lifespans: signs of impaired adaptive capacity termed “distress” by ILAR. To find out, we conducted a systematic review and meta-analysis to test the hypothesis that, compared to rats and mice in cages ‘enriched’ with resources to better met their needs, CH increases stress-related morbidity and all-

cause mortality. After a comprehensive literature search (which yielded 10,094 publications), 214 studies (within 165 articles, using 6,495 animals) were included in the review containing data on all-cause mortality plus five experimentally induced stress-sensitive diseases: anxiety, cancer, cardiovascular disease, depression and stroke. Of the included studies, 59.1% were performed on mice, 68.2% of the animals were male, and 31.8% of the animals were housed alone. 66.8% of studies did not adequately describe their CH, leaving us to infer it from local minimum standards; however, for those studies that did, conditions ranged from completely barren to ones containing a nest and shelter. For each study, we converted stress-sensitive disease data to standardized mean differences (SMD) to generate effect sizes (the magnitude of difference between CH and 'enriched' housing). We converted all-cause mortality data to hazard ratios and median survivals. Random-effects meta-analyses supported the hypothesis: CH significantly exacerbated disease severity with medium to large effect sizes: cancer (SMD = 0.71, 95% CI = 0.54-0.88); cardiovascular disease (SMD = 0.72, 95% CI = 0.35-1.09); stroke (SMD = 0.87, 95% CI = 0.59-1.15); signs of anxiety (SMD = 0.91, 95% CI = 0.56-1.25); signs of depression (SMD = 1.24, 95% CI = 0.98-1.49). It also increased mortality rates (hazard ratio = 1.48, 95% CI = 1.25-1.74; relative median survival = 0.91, 95% CI = 0.89-0.94). Meta-regressions indicated that such housing effects were ubiquitous across species and sexes, but could not identify the most impactful improvements to CH. Overall, CH thus has biological costs, causing distress. These results thus add to previous work showing that research rodents are typically CRAMPED (cold, rotund, abnormal, male-biased, poorly surviving, enclosed and distressed). This raises ethical questions regarding the ubiquitous use of CH, as well as questions regarding the generalisability of these rodents' data. Are CRAMPED rodents good models of a diverse array of humans (including fit and happy ones)? If not, would rectifying their housing improve not only their well-being, but also the validity of biomedical research? A new systematic review and meta-analysis from our laboratory seeks to address these questions, with hopes to find out if good welfare truly makes good science.

## **26 Effects of airspeed from fans located above freestalls on heat stress responses.**

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Lying time, a key indicator of cow comfort, decreases with heat stress. Previous research has shown effective strategies for reducing physiological responses to heat stress, such as spraying cows with water, but which fail to maintain lying times. Our objective was to evaluate how airspeed from variable-speed fans above the resting area affect heat stress responses. Lactating Holsteins (n = 8 groups of 16 cows each) were housed in deep sand-bedded freestall pens with 2 rows of 8 stalls each in a head-to-head configuration, with one fan aimed at each row and with no other on farm cooling. All groups were exposed to 3 treatments in a replicated crossover design (3 d acclimation, 4 d data collection): control (fans off,  $0.4 \pm 0.2$  m/s, mean  $\pm$  SD) vs. 60% ( $1.7 \pm 0.5$  m/s) and 100% ( $2.4 \pm 0.8$  m/s) fan power, 24 h/d. After data collection for each treatment, airspeeds were measured 0.5 m above the stall surface to represent

cow lying height in the first, fifth, and last stall in both rows and averaged for each group. Vaginal temperature (VT) and lying time (LT) were recorded at 1-min intervals with data loggers. Temperature Humidity Index (THI) in the pens was measured at 5-min intervals with a logger mounted at 2 m high on a central pole between the rows of stalls. Linear mixed-effects models were used to analyze fixed effects of treatment, maximum daily THI among the 4 d, and their interaction, with a random term for group of cows. We found main effects of treatment and treatment  $\times$  THI interactions for all dependent variables. Among all treatments, LT differed in a dose-dependent fashion (100% vs. 60% fan power vs. control: 14.3 vs. 14.0 vs. 13.3 h/d, respectively, SEM = 0.17 h/d;  $P < 0.036$ ). For every 10-unit THI increase, LT in the control decreased 0.4 h/d, whereas both fan treatments showed the opposite pattern (+0.3 and 0.7 h/d in the 60% and 100% fan power treatments, respectively;  $P < 0.046$ ). Maximum daily VT was lower in the 2 fan treatments (39.1°C in both the 60% and 100% fan power treatments, respectively, SEM = 0.06°C) relative to the control (39.5°C;  $P < 0.001$ ). For every 10-unit THI increase, VT increased 0.3°C in the control but was stable in both fan treatments (+0.0°C in both the 60% vs. 100% fan power treatments;  $P < 0.019$ ). Milk yield (MY) was higher in the 2 fan treatments (42.7 vs. 43.3 kg/d in the 60% vs. 100% fan power treatments, respectively, SEM = 0.4 kg/d) relative to the control (41.4 kg/d,  $P < 0.001$ ). For every 10-unit THI increase, MY in the control decreased 1.1 kg, whereas both fan treatments showed the opposite pattern (+1.1 vs. 1.9 kg in the 60% vs. 100% fan power treatments, respectively;  $P = 0.011$ ). In conclusion, high-speed air from fans over the freestalls directed at cow resting height was effective not only for maintaining vaginal temperature, but also for improving lying time and milk yield in heat stress conditions. Keywords: heat abatement, lying behavior

## 28 Withdrew

### 32 Effect of rearing environment and repeated testing on performance in depth and distance perception tasks for egg-laying hens.

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Multi-tiered aviary systems provide laying hens with opportunities for species-specific behaviors, including vertical and horizontal movement. Previous studies have suggested that floor rearing of pullets with minimal access to elevated structures may negatively impact adult laying hens' ability to navigate vertical space. Yet it is not clear whether this is due to deficits in physical ability, or, if differences in the complexity of vertical structures in the rearing environment influence the development of adequate spatial cognition. The effect of rearing environment and repeated testing on the performance in two novel distance and depth perception tasks was investigated in birds at 7-8, 15-16, and 29-30 weeks of age. Dekalb White pullets ( $n = 450$ ) were reared in three different environments from 0 until 16 weeks of age: floor (max. height of perch = 10 cm), single-tier aviary (max. height of perch = 30 cm), and two-tier aviary (max. height of perch = 120 cm). At 16 weeks, all birds were transitioned to a two-tier aviary

for the laying period. Distance perception was evaluated via a Y-maze task with a ratio of 1:3 or 1:1 in escape arm length and exit choice (short, long, no choice) was recorded. To evaluate depth perception, hens were placed on a perch in the center of the visual cliff table, facing the perceptual cliff. Each bird was tested with three trials with random assignment of cliff depth at 15, 30, and 90 cm below the perch. Birds were given the option of escaping by jumping to a platform suspended over the visual cliff. Ten birds per treatment (n=30) were tested at each time period in order to investigate the effect of repeated testing on performance. At 7 weeks old, a significant difference ( $\chi^2(4) = 11.81$ ,  $p=0.02$ ) in arm choice of the Y-maze was found; with 60% of floor and single-tier birds and only 20% of two-tier birds choosing the short arm. For all birds, there was a significant effect of age on latency to make an arm choice in the Y-maze ( $F(2, 52) = 4.7$ ,  $p=0.01$ ), with all birds displaying shorter latencies with each repeated test. For the visual cliff task, there was a significant main effect of age on the duration spent on-perch ( $F(2, 54) = 3.9$ ,  $p = 0.03$ ), with birds taking longer to move away from cliff at 16 weeks of age. In addition, birds which were quick to move away from the cliff at 16 weeks were likely to do so at 30 weeks (30 cm;  $r = 0.46$ ,  $p=0.01$ ). Taken together, these results indicate that the height and complexity of vertical structures in rearing environment for pullets does not necessarily impact a bird's ability to visually perceive depth and distance as they development, and instead, age and consistent individual differences in activity are more likely to impact a bird's performance in cognitive tasks which depend on locomotor responses.

### **37 Efficacy of a four-week education and training program on responses to petting in domestic cats.**

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Human-cat petting interactions are generally thought to provide a positive opportunity for human-cat bonding. However, not all cats enjoy petting and it can lead to negative outcomes such as aggression. Some approaches to petting (e.g., touching preferred areas, respecting individual limits), can improve cat responses to petting in the moment. We examined whether a 4-week training program incorporating these approaches improves petting tolerance in cats with a history of avoidance and threatening behaviour during petting. Cat owners were recruited through social media and for those who fit our recruitment criteria, cats were categorized as Accepting (always enjoys or accepts) or Resisting (sometimes shows avoidance or threats) petting. The pet and their owner were then randomly assigned to a Control or Training group. The Control group received no petting information, while the Training group was given general information for improving petting and a 12-step desensitization and counterconditioning petting program to follow. Participants provided videos of a petting session at the start and end of the study and completed a check-in survey about their cats' responses for each of the 4 weeks. A Wilcoxon signed rank test compared which 'petting step' owners made it to in Week 4 (W4) as compared to Week 1 (W1). A logistic regression model evaluated the

effect of W1 response and training treatment on cat responses to petting at W4 (positive: enjoyed or accepted petting; negative: resisted, warning signs, scratched or bit). Most of the Accepting group that completed the training program were able to complete all 12 'petting steps' in W1 ( $m_{1/2}$ =step 12) and W4 ( $m_{1/2}$ =step 12), with no difference detected between weeks ( $p>0.1$ ). However, the Resisting group showed improvement on the step they were able to get to in W4 ( $m_{1/2}$ =step 12) in comparison to W1 ( $m_{1/2}$ =step 9;  $p=0.005$ ), indicating that the training improved responses for this group. Step 12 involved 15 varied strokes whereas Step 9 involved 4 short strokes on the head and neck region. All owners ( $N=131$ ) had higher odds of reporting a positive petting response in W4 if cats had a positive petting response in W1 (OR:15.6, 95% CI:4.2-58.2,  $p<0.001$ ). We did not detect an effect of training, or an interaction between training and W1 response, suggesting that owners did not observe a change in response with training. Thus, training was effective at improving responses in the Resisting group, as measured by petting steps completed, but the overall improvement in response with training was not observed by owners. This discrepancy likely results from owners being instructed to end sessions when the cat showed resistance; even with 'petting step' improvement, this response might have been observed at the end of the session. Objective behavioural data from the petting videos at the beginning and end of the study will help to further evaluate changes in cat responses during the study. Overall, these findings will inform development of further strategies to reduce petting-related aggression and improve the human-cat bond.

