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Research Report

Use of location tracking to link egg production with toe pecking injuries, feather quality, body mass in laying hens

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SUMMARY

The current study sought to validate a proxy for egg laying that could be used within cage-free housing systems towards breeding programs of laying hens. Once validated, the proxy was then assessed against several common health and welfare traits (plumage, toe injuries, and body mass) to determine whether a relationship existed. We also estimated the heritabilities of the health traits and performed genome wide association studies to identify potential candidate gene, aiming to determine whether these phenotypes could be utilized in future breeding programs. Our effort used unique parental crosses with known pedigrees that allowed data within the cage free housing system to be compared with parallel family groups maintained in cages at the facility of our industrial partner. Findings indicated that the proxy of egg production was highly correlated with the production records of birds in cages. We also found that plumage in the cage-free housing system was positively related to the egg production proxy, whereas toe injuries were negatively related. We believe the findings support the position that phenotyping within cage-free housing systems can be a valuable component in efforts to breed hens that perform well in these systems.

Description of problem

Cage-free housing systems (CFHS) provide superior welfare over cage housing systems by offering a rich array of resources such as secluded nesting areas and litter for dustbathing that allow animals to express their natural behaviors (Blokhuis et al., 2005; Weeks and Nicol, 2006). Despite the clear superiority of CFHS regarding behavioral freedom, many unintended problems exist that severely compromise the actual health and welfare of housed animals. The problems vary widely in impact and range across many themes including those of a behavioral (e.g. feather (Cronin and Glatz, 2020) and toe (Gebhardt-Henrich et al., 2023b) pecking, piling (Gray et al., 2020; Winter et al., 2021)), physical (e.g., keel bone fracture (Harlander-Matauschek et al., 2015)), and disease (Hartcher and Jones, 2017) natures. The widespread and continued occurrence of these issues across the producers and nations that were early adopters of CFHS are a testament to the difficulty in resolving the problems and the limitations of available solutions. While adaptations of housing and management are and will remain essential in resolving these issues, a central problem rests with breeding methods that are poorly suited to CFHS. Current breeding paradigms for laying hens use cage housing systems with animals housed individually or in small groups. Such programs benefit traits that perform well within small groups of hens. Unfortunately, it is often difficult to use the same phenotyping methodologies to evaluate hens within large-scale, CFHS. As a consequence, while the applied methods have effectively delivered the high productivity of modern commercial operations, the available genetic hybrids are sub-optimal for CFHS leading to the discussed poor health and welfare. More simply, the current tools available to the providers of laying hen genetics do not allow desirable phenotypes to be observed, quantified, and selected for or against.

To overcome these challenges, phenotyping efforts need to take advantage of advances in sensor technology and computing (Siegford et al., 2016) towards breeding evaluations within large scale, CFHS (Ellen et al., 2019). Our group has previously been able to demonstrate dramatic variation in laying hen movement behavior using a commercial aviary outfitted for research (Rufener et al., 2018; Montalcini et al., 2023b; 2023a) with metrics that link to health states such as keel bone fractures (Rufener et al., 2019a; Montalcini et al., 2024). By combining traditional phenotyping with sensor-based tracking technology in specialized cage-free housing, our effort can provide the required

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information to identify appropriate phenotypes that can be used in breeding evaluation to yield hens that can perform robustly in CFHS.

The effort undertaken with this research focused on a specific behavioral routine (assessed via sensor technology that tracked the movement of individual hens) which we believe reflects egg laying. Although previous efforts were able to link production and movement by giving focal birds fat soluble capsules and then tracked (Rufener et al., 2019b), the technique was fairly invasive. In contrast, the current work provided far more detailed and extensive (i.e., daily) longitudinal records of egg laying. By using unique families of hens as part of a broader phenotyping effort, we attempted to validate our proxy of egg laying by comparing individual movement data from hens on our site with actual production data from cage-housed hens of the same families. We also compared the daily estimated total of our proxy with the actual pen-level data. Once the behavioral proxy was validated, we then related the proxy to multiple common health traits as a novel means to determine how hen health and productivity are related within CFHS. Although we do not intend to provide a causal link between egg laying and health status, we discuss potential mechanisms for future investigations. We also included genomic data of the laying hens to estimate genomic-derived heritabilties of the health and welfare traits to determine their utility for breeding.

Materials and methods

Ethical statement

The experiment was approved by the Veterinary Office of the Canton of Bern (BE4/2021) and met all cantonal and federal regulations for the ethical treatment of laboratory animals.

