257 Assessment of commercial Pekin duck welfare: A comparison of methods. Essam Abdelfattah*, Maja Makagon, and Giuseppe Vezzoli, *University of California, Davis, Davis, CA.*

Unlike methods that require the corralling, catching and handling of individual birds (IN), the transect walks (TW) method involves only visual inspection of the flock. During TW, observers walk the length of the barn. TW have been applied to broilers and turkeys. We aimed to assess the feasibility of using TW to evaluate the welfare of commercial ducks by determining 1) whether the TW method produces comparable results with the currently used IN method, and 2) whether the number of transects through the barn affect results of assessments. Six commercial 30-d-old Pekin duck flocks (5850–6300 ducks/flock) were evaluated. One hundred 50 ducks (IN) and 7 transects (TW) were evaluated per flock. During TW, ducks within approximately one meter in front of observer and for the width of transect were scored and recorded. Two observers recorded the incidence of ducks in the following welfare indicators: feather quality (featherless areas <5 cm^2 = FQ1; > 5 cm^2 = FQ2), feather dirtiness (dirty areas <5 cm^2 = FD1; > 5 cm^2 = FD2), blood on feathers (presence of blood = BF), eye condition (dirt or staining around the eye = E1; eyes sealed shut or blind = E2), nostril condition (nostril blocked from inside = N1; nostril opening blocked = N2), and lethargic birds ("sick" appearance = S). Data were analyzed using Proc Mixed in SAS (v.9.4) with barn as experimental unit. Obtained data were checked for observer effect then averaged between 2 observers. Lower proportions of ducks per flock were recorded in the FQ1, FQ2, FD1, FD2, BF, E1, and N1 categories using TW than IN ($P < 0.05$ for all indicators). Observed frequencies of indicators were not different across transect locations within a flock ($P > 0.05$), except for BF ($P < 0.0001$). Bootstrapping analyses (calculated expected mean and SE for different numbers of transects) of TW data revealed that similar results could be obtained using as little as 20% of information (2 transects per house). Method based differences in incidences of FQ and BF have also been reported for turkey flocks. There, the TW (but not IN) results corresponded to whole flock evaluations conducted during load out. It is not clear from our data whether TW or IN yield most accurate results for ducks. However, the similarities with previously reported results suggest that further research into the applicability of TW for on-farm duck welfare assessment is warranted.

Key Words: on-farm welfare assessment, Pekin duck, transect walks, individual sampling

258 Effect of bird density and bedding source on heavy turkey hens: Growth efficiency and litter composition. R. Michael Hulet*[1], Lisa Kitto[1], Sally Noll[3], Darrin Karcher[2], and Marisa Erasmus[2], [1]*Pennsylvania State University, University Park, PA,* [2]*Purdue University, West Lafayette, IN,* [3]*University of Minnesota, St Paul, MN.*

Turkey production efficiency and well-being are thought to come into conflict when bird density is studied. Studies have been sparse looking at variations in densities for heavy hens reared to market weight. Another factor that is affected by bird density is litter characteristics. Litter moisture and available nitrogen can be affected by bedding source. Therefore, a study (3X2 factorial; significant difference at $P < 0.05$) looking at 3 different bird densities (4.2, 5.3, and 7.1 birds/square meter, LOW, MEDIUM, and HIGH, respectively) on either pine shavings (PS) or Giant Miscanthus grass (MG) bedding was conducted. Hybrid poults (1056) were placed into either PS or MG bedding pens (8.18 m^2) with 35, 44, or 58 birds/pen. Birds were weighed at 0, 14, 28, 42, 56, 84, and 98 d of age and growth efficiency data (body weight, feed intake, and feed conversion) were collected. Litter samples were sampled in the pen at 0, 42, and 98 d of age. No significant interactions were found between bird density and litter source. Hens placed at LOW bird density had significantly greater body weight (10.76 kg) over hens placed at HIGH bird density (10.04 kg). Birds placed at MEDIUM bird density were not significantly different in body weight (10.43 kg) than the other 2 densities. Bird density showed no differences in feed intake, but pens with LOW density had significantly lower feed conversion (1.86) than the hens placed at HIGH bird density (1.98). Again the hens placed at MEDIIUM density did not differ in feed conversion (1.91) when compared with the other hen densities. No differences in body weight gain, feed intake, feed conversion nor mortality were found between SP and MG litter sources. However, HIGH density did have greater ammonium content of litter at 42 d and moisture content at 98 d than pens with LOW bird density. In summary, MG litter was equal in bird performance and litter composition to PG Litter. Birds reared at a LOW bird density had better performance than the hens reared on HIGH bird density, but no difference than hen reared under commercial MEDIUM bird density. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2016-67015-24457 from the USDA National Institute of Food and Agriculture.