### **39 Fear response and future performance: standardized personality testing is associated with performance in individually housed crossbred holstein x angus calves.**

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The aim of this study was to evaluate differences in behavioral responses in individually housed calves to standardized personality tests (novel person, novel object, and startle test) and investigate associations with performance. Crossbred Holstein x Angus individually housed calves ( $n=29$ ) had *ad libitum* access to water and calf starter. Body weight was measured weekly and calf starter intake was recorded daily from day of arrival at 8 d of age until 76 d of age. Behavior was recorded on days 13, 32, 53, and 67 using a camera and a pedometer (IceRobotics, Scotland). The feeding behaviors recorded were eating and drinking (muzzle in feed/water bucket), and oral manipulation (non-nutritive oral behaviors consisting of licking buckets or walls). The calves were subjected to 3 standardized personality tests in two blocks at approximately  $80.7 \pm 2.0$  d of age. Calves were subjected to the tests in the following order: novel person test, novel object test, and startle test. The novel person test introduced an unknown stationary person in the calves' home pen for 10 minutes. The novel object and startle test were combined into one test utilizing a remote-controlled car that was initially stationary and moved across the home pen when approached or at the lapse of 5 minutes with another 5-minute observation period after movement. During the

personality tests, 11 behaviors were recorded: grooming, licking, touching, interacting with the environment, inactive, attentive toward object or person, object/person play, locomotion play, latency to approach person or object, and latency to reapproach object. A principal component analysis yielded 3 factors that explained 76.1% of the variance and that were consistent responses across all calves during the tests. The factors were labeled as Factor 1 “fearful”, factor 2 “inactive”, and factor 3 “bold”. Spearman’s correlations were utilized to explore the relationship between individual calf performance, behavior, and the PCA factor scores. Total average daily gain had a negative association with factor 1 (“fearful”;  $F_{1,19} = 26.35$ ,  $P = < 0.001$ ) and average grain intake also had a negative association with factor 1 ( $F_{1,20} = 25.47$ ,  $P = < 0.001$ ). Average time spent licking, or non-nutritive oral manipulation of buckets or walls, had a positive correlation with factor 2 (“inactive”;  $F_{1,22} = 7.43$ ,  $P = 0.01$ ). Factor 3 (“bold”) had no significant association with any of the performance measures. These results indicate that calves that are more fearful, as measured during standardized tests, were associated with a lower average daily gain and calf starter intake. Additionally, calves that are inactive during the standardized test spent more time licking in the home pen. In conclusion, there is evidence to suggest that, as in group housed calves, personality scoring in individually housed crossbred calves can potentially be used to identify later individual performance.

#### **43 Welfare at the time of slaughter for farmed finfish in the United States.**

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*Jackson Gross, MSPH, PhD Assistant Professor of Cooperative Extension in Aquaculture, Department of Animal Science, UC Davis*

Current slaughter practices in the aquaculture industry have not been optimized for either animal welfare or product quality, due to insufficient research, education and investment. New research shows that there are many areas improvements can be made. This presentation will summarize commonly used fish slaughter techniques and the research surrounding their use and animal welfare. Globally, aquaculture is the fastest growing agriculture sector with over 50% of the seafood production originating through fish farming. This number is only expected to increase to meet the nutritional demands of a growing human population. It is estimated that 900- 2700 billion fish are slaughtered annually from capture fisheries and aquaculture. The AVMA Guidelines on Humane Slaughter recommend creating insensibility prior to slaughter to prevent suffering for any livestock or research animal. However, recommended techniques have not been shown to be effective at creating insensibility for all the species of farmed fish. In the US the ten most commonly farmed fish represent seven different taxonomic orders, with significant anatomic and physiologic diversity.

Commonly used slaughter techniques include blunt force trauma, ice slurry or a combination of the two for the slaughter of finfish. These techniques are sometimes followed by exsanguination, but without proper first step stunning fish can recover, creating welfare concerns. Most often a club, mallet or heavy pipe is used as a percussion stunner to cause brain death. These techniques can be difficult to master and often repeated hits are needed to sufficiently stun struggling fish. Some producers

have progressed to using non-penetrating captive bolt, however this technique is not standardized for placement and operating pressure for most species.

Electrofishing has been used for wild fish sampling for almost 50 years and is increasingly used in fish production and capture fisheries. Electrical stunning is gaining use as a first step method to create immobility and ideally insensibility yet stunning methods are regularly insufficient. When applied incorrectly this technique can cause tissue damage, broken spines and blood spotting and many fish will regain consciousness during the exsanguination phase. New studies are showing that some techniques that were previously thought to create insensate animals prior to slaughter may not work across all species. Utilizing modern techniques such as EEG monitoring we can measure brain activity throughout various slaughter techniques. However, because many fish species are so taxonomically and anatomically different, these studies will need to be conducted across many different genera.

#### **46 Olfactory and physical enrichment changes behavior and improves performance of growing pigs.**

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With over 60,000 swine farms in America, a new leading concern within the pork industry is the well-being of pigs on farms. Most farm pigs are housed with minimal environmental enrichment (EE) (toys or sensory stimulation) causing behavioral problems that may impact performance. Use of EE incurs a cost often with no economic benefit or return for farmers. Our objective was to determine the effects of two forms of physical enrichment with and without olfactory enrichment on pig performance and behavior. For olfactory enrichment we used the novel maternal pheromone (MP) recently discovered in our laboratory. Finishing pigs (N = 160) were randomly grouped in pens of eight and evaluated with one of five treatments: (1) control (CON) with only a metal chain (all pens had chains); (2) an operant sprayer control (SC); (3) a sprayer with MP (SMP); (4) a jack [large rubber jack-shaped toy] control (JC); and (5) jack with MP (JMP) applied daily. The sprayer was a device that allowed the pig to spray themselves when a lever was pushed. Behaviors summarized from video included aggression, feeding, lying, activity, and enrichment use during the first 24 h after grouping. Pigs were weighed at time zero and 30 d later. Pigs with JMP had a higher average daily gain (ADG, kg/d;  $p < 0.05$ ) than CON pigs. Aggression was lower in all enrichment treatments in comparison to CON; however, the largest reduction in aggression was in the SMP and JMP treatments (SMP:  $p = 0.019$  vs. JMP:  $p = 0.016$ ). Feeding behavior was not different ( $p = 0.718$ ) among treatments. Lying decreased ( $p < 0.05$ ) overall with EE (pigs were more active). MP increased activity and weight gain when used with the jacks or sprayers. The duration of lying was lowest in SMP and JMP ( $p = 0.015$ ), whereas SMP and JMP had the highest activity duration ( $p = 0.027$ ). Frequency of enrichment use nearly doubled compared to CON chain manipulation. Frequency of jack use was 5-fold higher than CON chain manipulation. Pigs given enrichment may be less aggressive due to an increased interaction with the enrichment device. MP

increased enrichment interactions and, when MP was added to physical enrichment, pig ADG was increased. Enrichment may reduce aggression, increase activity, and improve pig weight gain. Therefore, both physical and olfactory enrichments may improve pig welfare and performance to the point in which EE is economically attractive.

Key Words: Swine, Enrichment, Toys, Pheromone

#### 48 **Exploring the provision of resources and the cat-cat relationship in multi-cat households in the US and Canada.**

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There are an estimated 50 million cat households in Canada and the US, with approximately half of these owning 2 or more cats. Insufficient resources and agonistic interactions can have negative impacts on cat welfare in the home, lead to health and behavior problems, and may increase the risk of relinquishment. The following study aims to explore the relationship dynamic in 2-cat households by: (a) collecting information on owner-reported frequencies of cat-cat interactions in the home using videos as a reference, and (b) exploring the number and distribution of cat resources in the home. We surveyed primary owners of two adult, companion cats across Canada and the US. The five-part questionnaire developed included questions on owner demographics, individual cat information, resources provided, relationship between cats, and observed frequency of various types of cat-cat interactions. The selected videos included interactions ranging from extremely positive to extremely negative and were determined using an evidence-based ethogram. For each video, participants rated how often they observed their own cats displaying similar behaviors. We received a total of 6,529 (US: 6118, 94%; Canada: 411, 6%) complete responses. Descriptive statistics for Canadian and US participants were initially performed separately, but later combined due to their comparability. Participants were mostly female (72%), did not own a dog (76%), had no children (78%), and considered themselves very (45%) or somewhat (36%) knowledgeable about cat behavior. The majority of cats were fixed (99%), acquired at less than a year old (74%), from a shelter (60%), were domestic breeds (76%), and indoor only (67%). Most owners perceived their cats' initial physical meeting as somewhat negative (28%) or extremely positive (25%) and viewed their cats' overall relationship as extremely (40%) or somewhat (34%) positive. Most owners provided their cats with a single feeding area (59%), one litter box (57%), multiple scratching posts (2 posts: 26%; 3 posts: 22%) and multiple sleeping areas (83%) (example: cat beds, cat trees, owner's furniture). When owners reported how often they observed their own cats displaying similar behaviors as in the videos, nose-touching was observed often (44%), staring was observed sometimes (43%), sleeping with contact observed never (36%), resource guarding observed sometimes (35%) and allogrooming observed often (33%). In addition, owner's ratings of their cats' first encounter was associated with ratings of the cats' overall relationship ( $X^2(4, N = 5761) = 612.98, p < .001$ ). Overall, the results provide a better understanding of cat-cat interactions in 2-cat

households and suggest most cats are not provided their own separate feeding area and litter box. Further research should examine the influence of age and other parameters on cat-cat introductions, and how provision of resources in the home impacts cat-cat interactions and the impact on future health, behavior, and welfare.