Animals and management

The current experiment was part of a larger experiment with materials and methods previously reported (Makanjuola et al., 2024) but reproduced here with slight adjustments as needed. The hens used in this study were provided by Hendrix Genetics (5831 CK Boxmeer The Netherlands) and comprised 1,125 white laying hens. The hens arrived as hatchlings from crosses of pure lines on the site of the Aviforum, Switzerland. Each chick wore a wing tag that indicated the sire. The chicks were placed into eight pens with 600 animals each in an on-site rearing barn, stratified for sire with 100 sires in total. Four pens of the rearing barn contained the rearing aviary Landmeco Harmony, Landmeco A/S, Olgod, Denmark (4.89 × 4.55 m) and the other half had the rearing aviary Inauen Natura, R. Inauen AG, Appenzell, Switzerland (4.86 × 3.92 m). At approximately 11 weeks of age (WOA) they had access to a wintergarden with perches during daytime. Just before transfer to the production barn, at approximately 17 WOA, 1125 pullets from 25 of the 100 sires were fitted with a passive RFID tag (125 kHz) and a pen-specific color leg band. The allocation of pullets to five pens with 225 animals/pen in the production barn was stratified for sire and pen in the rearing barn and resulted in approximately 40 hens per sire across the five pens. At 18 WOA, the hens were transported to a production barn with a Bolegg Terrace aviary (Vencomatic Group, 5521 DW Eersel, The Netherlands). The entire barn was divided into 20 replicated pens with each containing 225 hens. The aviary system was a three-tiered aviary consisting of a top-level tier (TLT), nest box tier (NBT), and lower-level tier (LLT). In addition, the aviary had a floor littered area (LIT) and an attached wintergarden area (WG). The area of the litter immediately under the aviary as well as the entire WG were accessible from approximately 21 WOA onwards.

Feeding, vaccinations, duration of light, and other management procedures followed common guidelines and instructions for the Dekalb White hybrid. The maximum length of light was 15 hours from 0300 to 1700 and the natural light through windows was supplemented by artificial light, whereas access to the WG was between 10:00 and 16:00.

Recording locations

To monitor the (1) frequency of passage between and (2) duration of time each hen was located in the earlier defined zones of the aviary (i.e., TLT, NBT, LLT, LIT, WG), a Radio Frequency Identification System (RFID) (Gantner Pigeon Systems GmbH, 6780 Schruns, Austria) was used. Thirty-two 12-field SPEED antennas (75 × 35 cm) were distributed on the edges of all tiers on both sides of the aviary, in two rows on the littered floor, and either side of the pophole passage leading to the wintergarden. A previous set-up with only two rows of antennas on the floor was validated (Gebhardt-Henrich et al., 2023a).

Activity monitoring of the birds began five days after arrival in the aviary. The delayed start was to ensure that the hens adapted well into their unfamiliar environment and that the signals generated from the RFID were working appropriately. The last daily record collected from the birds was on day 290 (approximately 59 WOA) from the first day of arrival in the aviary. Duration of time was recorded in tenth of seconds. Birds that left a particular tier and returned later without being recorded in another location would be falsely attributed a wrong (i.e., longer) duration on that tier. Thus, all time duration records that exceeded 900 and 360 min of time spent in the different tiers and wintergarden, respectively, were removed from further analyses. In total, 426 (0.25 %), 619 (0.27 %), and 24541 records (10.8 %) were deleted due to an excessive duration in the WG, LIT, and TLT, respectively. Subsequent efforts from our group using a similar antennae setup quantified the non-detection period at 21 % of the lighted period (Perinot et al., 2025). Moreover, animals that died during the study (14, Table 1) or lost their RFID tags (4) were removed (1.3 %). Twenty-one days of unusual disturbances (e.g. catching birds for this or other projects in the barn, visitors, first registration after 05:00 or last registration before 16:00) were deleted (4.9 % of all records). In total, 1,108 animals with 937,740 daily collected movement records were retained for the data reported here.

As a proxy for egg-laying, we established criteria of a visit to the nest box tier of at least 15 min, but not longer than 90 min during the first eight lighted hours until 24 WOA. After 24 WOA, the criteria were a visit of at least 20 min but not longer than 90 min during the first six lighted hours. The proxy is based on consultation with Hendrix Genetics (Teun van der Braak, personal communication) as well as our own observations for patterns of use that manifest short and specific visits to the NBT at this time at regular diurnal intervals (Rufener et al., 2018; Montalcini et al., 2023b; 2023a). No further processing of RFID records were performed beyond the total durations for each zone mentioned above and the proxy of nestbox visits. We further validated the measure by correlating estimated egg production by proxy with actual total egg production within each of the five pens. The comparison with the actual pen-level production led us to exclude one day of a gross overestimation of the number of eggs and the deletion of weekend days

Table 1
Mortality of the 1115 birds across five pens.

Pen	Sire	Week of age	Cause
1	51	18	unknown
3	11	22	unknown
5	36	22	unknown
4	36	22	unknown
5	80	28	salpingitis
2	36	29	euthanasia due to injury
5	95	31	unknown
2	14	34	unknown
3	14	37	euthanasia due to weakness
3	58	42	unknown
5	88	44	unknown
1	26	48	euthanasia due to weakness
2	60	54	unknown
3	36	56	unknown
4	26	56	unknown

because egg numbers were significantly overestimated relative to weekdays (contrast weekdays vs. weekends: $F_{1,24} = 4.29$, $P = 0.049$).