Key Words: turkey hen, density, bedding source, litter composition

259 Effect of bird density and bedding source on heavy turkey hens: Behavior. Marisa Erasmus*[2], Kailynn VanDeWater[2], Darrin Karcher[2], Sally Noll[3], and R. Michael Hulet[1], [1]*Penn State University, University Park, PA,* [2]*Purdue University, West Lafayette, IN,* [3]*University of Minnesota, St Paul, MN.*

Environmental and management factors, such as the type of litter substrate, can interact with stocking density to influence turkey behavior and welfare. Few studies have examined the effects of stocking density and litter type on turkeys reared to market. This study examined the effects of 3 stocking densities and 2 litter types on the behavior of commercial turkey hens. In a 3 × 2 factorial design, hens were housed in groups of 35 (4.2 birds/m^2, LOW), 44 (5.3 birds/m^2, MED) and 58 birds/pen (7.1 birds/m^2, HIGH) and on either pine shavings (PS) or giant Miscanthus grass (MG) ($n = 4$ pens/treatment). Behavior of turkeys in each pen was video-recorded from 9:00–9:15 a.m. and 3:00–3:15 p.m. on one day at 13 and 14 wk of age (2 d total). From video recordings, 5 focal birds/pen were randomly chosen and the duration and occurrences of behavior (sitting, standing, walking, eating, drinking, feather pecking, preening, and number of disturbances) were determined. Results were analyzed using PROC GLIMMIX (SAS 9.4). Turkeys housed on MG spent less time sitting (137.8 ± 28.2 s, $P = 0.008$) and less time walking (83.0 ± 6.1 s, $P = 0.04$) vs. turkeys housed on PS (sitting: 154.8 ± 31.0; walking: 102.1 ± 7.3). Turkeys housed on MG also stood (21.9 ± 1.6, $P = 0.004$) and walked (11.1 ± 0.9, $P = 0.004$) less often vs. turkeys housed on PS (stood: 27.5 ± 1.6; walked: 15.1 ± 1.5). Turkeys at the HIGH density stood (21.3 ± 1.5; $P = 0.009$) and walked (9.4 ± 0.7; $P < 0.0001$) less often than turkeys at the LOW density (stood: 28.5 ± 1.9; walked: 17.1 ± 1.6). Stocking density affected both preening duration (LOW: 91.7 ± 10.6 s vs. HIGH: 63.8 ± 10.7 s, $P = 0.047$) and number of preening observations (LOW: 4.5 ± 0.4; HIGH: 3.2 ± 0.4; $P = 0.03$). Turkeys housed at the HIGH density (2.4 ± 0.36, $P = 0.03$) also experienced a greater number of disturbances vs. turkeys housed at the LOW density (1.34 ± 0.24). Apart from the number of walking observations (MED: 12.2 ± 0.9 vs. LOW: 17.1 ± 1.6, $P = 0.03$), behavior did not differ between LOW and MED or between MED and HIGH. Results indicate that turkeys on MG were less active compared with turkeys on PS. The highest stocking density resulted in reduced preening behavior and more instances of birds being disturbed by others in the pen, which may be detrimental to turkey welfare. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2016–67015–24457 from the USDA National Institute of Food and Agriculture.

Key Words: turkey, stocking density, litter type, behavior, welfare

260 Effect of on-farm hatching of broiler chickens on welfare and performance. Ingrid de Jong*[1], Sofie Cardinaels[2], Henk Gunnink[1], Kris De Baere[2], Ine Kempen[2], Johan Zoons[2], Theo van Hattum[1], and Lotte van de Ven[3], [1]*Wageningen Livestock Research, Wageningen, the Netherlands,* [2]*Experimental Poultry Centre, Geel, Belgium,* [3]*Vencomatic, Eersel, the Netherlands.*