## 50 Withdrew

## 52 Lying behavior and growth of pre-weaned dairy calves in alternative rearing systems.

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Recently, rearing systems where dairy calves are kept individually housed since very young age have raised public concerns in relation to the welfare of dairy calves, especially due to the limitation of social and maternal contact. Lying behavior is considered an important indicator of comfort and health in dairy cattle, but it might be affected by alternative rearing systems which offer calves the opportunity to socialize and engage in activities with other calves or cows. In this study, lying behavior and average daily gain (ADG) were compared for pre-weaned dairy calves housed individually, in pairs, in small groups, or with their mothers during the first 9 weeks of life. Lying behavior was recorded with ICETAG (IceRobotics, Scotland) sensors. One-hundred sixty-four Holstein (40.8 kg birth weight), ProCross (Montbéliarde, Viking Red, and Holstein, 39 kg birth weight) and GrazeCross (Normande, Jersey, and Viking Red, 36.2 kg birth weight) calves were evaluated during Fall 2020 and Spring 2021. At three days of age, calves were fitted with ICETAG sensors on the right hind leg and divided into four housing groups: individually housed ( $n=36$ ), pair housed ( $n=34$ ), group-housed (6 calves per pen;  $n=40$ ), and dam-reared (6 cow-calf pairs per paddock;  $n=49$ ). Body weights, lying time and number of lying bouts were recorded weekly. Statistical analysis using linear mixed models were performed with SAS 9.4. Analysis of lying time and lying bouts considered fixed effects as housing group, breed, and season. Random and repeated effects of calf and date were also considered in the model. Analyses of ADG included the fixed effects of season, breed, and housing group. Season ( $F_1=0.65$ ,  $P=0.42$ ) and breed ( $F_2=1.55$ ,  $P=0.21$ ) had no impact in lying behavior. Dam-reared calves ( $1.21 \pm 0.25$  kg/d;  $16.24 \pm 0.17$  lying hours/day) had higher ADG ( $P < 0.05$ ) and spent fewer hours lying ( $P < 0.0001$ ) compared with individually raised ( $0.98 \pm 0.13$  kg/d;  $17.91 \pm 0.19$  lying hours/day), pair-housed ( $0.96 \pm 0.14$  kg/d;  $17.37 \pm 0.19$  lying hours/day) and group-housed ( $1.04 \pm 0.17$  kg/d;  $17.24 \pm 0.18$  lying hours/day) calves. Individually housed calves spent more hours lying ( $P < 0.05$ ) than all other treatments. Group and pair-housed calves had similar daily lying hours ( $P=0.5711$ ). There was no difference in ADG among individual, pair and group-housed calves. There was no difference in number of lying bouts between all groups. The results suggest that social contact might affect lying behavior in dairy calves by increasing the opportunities to perform other activities. Individually housed calves spent more time resting, probably due to lack of social stimuli to perform other activities, whereas calves living with other calves in pairs or groups showed increased activity levels by spending less time lying.

Dam-reared calves had higher ADG than all other groups and spent more time performing other activities than resting, probably due to more space to roam and interaction with other cows and calves in the herd.

#### **54 Livestock Informatics Toolkit: User-friendly machine learning algorithms to visualize complex behavioral patterns in sensor data.**

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Sensor technologies enable ethologists to continuously monitor the behavior of many animals at the same time and over extended periods. This creates new opportunities to study livestock behavior in commercial settings, as well as new methodological challenges. The large and densely sampled datasets produced by these systems can be difficult to visualize using conventional Exploratory Data Analysis techniques, confounding efforts to build appropriate models for statistical inference. These challenges are further compounded where information from multiple sensors may be available, which prevents ethologists from building a more complete picture of an animal's behavior. The LIT toolkit is an open-source package that provides users, with basic proficiency in R, access to machine learning algorithms that are purpose-built to visualize complex nonlinear patterns within and across livestock data streams. The utility of this analytic pipeline is demonstrated using a dataset collected over a 6-month period from a closed group of 200 mixed-parity Holstein cattle on an organic dairy. Milking order, or the sequence in which cows arrange themselves as they enter the milking parlor, was recovered from RFID logs. Through an iterative clustering algorithm, it was revealed that some cows were remarkably consistent in their queue position over time - even through the shift to spring pasture access - while other subgroups groups shifted over time. Overall time budgets were also calculated from the output of an ear tag accelerometer system (CowManager©). Using minimally parametric ensemble simulation techniques that accounted both for the precision of the sensor and the relative plasticity of the behaviors, heatmap visualizations revealed clear differences between subgroups of cows in the behavioral tradeoffs made between time spent eating, ruminating, and nonactive. These patterns were discretized via a novel automated pruning algorithm, and the resulting clusters passed into a mutual information testing framework to recover nonlinear model-free associations between sensor platforms. As might have been anticipated, the cluster of cows that entered nearer the rear of the queue were significantly over-represented among cows with recorded health complications and subsequently among time budgets clusters with low time spent eating. A more unexpected result, however, was the detection of a small cluster of cows that always entered at the very rear of the queue. These "caboose cows" were significantly under-represented among time budget clusters with low time spent eating, and were not over-represented on the sick list. On the contrary, when sick cows were excluded from analysis, this queue cluster was over-represented among time budget clusters characterized by high time spent eating. This inverted pattern, which would have been overlooked by a linear model, has not been reported in previous

experimental work, and may be better explained by earlier observational studies that have suggested that cow herds may be led both from the front and the rear to prevent stragglers from being left behind.

### **57 The effects of the pre-weaning environment on the play behaviour and aggression of pigs in a social contest.**

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Play behaviour is expressed from a young age in all mammals and has a role in developing social skills that can be useful in subsequent social situations such as meeting new individuals. Mixing is common in intensive production systems, so it could be beneficial to support the development of social skills early in life to mitigate aggressive behaviours to improve animal welfare and production. We exposed pigs to five different treatments that stimulated play behaviour and/or social skill development and observed the influence of play experience on the behaviour of pigs toward an unfamiliar individual in a social contest. A total of 667 pigs were utilized in this study over five batches, with ten litters per batch. At five days of age, two litters per batch received one of five treatments: 1) control (standard farrowing pen conditions), 2) giving 6.00m<sup>2</sup> of extra space versus the regular 4.35 m<sup>2</sup> farrowing pen, 3) allowing adjacent pens of piglets to mingle, 4) straw provided daily on a mat, and 5) a weekly rotation of rubber dog toys. Pre-weaning play behaviour (social, object, and locomotor) was filmed for six hours a day on days 5, 10, 15, 20, and 24. Pigs were weaned at 4 weeks of age into pens with their littermates and a sub-sample were habituated to human handling and the social contest arena. At seven weeks of age, four pigs per litter were weight-matched (<14% difference) to four pigs in another litter of the same treatment, producing 20 matched pairs for contests per batch. Matched pairs underwent a 20-minute social contest that was filmed. Footage was viewed to record escalative, de-escalative, positive and neutral social behaviours. Procedures in this study were approved by the University of Saskatchewan animal care committee. An end point protocol was in place that is the social contest resulted in extreme stress to a pig, such as excessive bullying, the test was stopped. This abstract presents preliminary data from three of five batches. Counts of behaviours were analyzed by generalized linear mixed models to determine if treatment influenced the frequency of play behaviour pre-weaning and escalative or de-escalative behaviours expressed in social contests. The relationship between play and escalative behaviours was explored by spearman's correlation. Treatment influenced the amount of play pre-weaning ( $P < 0.05$ ): extra space, mingling and toy provision resulting in the greatest frequency of play, straw showing the lowest play and control intermediate. In social contests, the frequency of escalative behaviours was affected by treatment ( $p < 0.01$ ), with mingling piglets showing the least amount of behaviours ( $1.28 \pm 0.18$ , mean  $\pm$  SEM/ten mins of contest time), whereas straw performed the most ( $2.29 \pm 0.17$ ;  $P < 0.01$ ). Extra space, toys, and control were intermediate. There was no correlation between pre-weaning play and aggressive behaviours performed in social contests ( $r_s(110) = -0.06$ ,  $P > 0.5$ ). Treatments influenced

the level of pre-weaning play and the level of post-weaning aggression. However, no relationship between pre-weaning play behaviour and aggressive behaviour toward an unfamiliar individual indicates play is not the only factor influencing social behaviour: prior experience with social encounters and features of the pre-weaning environment also contribute to influence behaviour in subsequent social encounters.

# **ABSTRACTS FOR ORAL PRESENTATIONS**

**(In the order of presentation)**

# Friday AM

## 14 Evaluation of tube handling to improve the speed of post-shipment habituation in laboratory mice.

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Tube handling is a gentle handling technique where a mouse is shoed into a tube within its home cage so that they can be removed for scientific or husbandry purposes. This technique can reduce anxiety, stress, and human aversion compared to traditional tail handling methods. A recent study indicates that after habituation, tube handling a mouse just once can improve voluntary interactions with a human. However, it is unknown if applying this technique at arrival and weekly cage cleaning would show similar results and would aid in post-shipment habituation. We hypothesized that tube handled mice would be more likely to interact with a hand and those mice would show faster reductions in stress and would be less disturbed after cage cleaning (general activity). A factorial design (N=16 cages/32 mice) was used to assess the following variables: handling (tube vs tail), sex (male vs female), and strain (C57BL/6NHsd vs BALB/cAnNHsd). Mice arrived at the facility and were randomly assigned to a handling treatment (assigned to the cage), which was used to remove them from the shipping crate. Once housed, an initial approach test was video recorded (60s acclimation and 60s with a stationary hand present). Videos were coded for the duration of time engaging with the hand. After the approach test, the mice were anesthetized and an RFID chip was implanted subcutaneously to constantly record activity. Two hours after implantation, fecal pellets were collected for corticosterone metabolite evaluation (indicative of stress 8-10 hours before collection). The approach test and fecal collection were repeated at cage change on post-arrival day 7 and 14. Data were analyzed as general linear models. Mice that were tube handled spent more time engaging with the hand during the approach test than tail handled mice ( $F_{1,29} = 14.2$ ;  $P < 0.001$ ). Interestingly, this did not change over subsequent tests and experience ( $F_{2,29} = 1.3$ ;  $P = 0.3$ ). Neither corticosterone metabolites nor general activity were altered by handling treatment but both were affected by a time component ( $F_{2,24} = 6.2$ ;  $P = 0.007$ ;  $F_{4,264} = 23.4$ ;  $P < 0.001$  respectively). Corticosterone metabolites showed a negative linear decrease from arrival to 14 days ( $P < 0.05$ ). Activity was primarily affected during the inactive period, where mice were significantly more active on the day of cage cleaning compared to the 24 hours prior to cleaning ( $P < 0.05$ ). Although tube handling can increase voluntary interactions with the human handler, it does not appear to speed post-shipment habituation. However, the time needed to habituate after shipping may take longer than expected, regardless of handling method, and may be disrupted by basic husbandry procedures, potentially adding variability to scientific measures taken at these times.