Health assessments

All study birds were assessed at 40 and 60 WOA for toe injuries, plumage condition, and body mass by the same two people. Each person underwent reliability testing by scoring the same 10 hens independent from each other. Each health record included the assessor, hen identification code, as well as the health score. Severe toe injury and poor plumage condition at 40 WOA were too infrequent (i.e., almost all birds had excellent coverage) so were not analyzed further. Toe injuries and footpad health were scored on tagged visual analogue scales with photographs of toe lesions of varying severity and yielded a continuous variable between 0 and 100. The system for each was developed by our group in preparation for this effort. Scores above 49 were considered major and classified as the binary outcome of the variable toe injury. Plumage was assessed using a visual tagged analogue scale (continuous, 0–100) using photographs of white laying hens (adapted from Tauson et al. (2005) and took the complement to 100 so that higher scores are indicative of poorer feather condition (score 1: approx. 100–76 depending on the extent of damage; score 2: approx. 75–51; etc.) for each body part. Included areas were: breast, wings, cloaca, back, and neck. We combined all areas into a single value though also analyzed the breast as a separate variable as it had by far the greatest variability (Table 2) of all body areas. The repeatability for the continuous measure of toe injuries was 0.54 (95 % confidence interval (CI): 0.17, 0.73) and for the binary measure of severe toe injuries 0.82 (CI: 0.04, 0.94). The repeatability of plumage was 0.81 (CI: 0.35, 0.89). The repeatability for breast plumage was 0.88 (CI: 0.70, 0.92). Body mass was also measured for each bird by hanging it upside down from a shackle attached to a digital scale (Bat1, Veita Electronics, Czech Republic).

Field test comparisons

Field test data from hens of the same Pure Line matings (spread over two separate sites or replicated tests, one and two), but maintained in cage housing systems by our industrial partner (Hendrix Genetics) were accessed for comparison. Associated hens were housed with a combination of half and full sibs and a single male (i.e., the father) using cages containing eight or six hens per cage for tests one and two, respectively. The number of cages per sire was dependent on the number of daughters but varied from two to seven. Data included daily egg production from which total production up to 24 WOA and until 60 WOA, plumage condition (from the back only per Hendrix's internal protocol), and weekly mortality. Egg production data for the CFHS were adjusted to account for days of missed tracking on account of health assessments by adding six eggs to the total. Weekly mortality was not compared with the field tests because of too few incidences.

Table 2
Assessments of plumage, and footpad and toe health at 40 and 60 weeks of age (WOA). Toe injury and footpad health were measured using a visual tagged analogue scale developed by our group for this project. Feather quality was also assessed by a visual tagged analogue scale using photos adapted from Tauson et al. (2005). For plumage at the different body parts, 0 was the worst (no feathers) and 100 the best. For footpad and toes, 0 was the best (no damage) and 100 the worst. Body mass is given in g. STD – standard deviation.

Trait	Mean 40 WOA	STD	Mean 60 WOA	STD
Neck	94.3	12.4	66.77	25.8
Back	99.8	1.9	98.9	6.7
Wing	89.8	13.6	80.3	15.2
Tail	83.1	15.4	70.1	15.6
Cloaca	99.5	2.8	95.3	12.9
Breast	71.5	20	50.2	18.9
Plumage	538.7	35.7	461.3	56.6
Footpad	11.3	16.7	6.7	14.4
Toes	11.4	15.3	13.1	18.5
Mass	1727.8	110.6	1755.5	138.1

Genotype data

All birds alive at 30 WOA were genotyped using a proprietary 60 K SNP panel (Illumina Inc. 60 K). For the genotype quality control, only autosomal SNP markers with call rate greater than 0.95 and a minor allele frequency (MAF) greater than 0.05 were retained. After quality control, a total of 40,563 genome-wide SNPs were used for the downstream analyses. Genome-derived heritability estimates and genome-wide association studies (GWAS) of the health and welfare traits were performed using a polygenic model, as implemented in the R-package GenABEL (Aulchenko et al., 2007), accounting for assessor and pen as fixed effects in addition to the identical by descent derived kindship matrix. After quantifying heritability for the selected traits, the p-values of each SNP were extracted using *mmscore* procedure, whilst we considered genome-wide significance with p-values below the 5 % Bonferroni-corrected threshold for 40,563 independent tests ($p_{BONF} < 1.23 \times 10^{-6}$). Visualization of GWAS results were done with Manhattan and quantile-quantile Q-Q plots to account for the inflation of small p-values, hinting at false positive association signals. The effect of the best-associated SNP on the trait was evaluated graphically with boxplots to visualize differences in the trait according to the genotype information. Furthermore, we investigated which genes were located next to significant SNPs using the NCBI Genome Data Viewer, based on the Gallus_gallus-5.0 reference genome assembly. Candidate gene functions in human, chicken, and other livestock species were investigated in current published peer-reviewed literature. We have also assessed the heritability of movement using this dataset (Makanjuola et al., 2024) and candidate gene analysis (Hoeksema et al., 2024). The dataset was also used to consider genomic factors associated with keel bone fracture (Duenk et al., 2024).

Statistical Analysis

For all statistical analyses, only the 25 selected sires were considered. Nine hens falsely selected from eight sires outside the experiment were excluded from the dataset. The general(ized) linear models were analyzed with Proc Glimmix in SAS 9.4 (STAT 15.1). Residuals were plotted and checked. Random effects were tested with the Wald statistic for significance. Comparison of our egg production proxy and actual field test data from the genetic companies were made with a linear model using the proxy as the response and the field test data and test number as predictors. Health data was related to our egg production proxy for total production, but also 50 to 60 WOA as it was assumed that periods closer to the health assessment would be more relevant.