Farms are increasingly using on-farm hatching systems for broilers. Farmers report improved performance and health in on-farm hatched broilers as compared with broilers hatched at the hatchery. On-farm hatched chickens have immediate access to feed and water which might improve growth and stimulate gut development. We aimed to study whether on-farm hatching indeed positively affects broiler performance and welfare. Broiler chickens (Ross 308 as hatched) arrived from the hatchery and experienced standard hatchery procedures and transport (Control, C) or eggs (similar parent stock) were transported to the experimental farm at d18 of incubation and broilers hatched on-farm (F; using the X-treck system; Vencomatic, the Netherlands). Chickens were housed in pens in climate controlled rooms (±1150 chickens/pen); each room consisted of one C and one F pen. Per production cycle 4 rooms were used and 3 cycles were included in the study (n = 12 pens/treatment). Analysis of Variance or mixed models were used to test for treatment effects; production cycle and age were included in the model. F broilers were heavier as compared with C broilers from d0 up till slaughter (d0 41.3 vs. 47.1 g, sed 0.93, $P < 0.001$; d39: 2792 vs 2729 g, sed 21.1, $P < 0.05$). First week and total mortality did not differ between F and C. FCR1500 g was lower for F than for C broilers (1.038 vs 1.069, sed 0.01, $P < 0.05$) but total FCR did not differ. Litter dry matter% was higher in F than in C pens (62 vs 60% (sed 0.78) for wk 1–5, $P < 0.05$). F broilers had numerically better dropping scores than C broilers (0.56 vs 0.61, sed 0.15, $P = 0.11$). F broilers had less footpad dermatitis at d21 and d35 (backtransformed scores: d21 0.05 vs 0.08; d35 0.48 vs 1.01; $P < 0.001$) and less hock burn at d35 (back-transformed scores: 0.65 vs. 0.92, $P < 0.05$). Gait scores at d21 and d35 were equal for F and C broilers. Dissections at d28 and d39 showed no differences in dysbacteriosis and coccidiosis scores. Measures of (relative) length of gut segments between 1 and 5 wks of age, and villi height, crypt depth and v/c ratio in jejunum at d8, d14 and d21 did not indicate consistent treatment differences.This experiment showed that on-farm hatching improved aspects of broiler welfare and resulted in a higher body weight up till slaughter age. It is likely that early feeding plays an important role in these effects, although we could not find consistent differences in gut development between on-farm and hatchery-hatched chickens.

Key Words: on-farm hatching, broiler, welfare, performance, gut development

261 An intra-lab evaluation of performance in testing tonic immobility in broilers. Diego Martinez*[1], Elizabeth Cisneros[2], Cristian Uculmana[1], Erick Villegas[2], Jorge Tay[2], Fabiola Caqui[2], Ruth Yupanqui[2], and Carlos Vilchez[2], [1]*LIAN Development & Service, Lima, Peru,* [2]*La Molina National Agrarian University, Lima, Peru.*

The experiment was carried out to determine the precision of a trained team in testing tonic immobility time (TIT) in broilers. 240 male Cobb 500 chicks raised from 1 to 42 d were subjected to a Completely Randomized Design under a 2 × 2 × 6 factorial arrangement of treatments. Factors were: 2 dietary protein contents, SPR (standard) and LPR (low), 2 litter materials, NOLM (no material) and RHLM (rice husk litter), and 6 trained technicians, 3 men (YC, YT, YE) and 3 women (XL, XF, XK). Each of the 24 treatments had 10 replications with the bird as the experimental unit. At d 42, each bird was placed face up on a flat surface, restrained for 20 s (s) while standing close and making eye-to-eye contact. TIT was recorded as the time elapsed until the bird righted itself being 600 s the maximum time allowed. Procedure restarted up to 2 times if it was ≤ 10 s. TIT values were natural log-transformed to achieve normality. Variances were analyzed using the GLM procedure of SAS software and average differences were compared using *t*-tests by the LSMEANS option of GLM. The averages presented herein are the natural log-transformed LSMEANS TIT values. No significant main effects were found by technician on TIT (YC = 4.756 vs. YT = 4.596, YE = 4.841, XL = 4.876, XF = 4.618, XK = 4.632) ($P > 0.40$) nor litter material (NOLM = 4.723 vs. RHLM = 4.715) ($P > 0.90$); however, a lower TIT was seen in low protein diets (LPR = 4.605 vs. SPR = 4.835) ($P < 0.03$). No interaction effects were found between technician and litter material ($P > 0.17$), litter material and diet ($P > 0.88$) or among these 3 factors ($P > 0.51$). No difference was found between male and female technicians ($P > 0.20$). Coefficients of variation were low and similar among technicians (YC = 17.31%, YT = 15.95%, YE = 16.56%, XL = 18.85%, XF = 14.19%, XK = 16.80%). The average coefficient of variation of transformed TIT (16.91%) was lower than untransformed TIT (88.93%). In this regard, when data is not normally distributed non-parametric tests can be applied; however, this result indicates that log-transformation to achieve normality also reduced variability, which increases statistical power and test sensitivity. In conclusion, the evaluated team of technicians produced TIT values precise enough to detect differences due to one of the factors measured in the experiment but without differences among technicians. Natural log-transforming TIT values may be useful not only to achieve normality but also to reduce variability and improve statistical power.