## 22 Too much too soon? Risk factors for fear behaviour in kittens in foster care.

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Many companion kittens spend their sensitive period for socialization (~2 to 9 weeks of age) in foster care, and the quality of these early life experiences can impact behavioural development. This study aimed to improve early kitten care by using an online Qualtrics survey to investigate risk factors for fear behaviour in foster kittens prior to adoption (7 to 9 weeks of age) based on early management practices, foster parent personality traits, and evaluations of kitten behaviour. Worldwide recruitment resulted in 74 foster parent responses for a total of 241 kittens. Our main behavioural outcome was whether kittens showed fear behaviours during interactions with unfamiliar people, which included displays of avoidance, freezing, crouching, ears back, piloerection, and aggression. We also analyzed the impacts of being reported as fearful at intake into foster care, high vs. low amounts of gentling (i.e., general handling, mimicking handling during a vet visit, exposure to people), high vs. low amounts of stimulus exposure (e.g., to interactive toys, scratching material, etc.), and foster parent personality traits. Of the 241 kittens analyzed, 69 were reported fearful at intake, 109 received high gentling, and 95 received high stimulus exposure. Using a mixed logistic regression model, with foster parent as a random effect to account for foster home clustering, we found that kittens who were reported fearful at intake into foster care were more likely to display fear behaviours towards people than kittens who were not reported fearful at intake (OR=43.43 [95% CI=9.06, 208.26];  $p<0.001$ ). Kittens who received a high amount of stimulus exposure were more likely to display fear towards people compared to those who received a low amount of stimulus exposure (OR=15.90 [2.84, 89.06];  $p=0.002$ ), regardless of fear level at intake. Interestingly, we did not find a reduction in displays of fear behaviour towards people with increased gentling ( $p=0.17$ ), which was predicted based on previous literature. We also found that 48% of the variance in displays of fear behaviour toward people was at the foster-home level, suggesting this outcome was impacted by either litter effects or the foster parent's personality or approaches to socialization. Related to foster personality, kittens whose foster parent had a high score for emotional stability were more likely to display fear towards people compared to those who had a low score for emotional stability (OR=8.86 [1.66, 47.33];  $p=0.011$ ). However, this finding might reflect a bias towards emotionally stable foster parents being more willing to foster fearful kittens. Overall, these findings suggest that high amounts of stimulus exposure and gentling are not necessarily beneficial during early socialization. High gentling was ineffective at reducing fear levels in the current study, and high stimulus exposures appear to have exceeded kitten limits for optimal behavioural development, potentially due to flooding. Our previous research has shown that clear and concise training in identifying kitten behaviour improves human accuracy of rating fear in kittens—the current study reiterates the importance of accurately identifying when kittens are fearful and adapting socialization practices appropriately.

**10 Effects of caretaker interactions on the welfare of adult dogs (*Canis lupus familiaris*) in commercial breeding kennels.**

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A large portion of the demand for purebred dogs in the United States is met by commercial breeding (CB). The amount and quality of caretaker interaction has been demonstrated to affect welfare in livestock and laboratory animals, yet is widely understudied in kennelled dogs, especially those kept for CB, and warrants investigation. Thus, the aim of this study was to determine the effect of a regular, positive caretaker interaction on physiological and behavioral metrics of dog welfare in CB kennels. Adult bitches (n = 47) from two CB kennels received a daily, short interaction with a familiar caretaker. Half of the dogs (n = 23) received treats only (TO), and the other half (n = 24) received a 2-minute caretaker interaction with treats (CI). All other human interactions were limited to routine husbandry. Noninvasive physiological and behavioral metrics were collected at baseline (day 0), after two weeks of treatment (day 14), and two weeks after treatment ended (day 28). Behavioral change during treatment delivery was scored from video on days 1, 2, 8, 9, 13, and 14. General linear mixed models were used with treatment type and timepoint as fixed effects, dog nested within pen as random effects, and welfare metrics as dependent variables. The two facilities were analyzed separately. In facility 1 (n = 25), treatment type did not affect hair cortisol concentration (HCC) or fecal secretory immunoglobulin A (slgA). However, HCC increased significantly from day 0 to day 28 ( $X^2 = 5.83$ ,  $p = 0.016$ ) and slgA decreased significantly ( $X^2 = 21.52$ ,  $p < 0.001$ ) over all three timepoints. A non-significant decrease in affiliative response to a caretaker approach test was observed at each timepoint. In facility 2 (n = 22), there was no effect of treatment type on physiological metrics, however, HCC decreased in time ( $X^2 = 6.66$ ,  $p = 0.009$ ). During daily interactions, dogs from the TO group displayed increased affiliative ( $X^2 = 8.58$ ,  $p = 0.003$ ) and decreased ambivalent ( $X^2 = 10.42$ ,  $p = 0.001$ ) body language over time, while in the CI group, latency to approach the caretaker increased ( $X^2 = 4.38$ ,  $p = 0.033$ ). Patterns of change in physiological and behavioral data differed by facility and treatment group. Factors such as variation in treatment quality and prior caretaker-animal relationship may play a role. These preliminary results suggest that for some dogs unaccustomed to extended interactions with their caretakers, a 2-minute session may have resulted in increased physiological and behavioral stress; for these dogs, a more gradual introduction to human interaction may be more beneficial. Interestingly, dogs in the TO group in facility 2 showed improved behavioral metrics: this type of interaction may be perceived by some dogs as a less aversive initial approach. This study offers new insight on how to implement socialization, counterconditioning, and caretaker-dog interaction practices to maximize positive welfare in CB kennels. Future research is needed to confirm these findings.

## **25 The effects of sheltering and a behaviour modification program in cats (*felis catus*) from hoarding environments.**

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Animal hoarding includes an accumulation of animals that has exceeded capacity for meeting animal care needs. Cats entering shelters from a "hoarding environment" (HE) are commonly highly fearful, have high disease prevalence, and their resource-intensive care presents a challenge for shelters. No known research has investigated HE cats' in-shelter behaviour or behaviour modification (BMOD) progression.

This study has three aims 1) describe the behaviour and health of cats from HEs 2) report the progression of cats through a BMOD program and 3) assess associations between days since intake, BMOD, Cat Stress Score (CSS), and latency to emerge from hiding following shelter close (LTE). We predicted a high proportion of behavioural and health issues in HE cats on intake. Further, we predicted approximately 75% of cats would progress to BMOD completion within 40 days, and as days since intake increased, BMOD would increase, and CSS and LTE would decrease.

Cats were surrendered from three HEs in staggered intakes (n=36). Cats deemed fearful by a veterinary behaviourist (n=31) entered a six-step standardized BMOD program including increasing exposure to humans paired with food, culminating in a cat seeking human attention without treats. All cats received 1-4 BMOD sessions daily (consistent across cats) until program completion, and a mean daily BMOD score was calculated. A mean daily CSS was calculated after three in-person observations. LTE was scored from recorded video. All measures were collected daily until BMOD completion. A repeated measures correlation tested associations between days since intake, BMOD, CSS, and LTE for individual cats, with a Bonferroni correction (statistically significant  $p < 0.008$ ).

The mean ( $\pm$  SD) body condition (out of 9) was  $4.36 \pm 0.78$ . Common medical issues on intake included dental issues (23/36), ear inflammation or discharge (22/36), and eye inflammation or discharge (22/36). Most cats progressed to BMOD completion (n=27) in a median 13 days (range: 2-57) and were adopted. Other cats (n=2) were transferred (at BMOD stage 5), and n=2 were euthanized due to persistent fear. Results showed moderate-high negative correlations between CSS and BMOD [ $r_{(31)} = -0.69$ , 95% CI [-0.73, -0.64],  $p < 0.001$ ], and CSS and days since intake [ $r_{(36)} = -0.62$ , 95% CI [-0.67, -0.56],  $p < 0.001$ ]. A moderate positive correlation was observed between days since intake and BMOD [ $r_{(31)} = 0.58$ , 95% CI [0.51, 0.63],  $p < 0.001$ ]. Low negative correlations were observed between days since intake and LTE, and LTE and BMOD. A low positive correlation was observed between CSS and LTE.

While cats from HEs showed high prevalence of health and behavioural issues on intake, most cats were treatable. These results present evidence of consistent progression of cats from HEs through BMOD over days, and completion of BMOD quicker than predicted. BMOD progression, decreasing CSS, and reducing LTE in this population likely indicates acclimatization over time.

### **33 Evaluating behavioural implications of calf handling and restraint methods used for processing pre-weaned beef calves.**

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In the spring, many beef calves are processed at approximately 6-12 weeks of age, during which calves are individually handled and restrained to carry out various husbandry procedures such as castration and vaccination. Current best practices recommend use of calm, quiet handling techniques as well as proper restraint for safe and effective execution of husbandry procedures. The most common methods for handling and restraining in western Canada are roping and wrestling (W), roping and Nord fork (N), and use of tilt table (T), but the welfare implications of these different methods are unknown. Thus, the aim of this study was to evaluate the effects of these methods on calf behaviour. A total of 117 beef bull calves were assigned to 3 treatment groups (W, N, or T), consisting of 39 calves each (30 processed calves; 9 control group calves) and processed over 3 consecutive days. Each day, 13 calves per treatment group were processed by trained ranch staff. This involved oral meloxicam administration, vaccination, surgical castration, implant injection, and radio frequency identification (RFID) scanning. Control group calves were handled and restrained according to their treatment group but did not undergo any processing procedures. Video recordings of calves from the initiation of handling through to release from restraint were used to measure duration of handling and restraint, as well as to score vocalization (VR) and struggling (SR) rates (count/min), visual eye white (VEW; presence or absence), and gait score at release (walk, trot, or run). Durations of handling and restraint, and VR and SR were analyzed using the Kruskal-Wallis H-test, while Fisher's exact test was used to analyze VEW and gait scores. Both durations were highest for calves restrained by tilt table (median  $\pm$  IQR: handling = 23.4s  $\pm$  19.7 (T), 18.1s  $\pm$  3.6 (N), 14.7s  $\pm$  3.5 (W); restraint = 78.4s  $\pm$  7.4 (T), 67.5s  $\pm$  16.8 (W), 64.8s  $\pm$  22.7 (N);  $P < 0.05$ ). The VR was highest for calves restrained by Nord fork (11.7  $\pm$  12.3 (N), 5.0  $\pm$  7.8 (W), 1.6  $\pm$  7.8 (T) count/min;  $P < 0.05$ ) and SR was highest for calves restrained by tilt table (4.6  $\pm$  4.0 (T), 2.3  $\pm$  2.6 (W), 1.9  $\pm$  2.7 (N) count/min;  $P < 0.05$ ). There was no significant relationship between VEW and method of restraint, but there was a significant relationship between gait score and restraining method ( $P < 0.05$ ), with calves restrained by Nord fork having the highest occurrences of running upon release from constraint ( $P < 0.05$ ). The current study was limited to few behavioural indicators; however, based on preliminary results, calf behavioural responses did vary overall by method of handling and restraint. Specifically, calves restrained by roping and wrestling had lower rates of behavioural indicators that have been associated with stress. Completion of remaining analysis with more diverse indicators during and after restraint to assess calf responses to handling practices and potential welfare implications are underway.