Toe pecking leading to severe toe injuries occurred in mainly three out of five pens (Table 3). In the analysis of the binary trait of severe toe injury (above the median of the scale meaning broken skin with evidence of current or recent bleeding), no pens were excluded but pen was not included as a factor. Instead, sire was used as the random subject variable. In that analysis, birds with severe toe injuries were compared with birds without toe injuries excluding animals with minor lesions.

Table 3
Number of birds (% in brackets) with severe toe injuries involving past or current bleeding and those without any toe injuries in the five pens of the study. The last row shows the total number of hens in the respective pens including birds with minor toe injuries.

Pen	1	2	3	4	5
None	23	129	45	166	123
Toe injury	10 (30.3)	9 (6.5)	25 (35.7)	0	3 (2.4 %)
Total birds	208	215	218	218	213

The plumage / breast plumage at 60 WOA was used. All birds were included that were alive at 60 WOA including those with extremely low egg production. The statistical model was:

Estimated total egg production until 60 WOA = Health measure + $Sire_i + Pen_j + Rear_k$ + all two-way interactions + $error_{ijk}$ as defined by: health measure – either plumage (total, breast, or cloaca), toe injury, or body mass

Sire – sire, $i = 1$ to 25

Pen – pen in the production barn, $j = 1 – 5$

Rear – pen in the rearing barn, $k = 1 – 8$

We assessed movement data against our proxy for egg production by comparing each individuals': median total daily durations and frequencies of visits per day in the different zones (i.e., TLT, NBT, LLT, LIT, WG)), the tier with the greatest duration, the mean height per day (i.e. using the tiers with values of 3, 2, 1, and 0 for the TLT, NBT, LLT, LIT, respectively), as well as total number of daily transitions between 25 and 290 days in the production barn. The stated variables were also combined using Principal Component Analysis (PCA) of PROC Factor and minimum eigenvalues =1 and varimax rotation as selection criterion. When toe injury as a binary variable was used, egg production was logarithmically transformed after inspection of the residual plots. The repeatability of health scoring was calculated using the linear mixed-effects model "rpt.remlLMM" in R (Nakagawa and Schielzeth, 2010; Stoffel et al., 2017). All data and code, including with explanatory Readme file, are available at www.doi.org/10.17605/OSF.IO/9DW3Q.

Results and discussion

Comparisons with field test data

For the proxy of egg production in the period up to 24 WOA as a response variable, both field test data and test number were effective predictors (field data: estimate of the production: 0.78 ± 0.13, $t_{47} = 5.81$, $P < 0.0001$; $F_{1,47} = 33.8$, test: $F_{1,47} = 18.2$, both $P < 0.0001$; Pearson correlation coefficient: Field test 1: $r_p = 0.67$, Field test 2: $r_p = 0.62$) (Fig. 1). In examining the proxy for total egg production up to 60 WOA, only the number of field test eggs was an effective predictor ($F_{1,46} = 11$ $p = 0.002$) (Fig. 2). The correlation in both these conditions indicates that our egg production proxy could serve as an accurate prediction for actual egg production within CFHS and provide benefits towards general phenotyping efforts. Current methods of assessing egg production in individual hens using tracking systems are generally available, albeit not widely, for hens within CFHS (Thurner et al., 2006; Icken and Preisinger, 2009; Icken et al., 2010, 2011, 2013a; 2013b; Heinrich et al., 2014; Li et al., 2017; Bécot et al., 2023). However, these systems typically require that hens use the nestboxes singly, i.e., one hen at a time. When a hen is in the single nestbox, the laid egg is detected (e.g., rolling into a column where it can be registered) and linked with that particular hen. The hen can then leave the nestbox and allow another single hen to enter. These systems are fairly reliable and likely meet many of the industry's phenotyping needs. However, the required use of single nests represents a large departure from standard group-housed nestboxes with likely differences in nesting behavior. For instance, Heinrich et al. (2014) compared Lohmann Selected Leghorn and Lohmann Brown in a funnel nest system that identified individual hens with a family nest to compare nest visits and durations. Although nest visits were comparable, the Leghorn birds had nest durations that were 40 % higher in the family nests (i.e., 42 vs. 69 min). To the best of the authors' knowledge, no system exists that allows laid eggs to be linked with the individual hens without requiring a nestbox for single hens. Given the evidence that hens in large groups appear to move in a coordinated fashion (Sibanda et al., 2020; Gómez et al., 2022; Perinot et al., 2025) within CFHS and exhibit gregarious nesting (Appleby et al., 1984; Riber, 2012; Ringgenberg et al., 2015), the effect of different housing should not be a surprise. Although we are not suggesting that our nestbox proxy replace use of single nestboxes for the collection of phenotypic data, we believe our behavioral proxy represents a phenotype that can be used to complement directly collected, individual level, egg laying behavior. Furthermore, alternatives and adjustments to the proxy (e.g., when the hen reaches half of the time in the nestbox between 0200 and 0800, see Montalcini et al., 2023a) including the duration of visits, time window in which they are considered, and other key factors should be considered to improve accuracy.