Key Words: tonic immobility, variability, sensitivity, precision, stress

262 Effect of methionine restriction and intestinal challenge on tonic immobility in broilers. Diego Martinez*[1], Elizabeth Cisneros[2], Cristian Uculmana[1], Erick Villegas[2], Jorge Tay[2], Fabiola Caqui[2], Ruth Yupanqui[2], and Carlos Vilchez[2], [1]*LIAN Development & Service, Lima, Peru,* [2]*La Molina National Agrarian Unviersity, Lima, Peru.*

To determine the effect of growth rate and sanitary rearing conditions on tonic immobility (TI) as stress indicator, 240 male Cobb 500 broiler chicks were placed from 1 to 42 d in 40 cages (9.9 birds/m^2) and assigned to one of 4 treatments from a 2 × 2 factorial arrangement of treatments with 2 diets (standard diet, STD, according to genetic line nutritional guidelines, and methionine restricted diet, MRD, with 40% lower digestible methionine) at 2 challenge levels (no challenge, NCH, or intestinal challenge, ICH). Challenged birds were raised on rice husk litter and at d 21 were orally gavaged with 3 mL of an orally mixed inoculum of *Eimeria* spp. (≥29 × 10^5 live oocysts/bird) equivalent to 12× the dose of a commercial vaccine (Immucox for Chicken II). Body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), European efficiency index (EEI), carcass yield (CY), breast yield (BY), feather relative weight (FW) and TI were evaluated at the end of the study. A clinical evaluation of intestinal mucosa was also performed on a sample of birds at the end of the study to determine macroscopic coccidia damage. Data were analyzed using a Completely Randomized Design with a 2 × 2 factorial arrangement of treatments and 10 replicates each. TI values (seconds) were natural log-transformed to achieve normality. GLM procedure of SAS was used to analyze variances and average values obtained in each variable were correlated with TI by the CORR procedure of SAS. No interaction effect between diet and challenge was found in any evaluated variable ($P > 0.05$). ICH produced lower BW, BWG, FI, EEI ($P < 0.01$), higher FCR ($P < 0.04$) but showed no effect on CY, BY, FW, TI ($P > 0.40$). No visible lesions were found in the intestinal mucosa. MRD produced lower BW, BWG, EEI, TI and higher FCR (0.01), but no effects on FI, CY, BY, FW were found ($P > 0.09$). In addition, positive correlation coefficients to TI were found for BW (0.32, $P = 0.0310$) and CY (0.33, $P = 0.0274$); however, no other response variable was significantly correlated with TI ($P > 0.05$). In conclusion, severe methionine restriction led to slower-growing birds and lower production efficiency but also lower TI; in contrast, ICH reduced productive efficiency but did not affect TI. Even though the ICH was mild, it was consistent enough to reduce efficiency; however, it was not enough to increase TI.

Key Words: tonic immobility, intestinal challenge, growth rate, methionine restriction, stress

263 Assessing the welfare impact of on-farm euthanasia methods on broilers. Bethany Baker*[2], Stephanie Torrey[1], Tina Widowski[1], Patricia Turner[1], Susantha Gomis[2], Henry Classen[2], Jenny Fricke[2], Tennille Knezacek[2], and Karen Schwean-Lardner[2], *[1]University of Guelph, Guelph, ON, Canada, [2]University of Saskatchewan, Saskatoon, SK, Canada.*

This study used behavioral reflexes and pathology to assess the ability of 3 euthanasia methods to produce insensibility and death in broilers. The methods, cervical dislocation (manual (MCD) or mechanical with Koechner Euthanasia Device (KED)) with n = 30 for each of 7, 21 or 35d, and Zephyr EXL, a non-penetrative captive bolt (Z; n = 30 for 21/35d) were tested. Cull birds were obtained from 5 commercial farms. Efficacy at inducing insensibility and death was measured by time until loss of reflexes post-euthanasia; palpebral blink (PB), nictitating membrane (NM), pupillary light (PL), cloacal winking (CW) and convulsions (CVN). Damage was assessed by scoring pathology; skin tears, separation distance between vertebrae with cervical dislocation (SEP), spinal cord transection, subcutaneous hemorrhaging (hem) on dorsal surface of head, subdural hem, skull fracture, severity and number of vertebral fractures (VF). Reflex data were analyzed as a RCBD (block = farm) using Proc Mixed, and score data were log-transformed and analyzed as an assumed Poisson distribution in a CRD (SAS 9.4). Significance was considered at $P \leq 0.05$. Indicators of insensibility showed a treatment effect with loss of PL and PB occurring fastest in Z, then MCD and lastly KED, at 7, 21 and 35d. Time until loss of NM was shortest with Z usage, then MCD and KED at 21 and 35d. Euthanasia method also affected indicators of death. Time until CW end was shortest with use of MCD, then KED or Z at 7, 21 and 35d. Length of CVN was shortest in MCD, then Z or KED at 7 and 21d. Skin tears were lower with MCD (3%, 3%) than Z (22%, 63%) or KED (46%, 53%) at 21 and 35d respectively. For 21 and 35d, skull fracture, subdural and subcutaneous hem scores were highest with Z use, then MCD or KED. MCD had 98% of spinal cords transected and KED had 81%. SEP was higher with use of MCD vs. KED at 7 (2.97, 1.03), 21 (2.97, 1.40) and 35d (3.00, 1.27). At 7, 21 and 35d the VF severity was highest with use of the KED (1.93, 2.03, 1.83) then MCD (1.17, 1.10, 1.00) or Z (0, 0 for 21 and 35d respectively). The KED resulted in the highest number of VF at 21 and 35d. The results indicate that the Z induces insensibility fastest. MCD is the fastest at inducing death, while the KED took longest to induce insensibility and death, and had the highest scores for severity and number of VF.