## **Friday PM**

### **8 Hay provision affects 24h performance of normal and abnormal oral behaviors in milk-fed dairy calves.**

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Dairy calves often perform abnormal repetitive behaviors (ARBs) like tongue rolling and non-nutritive oral manipulation (NNOM) when opportunities to perform food acquisition and processing behaviors are restricted. Many US dairy farms limit access to milk, a well-studied risk factor for ARBs. However, farms also commonly do not provide forage to young calves, and the motor patterns of ARBs resemble those used in solid food processing. Our objective was to assess how access to hay from birth influenced time engaged in all oral behaviors performed across 24h. We used 49 Holstein heifer calves housed individually on sand bedding. Calves were fed ad libitum grain and water from birth (C, n=20) or had additional access to mountaingrass hay (H, n=29), and were bottle-fed 4.8-5.6L (55% of calves) or 5.7-8.4L (45% of calves) milk replacer/d step-up. Weaning began at d50. Feed and water intake were measured daily. At 4 and 6 weeks of age, oral behaviors (eating, ruminating, sucking milk, drinking water, grooming, tongue flicking, tongue rolling, and NNOM) were recorded using 1-0 sampling at 1-min intervals for 24h. We analyzed the proportion of intervals calves performed each behavior using a generalized linear mixed model with a beta distribution using week and treatment as fixed effects and calf as a random effect. H calves spent more observations eating solid feed (0.1 vs. 0.05 proportion of intervals,  $p<0.01$ ) and ruminating (0.27 vs. 0.17,  $p<0.01$ ) than C calves, though the high level of rumination shown by C calves may indicate sham or practice movements. Rumination occurred to a greater extent overnight, compared to during the day. H calves also spent less time performing NNOM (0.16 vs. 0.21,  $p<0.01$ ) and tongue flicks (0.16 vs. 0.18,  $p=0.03$ ) than C calves. While grooming did not differ overall (0.13,  $p=0.79$ ), individual calves varied in how much time they spent self-grooming (0.05-0.22). Performance of NNOM, tongue flicks, and grooming peaked around milk feedings, but all occurred across 24h. Polydipsia, or excessive water drinking, was also seen, with 3 calves consuming  $>10L/d$ . Overall there are 2 conclusions from this work: 1) hay can reduce commonly studied ARBs like NNOM, and 2) sham rumination behaviors, excessive grooming, and polydipsia, all reported in other species when highly motivated behaviors are thwarted, may reflect novel indicators of compromised welfare for dairy calves that can be overlooked when not scored across 24h.

Keywords: feeding behavior; forage; abnormal behavior; dairy

### **35 Isolation box test and its relationship with daily patterns of activity and feeding behavior measured by precision technology.**

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The isolation box test, placement of animals in a dark enclosed box with an electronic measurement of movement, has been successfully utilized to determine personality traits in sheep for decades and has recently been explored for use in dairy calves. The isolation box test has the potential to make personality testing more applicable for on

farm use. Additionally, the increased accessibility of precision technology grants the opportunity to explore behaviors that may be related to personality traits in a natural setting to dairy calves. Therefore, the aim of this study was to investigate the relationship between a personality trait measured via the isolation box test and precision technology measures of activity and feeding behavior. Holstein dairy calves ( $n = 23$ ) were enrolled into this study at 4d of age in group housing system, equipped with a leg accelerometer and fed milk and calf starter by an automatic feeding system. The leg accelerometer was utilized to determine total steps, activity index, lying time, and lying bouts from 4d of age until unenrollment at 81d of age. The automatic feeding system was utilized to determine the total milk consumption, calf starter consumption, and rewarded and unrewarded visits to the milk feeder. Calves were allotted 14 L/d of milk replacer until milk step down began at 45d of age. Calves were weaned at 67d of age and were kept enrolled for 2 additional weeks until 81d of age. Calves had *ad libitum* access to starter for the entire study period. Calves were weighed twice weekly to track average daily gain as a measure of calf performance. At  $24 \pm 3$ d of age calves were subjected to a 5-minute isolation box test. Agitation from the calf within the isolation box was measured via tri-axis accelerometers (HOBO Pendant g Data Logger, ONSET, Bourne, MA, USA; 2Hz frequency) attached to the box in 5 positions [left, right, back, top-back, top-front]. The total squared acceleration of the three axes through the test were summed for the 5 positions, to create a 'total movement index' (**TMI**) for each calf. Linear regressions were performed to view the relationship between TMI (explanatory variable) and the precision technology measures and performance (response variables). The F-value and P-values for each model were reported where  $P < 0.05$  was considered significant. The TMI had significant positive associations with both the total milk intake ( $F=5.66$ ;  $P=0.03$ ) and lying bouts ( $F= 6.56$ ;  $P=0.02$ ) for calves, indicating calves who had a more active response to the isolation box consumed more milk replacer and laid down more times than their less active counterparts. Additionally, TMI had a significant positive association with average daily gain throughout the study, so, calves that are more active within the isolation box grew more from 4d of age until 2 weeks post-weaning. The relationships between TMI and both performance and the daily patterns of behaviors of calves measured by precision dairy technology indicates that the stable differences of individuals are important from a production standpoint and can be measured and identified early in life in dairy calves. Additionally, the relationship between a broader range of personality traits in dairy calves with these precision technology measures is worthy of investigation.

#### **44 Can the supplementation of parental diets with omega-3 fatty acids increase the spatial memory of Shaver white and ISA brown chickens in a T-maze test?**

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Omega-3 fatty acids (n3 FAs) are pivotal for brain development, as deficiency leads to impaired cognitive outcomes, such as decreased spatial memory. Chicken feed is naturally low in n3 FAs. Supplementing breeder flock diets with flaxseed could improve brain development in embryos by ensuring an ample supply of n3 FAs are deposited

into the egg yolk. We hypothesized that chicks from flaxseed fed parents would perform better in a T-maze test than chicks from control fed parents. Shaver white and ISA brown parent flocks were raised in 16 pens and were fed either a control diet (n6: n3 = 14.7) or n3 FA flaxseed diet (n6: n3 = 5.3). Eggs were collected from the parent flocks at 30 and 36 weeks of age and incubated, resulting in two replicates of offspring. Female chicks were housed in four rooms with a total of 48 pens (12 per parental diet/strain). From each pen four chicks were randomly selected for a T-maze test (n=192) at 9-10 days of age. Two T-mazes were used, one maze had a blue left arm and green right arm, the other maze had the reverse configuration. Chicks were randomly assigned to a maze configuration and reward arm. Mirrors were used as a reward to simulate social reinstatement. Chicks were given three one-minute habituation periods during which three mirrors were placed in the T-maze, in each coloured arm and the end of the T. A training phase was conducted (mirror in reward arm only), where the chick was gently pushed into the reward arm and non-reward arm twice. During testing the chick had six one-minute trials to relocate the mirror. Chicks were scored from 0-6 for three possible outcomes: 'correct' (relocating the mirror), 'incorrect' (entering the arm without the mirror), or as 'no-choice' (not entering either arm). Data were analysed in R using a GLMER model (Poisson), with strain, treatment and their interaction as fixed effects. Pen, block, room and replicate, reward arm colour and maze configuration, and testing order and day of testing were nested as random effects. Models were reduced by removing random effects not contributing to variation in the data. This test showed that there was no effect of parental diet on the number of correct ( $\chi^2=0.3951$ ,  $p=0.5296$ ), incorrect ( $\chi^2=0.7530$ ,  $p=0.3855$ ) and no-choice ( $\chi^2=0.0378$ ,  $p=0.8459$ ) scores in this T-maze test. There was no significant interaction between strain and parental diet on correct ( $\chi^2=0.9976$ ,  $p=0.3179$ ), incorrect ( $\chi^2 = 0.0289$ ,  $p = 0.8650$ ), or no-choice scores ( $\chi^2=0.1717$ ,  $p=0.6786$ ). However, there was a significant effect of strain on the number of correct scores, white chicks made more correct choices than brown chicks ( $\chi^2=24.0412$ ,  $p<0.001$ ). Brown chicks received more no-choice scores than white chicks ( $\chi^2=70.2931$ ,  $p<0.001$ ). There was no effect of strain on incorrect scores ( $\chi^2=2.3642$ ,  $p=0.1241$ ). Contrary to our hypothesis these results show that strain, rather than n3 FA inclusion in the parental diet, had a greater impact on Shaver white and ISA browns' ability to relocate a reward in a T-maze test.