Egg production before and after 24 WOA

Egg production after 24 WOA was linked to egg production before 24 WOA (early production) and sire. The designated pens in the rearing and the production barn were not important (early production: $F_{1,163} = 7.33$, $P = 0.008$; sire: $F_{24,16} = 2.75$, $P = 0.02$, pen during rear: $F_{7,6} = 1.25$, $P =$

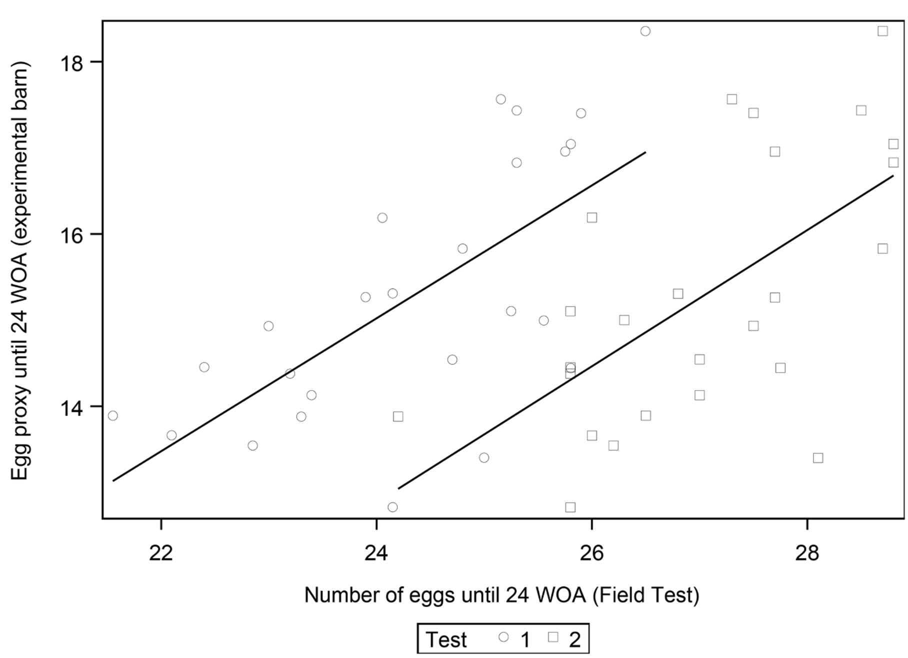

Fig. 1. Plot of number of eggs before 24 WOA in the field tests against our estimate of the number of eggs before 24 weeks of age using our egg laying proxy.

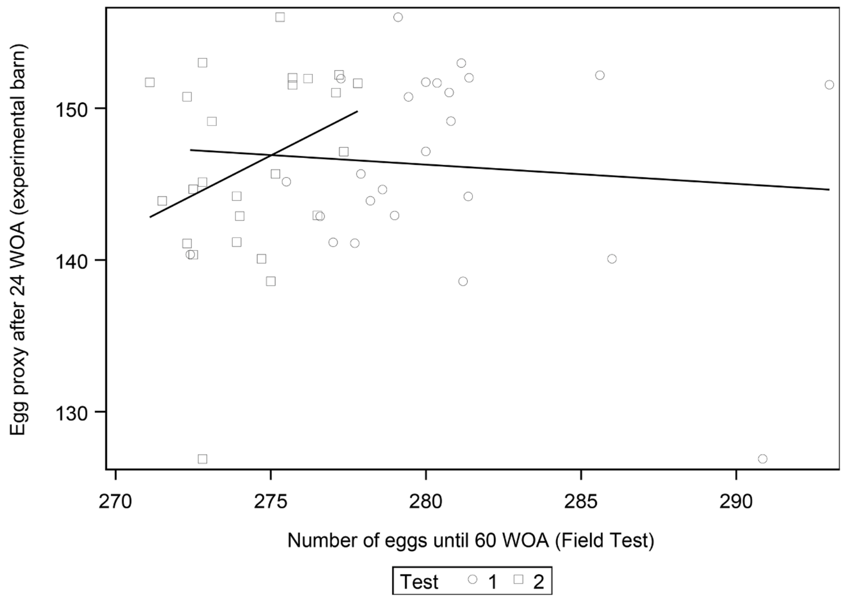

Fig. 2. Plot of number of eggs total egg production until 60 WOA in the field tests against our estimate of the number of eggs before 60 WOA using our egg laying proxy.

0.40, pen during production as random factor: $Z = 0.53$, $P = 0.30$).

