The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK
www.ufaw.org.uk

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Welfare Quality® parameters do not always reflect hen behaviour across the lay cycle in non-cage laying hens

C Daigle and J Siegford*

Animal Behaviour and Welfare Group, Michigan State University, 1290 Anthony Hall, East Lansing, MI 48824-1225, USA
* Contact for correspondence and requests for reprints: siegford@msu.edu

Abstract

The integration of outcome-based measurements to animal welfare assessment programmes can provide a new perspective on the individual animal's experience. Identifying variability in individual experiences can facilitate understanding of animals at the periphery of the welfare spectrum, compared to those at the average. Welfare Quality® physical measurements and behavioural observations were taken from the same fifteen non-cage laying hens throughout their production cycle. The average amount of time performing each of nine behaviours and the amount of variation in each behaviour's performance was compared at four different ages: 19, 28, 48 and 66 weeks. The same analysis was performed for all Welfare Quality® physical measurements. To identify associations between a hen's behaviour and her physical condition, a cluster analysis was performed for all ages, as well as on data collated from all ages. No differences were observed among the four ages for the average amount of time performing many of the behaviours, but the amount of variability differed for most behaviours observed. Physical measurements taken at 19 weeks differed from those taken at later ages. Bodyweight consistently clustered with time spent preening, yet the patterns of clustering differed at each age. These results highlight the importance of age when conducting welfare assessments. Auditors also should report not only the average, but the variability of responses; while the average response of the flock may appear consistent across time or treatment, differences among the hens within the same flock may vary drastically.

Keywords: *animal welfare, behaviour, group welfare, laying hen, outcome-based measures, Welfare Quality®*

Introduction

Traditionally, animal welfare audits and certification programmes have used resource-based measures (RBM), such as the availability of adequate food, water, veterinary care, and space, to assess how well a housing system meets animals' needs. However, using only an RBM approach, one could perform an on-farm audit without looking at a single animal. Therefore, the importance of outcome-based measures (OBM), such as behaviour or individual physical condition, is beginning to be emphasised as part of animal welfare assessment (Butterworth *et al* 2011). As the laying hen industry transitions from small conventional cages to group housing, the hens housed in these alternative systems may face different challenges than those historically faced by their conventionally caged counterparts. Thus, understanding the relationship between environment, behaviour and OBM is increasingly important.

Outcome-based measures of behaviour are typically reported at the group level by collating individual measurements to provide an average response for the group or an overall farm-level score (Johnsen *et al* 2001). However, many of the fundamental concepts of animal welfare are rooted in an individual's response to a situation, thus the concept of animal welfare inherently implies the importance of the individual experience (Duncan & Mench 1993; Dawkins 2003; Fraser 2008). Most theories of welfare apply to individuals because only individuals possess the characteristics (eg affective state, perception, needs, motivation) that make lives better or worse and individual variation with regards to genetics, experiences, and temperament, can impact how the individual perceives its current situation and ultimately determine its welfare.

Therefore, RBMs are extrapolated and weighed to infer how an animal is performing based upon its environmental conditions (Veissier *et al* 2011). However, the average condition of the group may not accurately reflect the condition of a specific individual and the condition of individuals within the same group may vary widely. Yet, issues of practicality limit the ability of on-farm assessors to examine each individual, and welfare must be assessed at the group level for there to be any chance of improving the welfare of an individual in a production system.

The Welfare Quality® assessment protocol for poultry (WQ) provides practical and accessible feedback to producers while examining individual hen physical condition by providing a combination of RBM and individual OBM to provide welfare information at the individual, pen, and farm level (Temple *et al* 2011, 2013). For a complete listing of the RBM and OBM measured as part of the WQ poultry assessment, please refer to

Figure 1

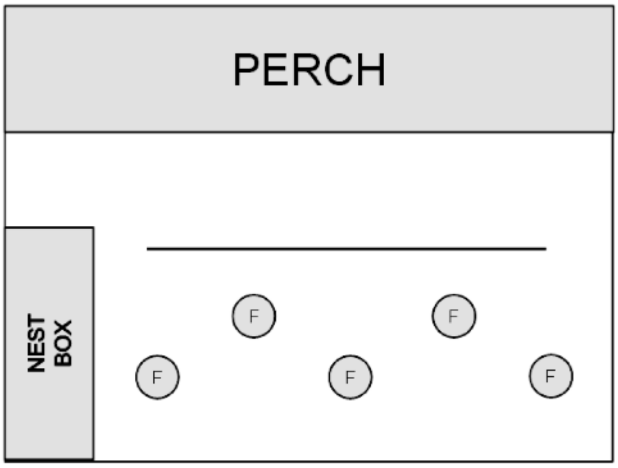

Diagram of the room in which hens were housed. F represents a tube feeder hanging from the ceiling. The horizontal line represents the water line.