Key Words: culling, cervical dislocation, death, insensibility

264 Effect of ventilation shut down (VSD) on changes in heat shock protein 70 and blood chemistry throughout depopulation. Kenneth Anderson*, James Petitte, Kimberly Livignston, Sanjay Shah, Michael Martin, Krista Eberle, and Ramon Malheiros, *North Carolina State University, Raleigh, NC.*

The 2015 highly pathogenic avian influenza (HPAI) outbreak highlighted the limitations of depopulation methods such as CO2 kill carts, CO2 infusion, and firefighting foam to asphyxiate the flock. The objective of this study was to examine the humane aspects and effectiveness of ventilation shut down (VSD) for depopulating laying hens in cage systems by measuring stress physiology via heat shock protein 70 (HSP70) and blood chemistry (BC). Four individual treatment/observation chambers were set up identically for VSD, VSD + Heat, and VSD + CO_2 treatments. For each treatment, 2 trials were conducted. The sampling sequence was time-based, derived from the established time of death (TOD) from a previous study; therefore, hens were sampled at time 0 to establish the baseline for HSP70 and BC, then hens were sampled at 1/4, 1/2, and 3/4 point of the duration to TOD and as close as possible to TOD. The total hens sampled were 30 (5 per treatment). At each sampling point, the hen was removed from the chamber, blood was collected for blood chemistry analysis within 60 s, euthanasia was performed via cervical dislocation, and brain tissue was collected for HSP70 analysis. Blood chemistry was analyzed using the *i*-STAT diagnostic system. The relative expression of HSP70 mRNA in total brain samples was measured using RT-PCR and reported as HSP70/18S. HSP70 data were transformed (HSP70/18S)-1 and analyzed using a one-way ANOVA. The mean comparisons for all pairs used Tukey-Kramer HSD Confidence Quantile ($q^* = 3.76082$; $\alpha = 0.05$). The blood chemistry was analyzed using GLM with means separated using Students-T Test. Relative expression of HSP70 decreased in the VSD and VSD + Heat environments from time 0 to TOD; however, the highest levels were observed with in VSD + Heat and VSD + CO_2 treatments. Blood PO_2 at 18.9 mmHg and Glucose 250.3 mg/dL was higher in the VSD+CO_2 than the VSD treatment. The HSP70 and BC differences may have been related to the speed to TOD than in the other methods. The hen's similar physiological responses to VSD, VSD+Heat, or VSD + CO_2 methods other than the duration of the processes appear to indicate no definitive differences. This would indicate equivalency between the methods as being humane poultry flock depopulation methods.

Key Words: depopulation, ventilation shut down, heat shock protein, blood chemistry

265 High and low feather-pecking lines of laying hens differ in their physiological responses to social stress. Patrick Birkl*[1], Peter McBride[1], Joergen Kjaer[3], Paul Forsythe[2], and Alexandra Harlander[1], *[1]University of Guelph, Guelph, ON, Canada, [2]McMaster University, Hamilton, ON, Canada, [3]Friedrich-Loeffler Institute, Celle, Germany.*