Linking health status and egg production proxy

Compared to actual egg production as a phenotype, the egg production proxy is likely beneficial in helping to explain the relationship between productivity and health status. In large commercial groups housed in CFHS, longitudinal observations of individual hens are generally difficult (Siegford et al., 2016; Rodenburg et al., 2019), preventing estimates of egg production and health status. As a consequence, it is difficult to determine how egg production and health status are related. Our effort, although conducted within quasi-commercial conditions, provides results that these processes are associated and meaningful. We identified a weak negative correlation between toe injury at 60 WOA and the total egg production proxy between 50 and 60 WOA, i.e., greater toe injury was associated with reduced egg production during the 10 weeks prior to the assessment of the toes based on our proxy (estimate $= -0.002$, SE$=$ 0.001, df $= 486$, $t = -2.29$, $P = 0.02$). Focusing on birds with severe injuries vs. birds without any toe lesions, birds with severe injuries laid fewer eggs in the period between 50 and 60 WOA (toe injuries: $F_{1,17} = 6.3$, $P = 0.02$; sire: estimate $= 2.1 \pm 0.93$, $Z = 2.26$, $P = 0.01$) (Fig. 3). Toe injuries are a significant welfare problem in white hybrids (Gebhardt-Henrich et al., 2023b), so these findings are relevant to commercial situations. Our previous study (Gebhardt et al., 2023b) examined possible risk factors, though there are no definite explanations as to the cause.

In terms of plumage, there was a weak positive correlation between overall plumage at 60 WOA and total egg production by proxy until 60

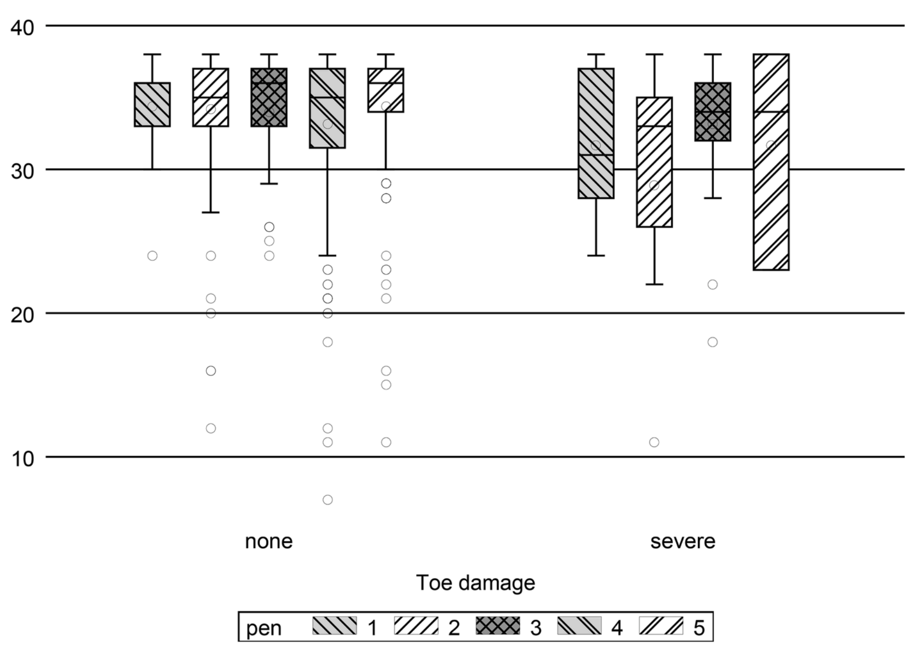

Fig. 3. Egg production based on our behavioral proxy for 50-60 WOA in relation to sire for hens with (right) and without toe injuries at 60 WOA. There were no birds with severely damaged toes in pen 4.

WOA (estimate = 0.002±0.0005502, t_{502} ==4.37, $P < 0.0001$, sire: $F_{24,96} = 3.9$, $P < 0.0001$). There was also an association for the breast plumage and production until 60 WOA (estimate = 0.02 ± 0.007, t_{502} = 3.0, P-value = 0.003; sire: $F_{24,96} = 2.23$, $P = 0.003$; interaction: $F_{24,502}$ = 1.43, $P = 0.09$). We found no relationship between our egg production proxy until 60 WOA and either body mass at 60 WOA or the gain in body mass between 40 and 60 WOA (body mass: $F_{1,139} = 0.68$, $P = 0.41$; gain: $F_{1,139} = 0.28$, $P = 0.60$). These findings are interesting as it suggests a de-linkage between production outcomes and health, i.e., that high production output is not necessarily a causal factor in poor health. Results of the current study support that high egg production, as indicated by our behavior proxy, was associated with better plumage, especially for the breast. Feather quality is generally considered an indicator of poor health and associated with poorer environments (see a recent meta-analysis by van Staaveren et al., 2021). We would expect that more productive hens would have to commit more resources to egg production and thus exhibit compromised health, however this was not the case. Further study is required to determine the true nature of the relationship between egg production and feather quality, ideally using more direct measures of health and production.

In contrast, we did find that toe injuries were associated with reduced production, although, as with feather quality, it is not clear if there is a causal relationship. In a related analysis, birds with toe pecking injuries at 60 WOA had reduced inter-tier transitions at the age of assessment (Gómez et al., 2024). Examination of transitions at earlier ages (up to 150 days earlier) when toe injuries where unlikely found no differences suggesting that the altered movement was a consequence of the injury rather than a cause. These findings suggest the nuanced and subtle benefits that can be extracted from detailed, longitudinal observations of hens using sensor technology (Rodenburg et al., 2019; Montalcini et al., 2023b; 2023a). As a parallel example of the benefits which can relate health and movement, we have also identified that altered movement is related to keel bone fractures (Rufener et al., 2019a; Montalcini et al., 2024).