## Saturday AM

### 5 Effects of a disease challenge on social networks of group-housed dairy calves.

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Social networks have proven useful for wildlife disease ecology and may provide a novel means to utilize social network position as an indicator of health in managed populations. In dairy calves, we found previously that individual calves initiated less social grooming following a respiratory disease challenge, suggesting disease may be associated with changes in social relationships. However, research examining social interactions at the group level following infection is lacking. We quantified social

networks for four groups of Holstein bull calves ( $n=6/\text{pen}$ ,  $6.6 \text{ m}^2/\text{calf}$ ) based on interaction data from an experimental disease challenge. Calves were assigned within pen to 1 of 2 treatments: 1) inoculation of *Mannheimia haemolytica*, a main component of bovine respiratory disease (MH;  $n = 12$ ,  $3/\text{pen}$ ), or 2) control, involving inoculation with phosphate buffered saline only (control;  $n = 12$ ,  $3/\text{pen}$ ). The experimental challenge was previously developed and induced only a short-term minor response; all procedures were approved by the institutional animal care and use committee. Continuous behavioral data were recorded from video on the day before challenge (baseline) and for 24 h following inoculation to describe social lying, defined as rest within one body length of another calf, and social contact between calves, noting identity of calves involved in each interaction. Social network position was measured using three centrality measures: degree, strength and eigenvector. To determine the possible effects of the disease challenge on social network position, we examined effects of treatment on individual centrality scores derived from directed interactions on challenge day only. Secondly, we tested whether an individual's social network position derived from undirected interactions differed significantly from baseline to challenge day and if this was related to the challenge. Using LMMs, we found challenged calves interacted with fewer penmates, spent less time in contact with others, and were less connected on challenge day. Overall, control calves were more central in their directed social contact network than would be expected by chance (eigenvector [ $t(24)=-4.57$ ,  $p<0.01$ ], degree [ $t(24)=-4.65$ ,  $p<0.01$ ], and strength [ $t(24)=-3.37$ ,  $p<0.01$ ]). Comparisons of individual centrality scores from undirected interactions on baseline and challenge day indicated a significant difference for strength (Wilcoxon signed-rank:  $t(24) = -4.18$ ,  $p < 0.01$ ) and degree ( $t(24) = -2.23$ ,  $p < 0.01$ ) in the frequency of all social contacts. Lastly, we calculated the differences in individual centrality scores from baseline to challenge day. These differences in scores for the frequency of all social contacts, represented as positive or negative values, were related to the challenge ( $p < 0.01$  for strength and degree), indicating reduced centrality and social connectedness for challenged calves. These results suggest that social network measures may be a useful tool for disease management in dairy calves.

## **21 Age and litter size influence puppy competitive behaviour during nursing in the domestic dog (*Canis lupus familiaris*).**

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While some level of competitive behaviour is normal in dogs, threatening and aggressive forms of resource guarding behaviour can impair owner health and safety, canine welfare, and the human-animal bond. Early experiences with competitive behaviours might influence later development of resource guarding behaviour, and this is an area that requires further study. However, little is known about competitive behaviour in young puppies. The objectives of this study were to: 1) identify and describe competitive behaviour displayed by puppies during the beginning of a nursing

bout and, 2) identify factors that influence the performance of competitive behaviour in young puppies. Publicly accessible nursing videos (N=376) were obtained from YouTube, with videos of 34 litters (N = 223 puppies total) meeting our inclusion criteria (i.e., 2 or more puppies, puppies visible, includes beginning of session, minimum 60s). An ethogram was developed, using existing literature and observation of nursing videos, of potential competitive and nursing behaviours. Videos were scored using BORIS (Behavioural Observation Research Interactive Software) starting when the first puppy latched to a nipple and continuing for 60s total. Competitive (paw push, body push, muzzle push and displacement) and nursing behaviours (teat seeking, headbutting nipple, latency to attach, and bitch and human interventions) were scored for each puppy in the litter. A mixed logistic regression model, with a random intercept to control for litter, was used to estimate an individual puppy's probability of displaying competitive behaviour during a nursing bout. Tested predictor variables included litter-size, age, nursing style (lying versus standing), time spent nursing or teat seeking and whether headbutting or intervention (human or bitch) occurred. In total, 62% (138/223) of puppies displayed competitive behaviour during the observation period, with 97% (33/34) of litters including at least one puppy displaying competitive behaviour. Puppies from litter sizes of 7-8 had a higher odds of displaying competitive behaviour when compared to litter sizes of 2-4 puppies (OR: 2.8, 95% CI: 1.24-6.35,  $p = 0.013$ ). Puppies who were 3-4 weeks-old had a lower odds of displaying competitive behaviour when compared with 0-2 week-old puppies (OR: 2.2, 95% CI: 1.06-4.64,  $p = 0.035$ ). No other recorded variables significantly predicted competitive behaviour. As predicted, puppies belonging to larger litter sizes displayed increased competitive behaviours, likely due to more limited space and nipple availability. Puppies who were 3-4 weeks-old displayed decreased competitive behaviours, perhaps due to introduction of solid foods that typically occurs around 3 weeks-of-age decreasing dependence and motivation for milk. Alternatively, increased autonomy and locomotion at 3-4 weeks-of-age might result in more staggered feeding times, decreasing the need for competitive behaviours to attain milk. This research indicates that young puppies do, in fact, show competitive behaviour during nursing. Next, further longitudinal research is needed to assess how this early competitive behaviour impacts development of resource guarding later in life.

### **30 Are infrared thermography and automated feeding systems capable of identifying group-housed sow social hierarchies?**

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Identifying the social hierarchy of group housed sows could offer producers an advantage in managing sow welfare and reproductive success. While not currently practical for producers, social hierarchy detection within a group of sows may be possible with the introduction of precision technologies, such as infrared thermal imaging (**IRT**) and automated radio-frequency identification (**RFID**) feeding technology. Therefore, the objective of this preliminary study was to investigate the use of IRT characteristics and feeding behavior traits obtained from an automated RFID feeding system for detecting the social hierarchy within three groups of weaned sows ( $n=14$ ,

n=12, and n=15 for groups 1, 2, and 3 respectively). Additionally, the relationship between observed social hierarchy, body condition score (**BCS**), backfat depth (**BF**), and reproductive performance were assessed. Aggressive and submissive behaviors occurring within 12 hours after re-introduction to group gestation housing were evaluated to determine the sow social hierarchy using a common dominance index (**DI**) method. Sows with greater DI were assumed to rank higher within the group, with rank of 1 representing the sow with the greatest DI. Individuals within groups were then categorized into four rank quartiles (RQ) based on DI, where the 1st quartile (RQ1) represented the highest-ranked sows (3-4 sows with the greatest DI depending on overall group size) and the 4th (RQ4) represented the lowest-ranked sows. Infrared thermal images taken at the base of the ear were captured from each sow on d 3, 15, 30, 45, 60, 75, 90, and 105 post-mixing. Feeding behavior traits were collected throughout the gestation period using an RFID electronic sow feeding system. Body condition score on a 3- and 6-point scale (**BCS3** and **BCS6, respectively**) and BF were collected on d 15, 30, 45, 60, 75, 90, and 105 post-mixing. After approximately 105d of gestation, sows were moved from group gestation pens to individual farrowing stalls for farrowing. Reproductive performance of each sow was documented after the completion of farrowing. Infrared thermal imaging characteristics (*i.e.* mean, minimum, and maximum temperature), BCS3, BCS6, and BF were analyzed in relation to RQ using the GLIMMIX procedure in SAS (v. 9.4; SAS Institute, Cary, NC). Pearson correlation coefficients between DI value, feeding behavior traits, and reproductive performance traits were analyzed using the CORR procedure in SAS. Rank quartile was not associated with IRT characteristics, BCS3, BCS6, or BF ( $P > 0.05$ ). Dominance index tended to correlate negatively with the number of visits per day with feed ( $R = -0.38$ ;  $P = 0.07$ ) and correlate positively with feed intake per meal with feed (kg/meal;  $R = 0.39$ ;  $P = 0.06$ ). Additionally, DI tended ( $P = 0.08$ ) to correlate negatively with the number of piglets born at 1 kg or less ( $R = -0.35$ ). These preliminary data show that sow position within the social hierarchy cannot be identified with IRT, is not strongly correlated with RFID feeding activity, or reproductive performance. Further, sow social hierarchy was not associated with BCS or BF throughout the gestation period. Therefore, the use of IRT and RFID feeding activity for detection of sow social hierarchy may not be a useful precision method for on-farm sow welfare monitoring.

### **17 Wound healing following caustic paste disbudding in dairy calves.**

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Disbudding is a common procedure used on 94% of U.S. dairy cattle farms to prevent horn growth by creating permanent damage to the horn bud tissue either with a hot iron (heat) or caustic paste (chemical). Caustic paste is increasing in popularity on commercial dairy farms, but little research is available on the associated pain and wound healing. Studies have shown it takes 6.0 to 13.0 weeks (mean = 8.3) for hot-iron disbudding wounds to re-epithelialize in dairy calves, while caustic paste wounds in goat kids are present for at least 6 weeks. Our objective was to describe wound healing

following caustic paste disbudding in dairy calves. First, we developed a scoring system to evaluate the presence of wound tissue types based on a previously used model for hot-iron disbudding. Wounds were scored for the presence/absence of 8 categories: fresh paste, attached necrotic tissue, detaching necrotic tissue, exudate, granulation tissue, crust, new epithelium, or fully healed. To establish inter-observer reliability, 80 photos were evaluated for binary presence of each tissue type. Scores were compared to a gold standard (A. Drwencke) using Cohen's Kappa. Observers needed to reach a score of [Equation]0.9 for each category to collect data. Jersey and Holstein female calves were disbudded using Dr. Naylor's caustic paste at 3 days of age (n=18). Prior to disbudding, calves received meloxicam at a rate of 1 mg/kg of body weight and a lidocaine cornual nerve block with 5 ml per horn bud. Calves were checked for horn bud sensation prior to paste application, an additional 2 ml of lidocaine was provided if calves were responsive to a pin prick. For calves [Equation]34 kg, 0.3 ml of paste per horn bud was used, whereas calves <34 kg had 0.25 ml applied per bud. Paste was rubbed into unshaved horn buds. Following disbudding, wounds were scored live for tissue type presence 2 times per week until healed. Wounds were slow to re-epithelialize (mean =14.1; min to max: 6.3 to 19.0 weeks; n=23 horn buds) and to fully contract and be considered healed (mean =15.2; min to max: 8.7 to 19.6 weeks; n=27 buds). Indeed, these data only represent a subset of the entire sample size (n=36 horn buds) because the remaining calves still have unresolved wounds that we are still monitoring. These data indicate that wounds from caustic paste disbudding can take twice as long to heal, compared to cautery methods.