When an unfamiliar hen encounters a stable social group of conspecifics, behavioral and physiological changes may follow. To test whether for genetic differences in response to social stress, 2 lines selected for high (HFP) or low (LFP) feather-pecking activity and an unselected control line (C) were used. We predicted that HFP birds will display more changes in their growth rates; alterations in amino acid ratios indicative of a stress response (phenylalanine: tyrosine, PHE: TYR); and more abnormal behavior (feather pecking) in response to social stress. One hundred sixty laying hens were housed in mixed groups of 16 individuals (HFP, n = 4;LFP, n = 3;C, n = 9;10 pens in total) in floor pens. We disturbed the social order within 5 pens of birds at 16 weeks of age by mixing individuals with unfamiliar birds to simulate social stress (to intensify the simulated social stress, we performed the mixing again after 2 d). Groups were mixed by assigning the 16 individuals into 4 sub-groups of 4. Each sub-group was then transferred into a new pen, wherein they encountered 3 other sub-groups for the first time. Body weight was measured before mixing and 3 weeks after the second mixing, to assess weight-gain. Blood plasma was collected one day before mixing and 2 d after the second round of mixing and was used to determine amino acid concentrations for calculating PHE/TYR ratios. Aggressive pecking and feather pecking were recorded on an all occurrence basis, before mixing (baseline), 2 min after mixing, one hour after mixing, and 24 h after mixing, during 10-min observation periods per pen and time point. Data were analyzed using a GLIMMIX procedure in SAS. We identified that PHE/TYR ratios were different between all 3 lines (HFP:0.75 ± 0.01 vs LFP:0.62 ± 0.01 vs C:0.65 ± 0.01). In HFP birds, PHE/TYR ratios were affected by the social stress treatment, and stressed HFP birds had significantly lower PHE/TYR ratios than unstressed birds (HFP unmixed:0.75 ± 0.02 vs HFP mixed:0.70 ± 0.02, $P < 0.02$). In the short term, social stress did not affect feather pecking, however, weight-gain showed increased variability in stressed groups in comparison to control groups (mixed: 15.1% ± 0.9 vs unmixed: 4.9% ± 0.9, $P < 0.001$). Our results showed that HFP birds responded more sensitively to social stress than LFP or C birds. Also, altered growth of individuals within stressed groups demonstrated a measurable physiological consequence of social stress.

Key Words: social stress, feather pecking, laying hen

266 Effects of infrared beak treatment on early pullet behavior, pecking force, and beak length. Sarah Struthers*, Henry Classen, Susantha Gomis, and Karen Schwean-Lardner, *University of Saskatchewan, Saskatoon, SK.*

The effects of infrared beak treatment (IRBT) on behavior, pecking force (PF) and beak length were examined using Lohmann LSL-Lite (LW) and Brown (LB) pullets. IRBT settings were adjusted to create specific beak shapes: shovel (SHV) (top much shorter than bottom), step (ST) (intermediate differentiation), standard (STAN) (small differentiation) and a sham (C) untreated control. Treatments (trt) were arranged in a 4 × 2 factorial design, in a completely randomized design. Birds (n = 160) were housed in cages (8 reps per IRBT and 16 reps per strain). Photographs of 10 beaks per IRBT × strain were taken at 0 and 4wk for beak length calculation. PF was tested at 1, 2, 3, and 4wk using 4 birds per IRBT × strain. Ten birds per IRBT × strain were video recorded from 9 to 23d for 8h per day, then behavior analyzed using scan sampling at 15-min intervals. Mortality was recorded daily. Data were analyzed using Proc Mixed (SAS 9.4), with Tukey's test to separate means. Differences were significant when $P \leq 0.05$. IRBT did not alter the force with which a bird could peck or mortality. C birds spent significantly more time at the feeder than ST and STAN birds at 9, 11, 13, and 15d. C birds spent less time at the drinker than ST birds at 9, 11, and 17d. At 9d, C birds spent less time standing than STAN birds (26 vs. 32% of time). IRBT did not affect other behaviors. Differences in bottom beak length were significant at 4 wk with C birds having longer beaks than SHV, ST, and STAN birds (10 > 9 > 8 > 7mm). Strain had a significant effect on drinking at 13, 19, and 21d, with LW spending more time at the drinker than LB. Strain had an effect on standing, with LW standing less than LB at 9d (29 vs. 34% of time) and at 19d (35 vs. 37% of time). Mortality from 0 to 4wk was higher for LW compared with LB (3 vs. 0.3% of birds placed). An interaction was seen between trt and strain on PF at 4wk. For STAN trt, LB pecked with more force than LW. An interaction was seen for standing at 11 and 21d. At 11d, LW C trt stood less than other trt. At 21d, LW SHV trt stood more often than C trt. Overall, the results suggest that IRBT does not negatively impact PF and is effective at shortening beak length. The results also suggest that IRBT may alter behavior slightly in early life, with treated birds spending more time at the drinker or standing, and less time at the feeder.