Although interesting, our hens were generally healthy with little variation in terms of feather coverage and plumage (Table 2). Future work should consider older birds where health outcomes would be expected to be more severe and variable to determine if the associations remain and/or change quantitatively (e.g., greater in magnitude) or qualitatively (e.g., new relationships emerge).

Linking behavior and egg production via proxy

Our proxy for total egg production was not linked with duration in any of the aviary zones as independent metrics, i.e., separate models $p >$ 0.41. The PCA identified two factors with one factor positively correlated with number of transitions, time spent in the WG, LIT, LLT, and negatively correlated with time spent on the TLT. Factor 2 was positively correlated with the time spent on the NBT, and weakly with the time spent in the WG, on the TLT, and the number of transitions, and was negatively correlated with the median number of visits to the NBT, and duration on LLT. As the second component included duration in the nestbox, it is confounded with the proxy and was not pursued. Focusing on the first component, when the entire dataset is included with birds with very low production, there were two-way interactions between factor 1 and pen ($p = 0.025$) and sire ($p = 0.001$) in relation to the total egg production proxy.

The influence of both pen and sire on egg production highlights the importance of environmental factors within CFHS. Cage housing systems are relatively simple by design with smaller groups and consequently reduced variation in social interactions. Compared to CFHS, caged animals cannot interact with external environments (e.g. pasture) or resources distributed throughout the aviary (e.g., litter) that will likely affect their health and welfare. Even though the five pens used in the current study where adjacent and occupied the same 11.5 × 7.0 meter space, there were still statistically valid variations across pens. There are infinite sources to explain the variation that are nonetheless quantifiable and relevant for breeding, including social genetic effects (Ellen et al., 2014). We believe the results of the current study demonstrate the variation that is present and can be quantified towards the improvement of animal health and welfare. Future work should examine the relative benefit of these traits, including the egg production proxy, within the larger goals of breeding programs.

Heritability and Genome-wide association studies

In addition to detailed, longitudinal behavior and health assessments, the inclusion of genomic information also provided important information as to the causes of variation. Genome-derived heritability estimates for all health assessment metrices at 40 and 60 WOA are summarized in Table 4. Overall, heritability estimates at 60 WOA were higher compared to those at 40 WOA. Body mass had the highest heritability, with an estimate of 0.73 at 60 WOA, whilst the other traits showed low to moderate heritability estimates. Manhattan plots revealed significant associations for body mass at 40 and 60 WOA (Fig. 4) and for cloaca at 40 WOA, though did not reveal any associations with other health assessment measures.

The top associated SNPs for body mass on chromosomes 6 and 9 were located next to the genes A1CF and WNT9B, where the A1CF is a candidate gene for residual feed intake in beef cattle (de las Heras-Saldana et al., 2019) and WNT9B for bone breaking strength in laying hens (Jansen et al., 2021). Wnt genes are known to impact bone mass and therefore likely influence body mass. It was also interesting to see that, for body mass at 40 WOA, the hit on chromosome 27 was more significant compared to 60 WOA, although the health metrics were generally poorer at 60 WOA. Future work should consider this finding in terms of quantifying environmental effects on the phenotype. The top associated SNP for the cloaca at 40 WOA was located in the gene SGK3, which is also a candidate gene for human alopecia conditions (Hytönen and Lohi, 2019). If the association is viable, it may indicate cloacal coverage would serve as a poor indicator of health. Interesting was the lack of a relationship with the metrics that were linked with our behavioral proxy of egg laying. For instance, the low heritability of toe injuries and lack of associated candidate genes indicates a large environmental component in the observed variation. Consequently, solutions to resolve toe pecking will likely involve management and housing interventions. Moreso, our finding of a negative association between toe injuries and total egg production suggest reduction of the former would benefit hen productivity. In contrast, heritability of breast plumage was relatively high at both 40 and 60 WOA. Given the positive relationship with total egg production, the correlation between these traits should be evaluated and considered for breeding programs in CFHS.

Conclusion and applications

1. Using detailed movement data as a proxy for egg laying could provide a valuable proxy to assess the relationship between production

Table 4
Heritability values of all health assessment metrics at 40 and 60 WOA.

	Age	
Health Metric	40	60
Neck	0.1328	0.2006
Back	1.11E-08	0.1811
Wing	0.0917	0.0957
Tail	0.1423	0.277
Cloaca	0.0685	0.277
Breast	0.35	0.4045
Plumage	1.25E-08	0.3353
Footpad	1.14E-08	0.1621
Toe Injury	0.0678	1.01E-08
Body Mass	0.6662	0.7278