Keywords: Disbudding; caustic paste; wound healing

## **18 Effects of willow bark (*Salix*) on pain and stress in recently disbudded organic dairy calves.**

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Disbudding is a common painful procedure on US dairy farms, yet organic producers have limited options for providing pain relief. The local anesthetic lidocaine (LID) is a synthetic substance approved for use in organic production; however, it can be difficult to administer successfully. With few alternatives available, many organic producers forgo the use of either anesthesia or analgesia for disbudding pain, necessitating the search for organic pain relief options. White Willow Bark (*Salix*) has been used to alleviate inflammatory pain in disbudded calves under organic management, but there is no scientific evidence that willow bark (WB) has an analgesic effect in cattle. The purpose of this study was to evaluate the effect of an oral WB bolus on heart rate, ocular temperature, salivary cortisol, and lying behavior of 42 disbudded Holstein and crossbred heifer calves. Calves were disbudded with a hot iron between 4 to 7 weeks of age (Mean = 42 days). Calves were randomly assigned to receive either LID, oral WB, or a sham, which involves the use of a cold iron to simulate disbudding (n = 14 calves per treatment). Thermal images of the eye and saliva samples were collected 1 hour before disbudding, at the time of disbudding, 5 min, 10 min, and every 30 min until 240

min after disbudding. Polar H10 heart rate monitors recorded the heart rates continuously throughout the 5-hour study period. The HOBO loggers recorded lying behavior during the study period and the following two days in 1 min intervals. Results indicated that the mean heart rate of sham calves was lower than both LID ( $P < 0.05$ ) and WB calves ( $P < 0.001$ ) by  $11.44 \pm 4.13$  bpm and  $14.71 \pm 4.21$  bpm, respectively. Although WB and LID calves did not differ overall, WB calves' heart rates were  $11.64 \pm 5.78$  bpm higher than LID calves during the 5 minutes immediately following disbudding ( $P < 0.05$ ) and  $17.00 \pm 5.72$  bpm higher in the next 5 minutes ( $P < 0.05$ ). The WB calves' heart rates increased from baseline immediately after disbudding ( $P < 0.001$ ) and in 5-minute intervals coinciding with sampling at 60min ( $P < 0.001$ ), 120 min ( $P < 0.05$ ), 150 min ( $P < 0.05$ ), 180 min ( $P < 0.001$ ), and 210 min ( $P < 0.05$ ). A similar pattern was observed in LID calves, but not in sham calves, indicating that WB and LID calves were more reactive to further handling after disbudding than sham calves. No differences were observed for ocular temperature. Cortisol concentration was affected by treatment ( $P < 0.005$ ) and LID (Mean =  $104.50 \pm 3.29$  pg/mL) and WB (Mean =  $108.03 \pm 4.28$  pg/mL) calves had higher cortisol concentrations than sham (Mean =  $87.44 \pm 2.31$  pg/mL) calves. Cortisol of WB calves peaked at 30 min (Mean =  $147.13 \pm 27.43$  pg/mL;  $P < 0.001$ ) and remained above baseline at 60 min ( $P < 0.05$ ), 90 min ( $P < 0.001$ ) and 120 min ( $P < 0.05$ ) after disbudding. The LID and sham calves did not differ from their respective baseline cortisol levels. Lying time and bouts did not differ across treatments. These results indicate that neither WB nor LID alone are sufficient for relieving disbudding-related pain in organic dairy calves.

## **56 Evaluation of firocoxib, delivered transmammary to piglets, to alleviate pain associated with elective husbandry procedures.**

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Piglets raised in commercial production systems in the U.S. undergo painful management procedures without analgesia or anesthesia provision. This is a significant animal welfare issue, affecting millions of piglets each year. The objective of this study was to assess the efficacy of firocoxib, administered to the sow and delivered transmammary to her piglets, and a vapocoolant spray, to reduce pain associated with piglet processing procedures. One hundred and twenty-eight Yorkshire x Landrace piglets (5 days old) across 16 litters were used. The study was split into a winter cohort and a summer cohort, with an equal number of piglets represented in each group. Seven hours prior to processing piglets, half of the sows received an intramuscular injection of 2.0mg/kg firocoxib (for transmammary-delivery to piglets). Piglets were assigned to one of four treatment groups: firocoxib + ethyl chloride spray, firocoxib only, ethyl chloride spray only, or no treatment. The ethyl chloride spray was applied to the

ears, tail, and scrotum of the piglets immediately before ear notching, tail docking and surgical castration, respectively. The outcome variables included assessment of firocoxib concentration in piglets, behavior, cranial and eye temperature (using infrared thermography), and plasma cortisol concentrations. The addition of the ethyl chloride spray did not lead to significance for any of the outcome measures; therefore, treatments were collapsed into two groups: FIRO piglets who received transmammary-firocoxib prior to processing; CON piglets who were processed without analgesic provision. The concentration of firocoxib in piglets was significantly higher in the winter cohort compared to the summer cohort ( $P=0.0008$ ). The greatest concentration of firocoxib found in piglets was ~30h post-administration to the sow ( $P<0.0001$ ). Piglets in the summer cohort also had significantly higher cranial and eye temperatures, and significantly lower cortisol concentrations, than piglets in the winter cohort ( $P<0.0001$ ). There was no significant difference in proportion of time piglets displayed pain behavior between treatment groups; however, FIRO piglets engaged in significantly less agonistic behavior and significantly more play behavior across the observation period compared to CON piglets ( $P=0.04$  and  $P=0.002$ , respectively). Irrespective of treatment, piglets were observed displaying significantly more pain behavior at 24 and 30h post-processing compared to all other time points ( $P<0.0001$ ). Transmammary-delivery of firocoxib may have provided some benefit to piglets in this study; however, the concentration of analgesic at the level of the piglet was not sufficient to significantly reduce pain. Season (e.g., temperature of the farrowing room) affected drug delivery and pain outcome measures in this study and should be accounted for to determine the optimal drug dose and treatment regimen in future work.

### **19 Developing a Goat Grimace Scale to assess pain in goats.**

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Validated grimace scales have successfully detected post-procedural pain in both human and non-human animals, and are an ideal tool for pain assessment given they are easy to use, non-invasive, and provide rapid results with high specificity. A Goat Grimace Scale (GGS) may limit the impact on animal welfare as a practical solution to accurately detecting and quantifying pain in goats, which is necessary for appropriate development of pain management for this species. The objective of this preliminary study was to develop a GGS to detect pain in goats with experimentally-induced lameness. Twenty-four goats of mixed breed and sex were randomly assigned to one of four lameness induction treatments ( $n=6$  goats/treatment group): saline (CNTL; 1ml), high dose/high volume (HH; 10mg: 1ml), high dose/low volume (HL; 10mg: 0.5ml), or low dose/low volume (LL; 5mg: 0.5ml) of Amphotericin B (Amp-B). Goats were then randomly allocated into one of two cohorts ( $n=12$  goats/cohort) with equal representation of treatments. Treatments were administered by intraarticular injection of Amp-B of the left rear digit. Goats were video recorded walking across a pressure mat

at baseline and at 4, 6, 12, 24, 48, and 72 h post-induction. A total of 351 goat facial images were collected from 168 videos. Images were scored using four facial action units (FAUs): orbital tightening, ear positioning, cheek tightening, and nostril shape and dilation. Following the same timepoints as GGS image capture, a Visual Analog Scale (VAS) and Visual Lameness Score (VLS) were also assessed given this study was part of a larger study assessing goat response to lameness. The VAS was collected using a tick mark on a linear scale ranging from 0cm (not lame) to 10cm (lame), while the VLS used a scale ranging from 0 (no lameness) to 4 (severe non-weightbearing lameness). Scoring of GGS images, VAS, and VLS were performed by two different observers blinded to treatment and time point. Irrespective of treatment, Observers 1 and 2 found goats grimaced more at 24 h post-induction compared to baseline ( $P < 0.05$ ). Observer 1 found that Cohort 1 and the HH group grimaced more than Cohort 2 and the LL group, respectively ( $P = 0.1$  and  $P = 0.2$ , respectively). The HH goats also tended to grimace more than CNTL goats ( $P = 0.1$ ). Additionally, Cohort 1 had higher VAS scores than Cohort 2 ( $P = 0.02$ ). Lastly, the VLS scores found a higher proportion of lame goats in the HH and HL groups ( $P = 0.0009$ ). The GGS developed in this study correlated well with VAS and VLS, which are established pain outcome measures. This GGS may be a useful tool in detecting pain in lame goats and improving on-farm goat welfare.

## Saturday PM

### 36 Laying hen welfare in furnished cages: Effect of sham dustbathing and rearing experience on motivation to dustbathe on peat.

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Dustbathing in laying hens is a motivated behaviour that is difficult to accommodate for in caged-housing systems. Furnished cages are intended to support dustbathing, but often only allow for sham dustbathing on wire floors. We asked how sham dustbathing affects a hen's motivation to dustbathe on suitable substrate, and whether early experience (with or without substrate) affects motivation to dustbathe in adulthood. In this study, Lohmann LSL-lite chicks were assigned to 1 of 2 rearing treatments: standard conventional cage (C), or rearing aviary (A, 6 perches, elevated platform, litter area with wood shavings). At 17 weeks of age, hens were transferred to furnished cages (4 perches, scratch mat, nest box), equally distributed across 2 rooms, with each cage housing 30 hens from the same rearing treatment (4 cages, 2 replicates/treatment, 120 hens total). Sixty hens (30/treatment) were randomly selected for 3 rounds of testing. In round 1, hens were observed sham dustbathing before being transferred to an arena with peat moss, a suitable dustbathing substrate. In round 2, the same 60 hens were subjected to a control where they were placed in the arena after a minimum of 30 mins without sham dustbathing in their cage. If a hen did sham dustbathe, the test was aborted and attempted another day. In round 3, 20 of the 60 hens were randomly selected for a repeat of round 1 to examine effects of previous exposure to peat. For

those that dustbathed on peat, latency to dustbathe and dustbath duration were recorded, as well as the occurrence of key dustbathing elements.