Key Words: Lohmann, shovel beak, mortality, activity

267 The unforeseen consequences of a "bad hair day" in laying hens. Irene Campderrich, Guiomar Liste, and Inma Estevez*, *Neiker-Tecnalia, Vitoria-Gasteiz, Alava, Spain.*

Genetic selection and management practices are designed to promote laying hen performance and flock homogeneity. However, injuries, disease, feather pecking, or delay in egg laying can alter the phenotypic appearance of some individuals. These birds may attract a higher level of aggressive interactions, sometimes with fatal consequences. The present study assessed the effects of the artificial manipulation of phenotypic appearance (PA) in groups of adult Hy-line Brown laying hens reared at 3 different group sizes (GS: 10, 20 or 40; 8 birds/m^2). PA was altered in a proportion of birds (0, 30, 50, 70 or 100% in each GS) at 1 d old by applying black dye to feathers at the back of the head. This treatment resulted in homogeneous groups (100U, 100M) in which all group-members had the same PA, marked (M) or unmarked (U), and heterogeneous groups (30M/70U, 50M/50U, 70M/30U) where the 2 phenotypes (M and U) coexisted in the same pen at different proportions. The groups remained unchanged until 33 weeks of age (T0). Homogeneous groups were then sequentially altered by progressively marking or un-marking 30, 50 or 70% of hens at 34 (T1), 38 (T2) and 44 (T3) weeks of age. Aggressive interactions in altered groups at T1, T2, and T3 were compared with those observed in heterogeneous groups that remained unchanged from d 1 and were used as controls. No differences were detected across PA and GS treatments for T0 ($P > 0.05$). However, when PA was sequentially altered in homogeneous groups (100M and 100U) at T1, T2 and T3, recently altered groups presented higher aggression rates than their controls ($P < 0.05$, all cases). Aggression decreased as the proportion of altered birds increased (from 30% to 70%). No effects of GS were found except at T3, were GS 40 presented higher aggression rates than GS 10 ($P < 0.05$). Aggressive interactions in altered groups were directed from unaltered toward recently altered birds, irrespective of their original PA. This directionality was observed at T1 and T2 but disappeared at T3 when aggression levels diminished considerably. These results show that a sudden change in PA can seriously alter group dynamics, leaving altered birds exposed to aggressive attacks even when in small groups, where the assumption of a stable social system and hence low aggression is presumed.

Key Words: laying hen, phenotypic appearance, aggressive interactions, group size, social dynamics

268 Do laying hens avoid dirty scratch pads in enriched cages? Bishwo Pokharel*, Luxan Jeyachanthiran, Ilka Boecker, and Alexandra Harlander, *University of Guelph, Guelph, ON, Canada.*

Enriched cages for laying hens provide scratch pads for foraging on the wire mesh floor. Not only do birds forage on scratch pads, they also defecate on these pads causing them to become dirty. How a bird perceives a dirty scratch pad and what this means to them from their point of view has never been tested. The aim of the present study was to determine the relative preference for clean or dirty scratch pads of laying hens. In our study, a total of 288 laying hens were housed in 16 enriched cages (18 hens per cage), with each cage having 2 identical compartments joined by a pop hole to allow free movement of hens between compartments. On a daily basis, half of the scratch pads [piece of artificial turf] (1 in each compartment, which accounted for approximately 14% of the total cage surface) was removed and cleaned, while the other half was cleaned and then covered with 550 g of conspecific feces. Clean (C) and dirty (D) pads were then put back into the cages in a random order, avoiding side bias. Feed as litter substrate (~5 g per delivery per scratch mat per hour) was delivered automatically onto the scratch pads by a spiral conveyor pipe. After identifying the time when hens were most active using video recordings (mid-day), the number of visits and the total time spent scratching/foraging on C and D pads were video recorded for 10 min/day, 3 times a week, over a period of 4 weeks. The observation period started when the feed was delivered on each scratch pad. Laying hens showed a relative preference for D scratch pads. Birds visited more frequently ($P < 0.01$) and spent more time foraging on the D than on the C scratch pads. Interestingly, laying hens did not avoid dirty (D) scratch pads. Scratching/foraging on D scratch pads to get feed required more effort to obtain the feed and might explain why laying hens kept in enriched cages clearly showed a relative preference for foraging on scratch pads where feed was delivered on top of the feces (D pads).

Key Words: cleanliness, foraging, preference, laying hen, scratch pads

269 Keel bone damage: The role of behavior and impacts experienced at the keel. Maja Makagon*[3], Sydney Baker[3], Cara Robison[1], Darrin Karcher[2], and Michael Toscano[4], [1]*Michigan State University, East Lansing, MI,* [2]*Purdue University, West Lafayette, IN,* [3]*University of California, Davis, Davis, CA,* [4]*Research Centre for Proper Housing: Poultry and Rabbits (ZTHZ), Division of Animal Welfare, VPH Institute, University of Bern, Bern, Switzerland.*