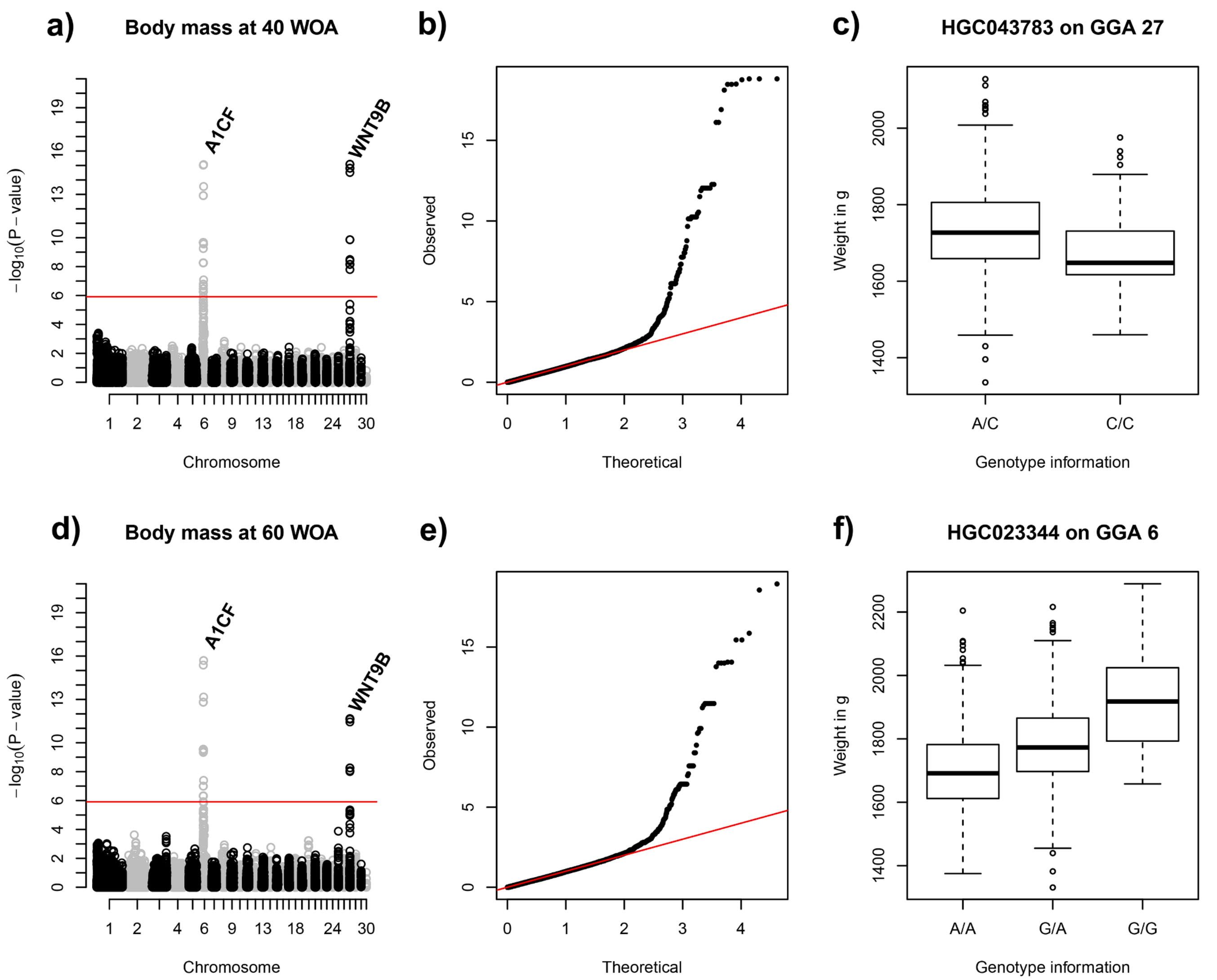

Fig. 4. Genome-wide association study (GWAS) results for body mass at 40 and 60 weeks of age (WOA). **a)** Manhattan plot for body mass at 40 WOA with the red line representing the 5 % Bonferroni-corrected threshold for 40,563 independent tests ($p_{BONF} < 1.23 \times 10^{-6}$). **b)** Quantile–quantile (Q–Q) plot for body mass at 40 WOA with the observed *p*-value plotted against the expected one. (**c**) Boxplots representing the genotype effect of the top associated SNP on chromosome 27 on the body mass at 40 WOA. The horizontal line shows the median, the box extends from the lower to the upper quartile, and the whiskers to 1.5 × the interquartile range above the upper quartile or below the lower quartile. **d)** Manhattan plot for body mass at 40 WOA with the red line representing the 5 % Bonferroni-corrected threshold for 40,563 independent tests ($p_{BONF} < 1.23 \times 10^{-6}$). **e)** Quantile–quantile (Q–Q) plot for body mass at 60 WOA with the observed *p*-value plotted against the expected one. (**f**) Boxplots representing the genotype effect of the top associated SNP on chromosome 6 on the body mass at 60 WOA.

and health, as well as other variables of interest. Based on the observed correlations, egg laying did not seem to be a factor in reduced feather quality though may have been reduced following toe injuries.

2. Genome-derived heritability of several health assessment traits was demonstrated and provided information on their utility within health assessments. Most notably, the identified candidate genes for body mass warrant future exploration in relation to other health and movement traits in laying hens.

CRediT authorship contribution statement

M.J. Toscano: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **M. Neuditschko:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **S.G. Gebhardt-Henrich:** Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Michael J Toscano reports equipment, drugs, or supplies was provided by Hendrix Genetics. The hens were provided as an in kind contribution by Hendrix Genetics. Hendrix Genetics had no influence on the interpretation or presentation of research findings. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.japr.2025.100603.

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