We analyzed the likelihood to dustbathe depending on rearing and prior sham dustbathing by using a generalized linear mixed model (GLMM) with a binomial distribution. GLMM was also used to analyze the effects of rearing and sham dustbathing on the latency to dustbathe on peat, and the effects of rearing on sham dustbath duration. Fixed effects were rearing treatment and sham dustbathing (yes/no); random effect was room and hen ID was nested in cage. Descriptive statistics were generated for dustbath duration depending on rearing and sham dustbathing. Rearing treatment affected likelihood to dustbathe on peat ( $F= 14.22$ ,  $P= 0.0002$ ). A hens were 5.4 times more likely to dustbathe on peat compared to C hens (95% CI 2.23-13.10). Sham dustbathing prior to testing did not affect likelihood to dustbathe or dustbath duration but did increase latency to dustbathe on peat ( $F=55.79$ ,  $P= <0.0001$ ), suggesting that it reduces motivation to some degree. Rearing did not affect sham or peat dustbath duration.

Although A hens dustbathed more, they did not have shorter latencies or longer dustbath durations than C hens. Overall, it is unlikely that the hens dustbathed out of “need” to relieve a build-up in motivation that sham dustbathing did not satisfy. Rather, we suggest that the hens dustbathed based on opportunity, and that aviary-reared hens better recognized this opportunity due to early experience.

#### **41 Do housing-induced changes in brain activity cause stereotypic behaviours in mice?**

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Repetitive stereotypic behaviours (SBs) are common in farm, zoo and laboratory animals, and indicate poor welfare. Caused by barren housing, some suggest they reflect brain dysfunction akin to changes implicated in humans with Obsessive-Compulsive Disorder or Autism. Using mice as models, we therefore tested two sets of hypotheses: that individual differences in SB reflect housing-induced changes in I) cortico-striato-thalamo-cortical circuits responsible for motor, cognitive and motivational aspects of behavioural control, and/or II) the relative activity of basal ganglia pathways thought to be inhibitory or excitatory on behaviour. Subjects were 35 females from DBA/2 (DBA:  $n = 17$ ) or C57/BL/6J (C57:  $n = 18$ ) strains, kept in conventional housing (CH: barren ‘shoebox’ cages;  $n = 21$ ) or well-resourced housing (WR: large cages containing preferred ‘enrichments’;  $n = 14$ ) for 17 months. SBs -- largely route-tracing (RT) or bar-mouthing (BM) -- were recorded monthly, quantified as proportions of total visible observations, and averaged over the lifetime. As expected, CH mice were more stereotypic than WR, and DBAs were more stereotypic than C57s. After being humanely killed, brains were extracted, sectioned, and stained with cytochrome oxidase histochemistry, to index local oxidative metabolism reflecting long-term neuronal activity. Optical density (OD) per unit area was measured via ImageJ for 23 regions

within the cortex, striatum, basal ganglia and thalamus; darker staining indicated greater neuronal activity. Generalized linear mixed models then tested the hypotheses. Inhibitory/excitatory pathways hypotheses (set II) were not supported: housing affected none of the relevant regions. CH reduced activity in the ventral anterior cingulate cortex ( $F_{1, 31} = 4.38$ ,  $p = 0.04$ , Cohen's  $d = 0.73$ ) and a striatal area receiving input from the 'mouth region' of the motor cortex ( $F_{1, 30} = 5.58$ ,  $p = 0.02$ , Cohen's  $d = 0.85$ ). For both, activity levels also tended to be lower for DBA mice with high BM (respectively  $\beta = -0.30$ ,  $p = 0.06$ ; CH subjects only;  $\beta = -0.26$ ,  $p = 0.09$ ). For BM, these results thus partially support two 'circuit' hypotheses (set I), by implicating regions of the prefrontal cortex and striatum that play roles in respectively the cognitive and motor control of behaviour. CH also significantly reduced activity in medial prefrontal and motor cortices, other striatal regions and the nucleus accumbens. However, for these, activity showed either no relationships with SBs or unexpectedly positive ones: at odds with housing-induced reductions causing SB. Overall, results do not yet fully identify the causal bases of SB. However, they show that for mice, conventional caging has many effects on the brain; several brain regions unaffected by housing still covary with SBs, suggesting individual-level risk factors; and RT and BM have different neurological correlates, confirming the heterogeneity of SB. Our next steps are to opportunistically use model-fitting to identify combinations of housing effects and covariates of SB that best predict RT and BM, to develop new hypotheses for future investigation.

#### **42 Measuring the amount of vertical space needed for laying hens to flap their wings.**

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As of January 2022, nine U.S. states passed legislation regarding the housing of commercial laying hens. Many of these regulations require producers to use cage-free systems, like multi-tiered aviaries, and to follow spacing guidelines as stipulated by United Egg Producers. However, several states also mandate hens be able to fully extend limbs without touching their enclosure. This requirement is ambiguous and does not specify if hens must be able to extend limbs horizontally or vertically as well. Little research has looked at how much vertical space a hen occupies while extending her limbs (i.e., flapping her wings); however, this information is important to investigate, especially considering the configuration of aviaries, which are generally composed of several vertical tiers atop one another. Therefore, this study looked to establish a fast and reliable method to measure the maximum height reached while wing flapping. Subjects used were 28 individually caged Hy-line W36 hens (age: 45 weeks). Physical measures taken from each hen, like body weights and lengths of extended left wings, resulted in averages of  $1.62 \pm 0.17$  kg and  $34.47 \pm 2.56$  cm, respectively. A ceiling-mounted Intel RealSense Depth Camera D435 was centered approximately 250 cm above the surface of a black plywood board (121.9 x 121.9 cm) affixed to the floor. The depth camera was calibrated to that surface before a foldable test pen (121.9 x 121.9 x 121.9 cm) was placed around the plywood. A tripod-affixed camcorder filmed the test pen from a side view. Prior to data collection, all hens were habituated to handling and testing procedures at least twice. Hens were removed from home cages in groups of

four and were placed individually in one of four hard-sided pet carriers (45.5 x 29.2 x 30.5 cm) surrounding the test pen; this was done to allow visual contact among hens, to keep track of each hen, and to encourage wing flapping once hens were tested. During testing, one hen at a time was placed in the test pen and video recordings started. Recordings stopped once each hen flapped her wings. From depth footage for each hen, frames were cropped and processed to increase image quality and fill missing/dead pixels. A binary mask was created to separate hens from background and the minimum distance between pixels was obtained for each frame. The maximum height reached by each hen was computed. The binary mask was then overlaid on depth footage, superimposing a dot on the highest point reached in each flapping event, to visually verify that the computed maximum height corresponded to the highest position for each hen. Initial results yielded an average maximum height during wing flapping of  $51.02 \pm 4.7$  cm; these results are being corroborated using analysis of side-angle camcorder footage with ImageJ. Hens used in this study were from a single strain, old enough to have keel damage or poorer feather condition and were cage-reared and housed (likely with minimal muscular strength or experience wing flapping), limiting our ability to generalize our findings to a vertical height standard for cage-free hens. However, these methods may provide a useful approach for others to measure space requirements in laying hens of varying strains, ages, and rearing/housing methods.

### **31 Impact of stocking density and exercise on the social and stereotypic behaviors of developing beef heifers.**

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To examine the effects of confinement stress and restricted physical activity on the social and stereotypic behavior of heifers reared in high stocking densities, 90 Angus ( $\frac{3}{4}$ ) x Brahman ( $\frac{1}{4}$ ) heifers were ranked by age and BW that was measured approximately 15 d after weaning (7 months of age) and allocated to: a) 1 of 6 drylot pens (10x14 m pens; 10 heifers/pen) resulting in a stocking density of 14 m<sup>2</sup>/heifer (DENS), or b) 1 of 3 paddocks (2-ha paddocks; 10 heifers/paddock) resulting in a stocking density of 2,000 m<sup>2</sup>/heifer (CON). After two weeks of acclimation, 3 DENS pens were randomly selected to utilize an exercise area during the experimental period (DENS-EX). Heifers were allowed to enter the exercise area (a 30 x 150 m narrow paddock with no forage) 3 times/wk (Mon/Wed/Fri) for 1 h. Live behavior observations, conducted on two consecutive days (Sat/Sun), recorded the frequency of social and stereotypic behavior using continuous observations from 08:00 to 17:00 during wk 0 (prior to exercise implementation), and wk 1, 3, and 6 after exercise regimen began. Daily pen totals were averaged by week and the impact of treatment, week, and their interaction was evaluated using a Generalized Linear Mixed Model (PROC GLIMMIX) with differences evaluated using Least Squared Means with a Bonferonni correction. When an interaction was detected, differences among treatments were evaluated within week. Tongue rolling (TR) differed by treatment ( $P=0.02$ ) and increased over time

( $P=0.01$ ). CON cattle performed fewer TR bouts per day ( $0.3\pm1.6$ ) compared to both DENS ( $9.3\pm1.6$ ) and DENS-EX ( $7.6\pm1.6$ ) cattle. Allogrooming (AL) differed by treatment ( $P=0.005$ ) and increased over time ( $P=0.0002$ ). CON cattle performed fewer AL bouts ( $12.6\pm3.36$ ) than either DENS ( $33.8\pm3.36$ ) or DENS-EX ( $35.5\pm3.36$ ) cattle. An interaction between treatment and week ( $P=0.002$ ) was observed for bar licking (BL) bout frequency. Treatments were similar for BL during wk 0; however, treatments differed during wk 1 ( $P=0.002$ ), wk 3 ( $P=0.005$ ), and wk 6 ( $P<0.001$ ). During wk 1 and wk 3, DENS and DENS-EX cattle performed similar rates of BL and both DENS and DENS-EX cattle performed more BL than CON cattle. During wk 6, all treatments differed in BL with CON ( $4.7\pm3.32$ ) cattle performing fewer BL than DENS-EX ( $23.0\pm3.32$ ) and DENS-EX performing fewer BL than DENS ( $41.3\pm3.32$ ). Irrespective of treatment, daily head butt frequency increased over time ( $P=0.006$ ). Neither treatment nor time impacted mounting or bunk displacement frequency. Confinement stress resulted in the increased performance of oral behaviors including TR, AL, and BL. The implementation of an exercise regimen may alleviate stressors associated with the performance of BL but may not be effective in mitigating the performance of TR or AL rates. These results illustrate that confinement is stressful for cattle and results in an increased performance of stereotypic and social behaviors. Thus, motivation-specific management strategies are needed to mitigate the performance of stereotypic behaviors, and social behaviors should be evaluated in context. Supported by USDA-NIFA #2021-67015-34083.