In addition to raising animal welfare concerns, keel bone damage (KBD) in laying hens can increase hen mortality, and decrease egg quality, and carcass value. We aimed to determine the causes of KBD - fractures and deviations - in laying hens housed in enriched colony systems. We evaluated 1) the impact energy experienced at the hens' keels as they navigate their environment, 2) behaviors and cage locations associated with these impacts, and 3) how impacts and behaviors relate to KBD development. Data collection focused on 10 of 60 Hy-line W-36 hens housed in each of 12 environmentally enriched cages (4 rooms of 3 cages). Changes in keel bone integrity was evaluated for each of the 120 focal hens from CT scans taken at the start and end of 2 3-week data collection periods (4 scans per hen). The impacts sustained at the hens' keels were evaluated using tri-axial accelerometers; behavior was transcribed from video recordings made continuously over the course of the study. In total, 14,516 impacts were recorded. Of these, 52.37% had summed accelerations under 20G, 22.27% between 20 and 40G, 9.3% 40–60G, 4.94% 60–80G, 3.03% 80–100G, and 7.99% > 100G. Video recordings were matched with 7,887 impacts. The majority of impacts under 20G were linked with maintenance behavior (e.g., grooming), unlikely to cause major KBD. Collisions accounted for 80.22% of impacts with summed accelerations over 20G. Aggressive interactions among hens, scattering behavior, grooming and wing flapping accounted for 10.48%, 3.20%, 3.06% and 2.48% of impacts over 20G, respectively. Hens collided with the perches (74.35%), the wire floor (11.44%), other hens (6.92%), support beams (4.14%), the feeder (2.09%) and cage walls (1.06%). Collisions were most often the result of hens trying to navigate onto a perch, or being pushed by a cage mate. Binary logistic regressions (R, v.3.3.2) were used to determine the relationships between keel bone damage, number of collisions, and number of impacts < 20G experienced at the keel. No relationships were found, though statistical trends were found between the number of collisions a hen experienced and the incidence of keel bone fractures ($P = 0.061$) as well as the incidence of overall damage ($P = 0.054$). We conclude that issues pertaining to perch navigation are a key risk factor for keel bone damage, particularly the development of fractures, sustained by laying hens housed in enriched colony cage systems.

Key Words: keel bone, accelerometer, laying hen, behavior, welfare

270 Two-dimensional space use by 4 genetic strains of laying hens in an aviary system. Ahmed Ali*[1], Elizabeth Riddle[1], Dana Campbell[2], and Janice Siegford[1], [1]*Michigan State University, East Lansing, MI,* [2]*Armidale, NSW, Armidale, New South Wales, Australia.*

The laying hen industry is implementing aviary systems intended to improve welfare by providing hens with more space and resources to perform species-specific behaviors. However, knowing how much space hens need to perform the various behaviors encouraged by aviary resources is necessary before determining stocking densities for new housing systems. To date, limited research has examined spatial requirements of laying hens for performing key behaviors or differences in space use between laying hen strains, and none has been conducted within an alternative housing system. This study investigated the amount of space used by 4 strains of laying hens (Hy-Line Brown [HB], Bovans Brown [BB], DeKalb White [DW], and Hy-Line W36) to perform 5 different behaviors in the litter area of a commercial-style aviary. Ceiling-mounted video cameras recorded hens standing [S], lying [L], perching [P], wing flapping [WF], and dust bathing [DB] on the open-litter area that included an outer perch, from 12:00 to 14:00 at peak lay (28wk). For each behavior, 16 hens per strain (4 hens/unit × 4 units/strain). Still images were captured from video footage, digitized, and analyzed using ImageJ software. Maximum hen length and width were measured with ImageJ and used to calculate total area per hen for each behavior. For DB and WF, several measurements were taken throughout the behavior but only maximum hen length and width were used. Space use (area) was compared among strains for each behavior using ANOVA in R 3.3.1. HB and BB hens required more space for S, P, and L than DW and W36 ($P \leq 0.05$), while DW and W36 used more space during WF and DB than HB and BB ($P \leq 0.05$). On average, brown hens required 100cm^2 more space for S and 87 cm^2 more space for L compared with white hens. Whereas, on average white hens required 796 cm^2 more space for WF, and 190cm^2 more for DB than brown hens. Hens of all strains were, on average, wider while perching than the commonly used standard of 15cm (DW: 18.3; HB: 22.2 cm), and brown hens required 3.7cm more perch space than white hens. Overall, brown hens weighed more (HB: 2.0, BB: 1.9, DW: 1.6, W36: 1.5 kg) and physically occupied more space in static postures P, S, and L. However, white hens used more space to perform dynamic behaviors WF and DB. Differences in space requirements between brown and white hens should be considered during the planning and stocking of laying hen facilities.

Key Words: laying hen, welfare, behavior, space, strain