Welfare Quality® (2009). The RBM and OBM in the WQ assessment emphasise four primary areas: good feeding, good housing, good health, and appropriate behaviour relevant to the Five Freedoms developed by the Brambell Committee (1965).

Behaviours made at an early age can also influence future body condition and future behaviour. When animals are housed in more complex and stochastic environments (compared to conventional housing systems), they have opportunities regarding when, where, and how they access resources and perform natural behaviours. These opportunities can create situations that impact their physical condition (eg landing from a perch), and conversely their physical condition can impact the behaviours they perform (eg perching) with a cause and effect relationship that is difficult to untangle. An animal's coping style (or personality, temperament, axes, constructs, or behavioural syndrome) has been linked to behavioural and physiological characteristics such as exploratory behaviour and boldness (Stowe *et al* 2010; van Oers *et al* 2011) and can be consistent over time and across contexts (Sih *et al* 2004; Re'ale *et al* 2007). Because behaviour can be shaped by experience, and experiences shape future behaviours, understanding the cyclic relationship between the body and behaviour is becoming increasingly important — especially as regards animal welfare.

Therefore, the objectives of this study were to assess how WQ scores, behaviour, and resource use of individual laying hens changed as they aged, assess whether the variability of WQ scores, behaviour, and resource use changes among individual hens as they age, and identify relationships between hen WQ scores and behaviour. We anticipated that hens would become more variable in their behaviour and physical condition as they aged, even though average conditions will stay unchanged. Furthermore, we anticipated that WQ parameters that measure a specific body part (eg foot condition) would be associated with behaviours requiring use of the respective body part (eg walking, foraging).

Materials and methods

Study animals and housing

Data were collected from laying hens housed in an experimental non-cage system at the Michigan State University Poultry Teaching and Research Center (MSU-PTRC). Prior to the start of the study, all protocols were submitted to and approved by the Michigan State University Institutional Animal Care and Use Committee.

Three identical rooms (6.0×4.5 m; length $\times$ width) at MSU-PTRC were used. Each room was furnished in the same configuration with nest-boxes, perches, tube feeders, and a water line with nipples. Sixteen nest boxes (each $0.4 \times 0.3 \times 0.3$ m; length $\times$ width $\times$ height) in an 8×2 configuration were mounted 0.3 m above the ground on one wall. Perches consisted of a three-level wooden rail structure (with each rail 6 m long and ~5 cm in diameter with a flat top and rounded sides and bottom) and mounted over a 1×6 m slatted area at a height of 0.53, 0.76, and 0.99 m from the ground. The perches were mounted to the wall at a slope of 45° with a 40 cm distance between each wooden rail. Room floors were covered with ~8 cm of wood-shavings at time of data collection. Wood-shavings were exchanged for clean shavings as needed (approximately every 12 weeks beginning when the hens were 30 weeks). Food and water were provided daily *ad libitum*. Daily care, including egg collection, feeding, and hen inspection, occurred at least once a day. Two incandescent light bulbs (60 lux at bulb level) on an automatic timer provided light 15 h per day in each room. Temperature was maintained between 16–22°C using a ventilation fan and forced air heating.

Hy-Line Brown laying hen pullets (n = 405; 135 hens per room) were reared in each of the rooms as described above with accommodation made for their size (ie smaller perches, which were removed at six weeks of age) and immaturity (ie access to nest boxes was not granted until ten weeks of age). Each room provided 0.21 m^2 floor space, 17.8 cm of perch space, 0.01 m^2 of nest-box space, and 4.83 cm feeder space per hen (Figure 1). Thirteen nipples provided drinking access at a ratio of 10.3 hens per nipple. All room parameters met or exceeded United Egg Producers and the Federation for Animal Science Society's housing requirements for non-cage laying hens (FASS 2010).

At ten weeks, we weighed all hens and fitted them with uniquely numbered leg bands. At 11 weeks, ten hens (7.4% of the population) per room were selected and fitted with a harness for unique and individual visual recognition. The harnesses provided an opportunity to visually monitor individual hens across time, which allowed us to make observations on the same individuals throughout the production cycle. We selected hens from a different part of the spectrum of bodyweights because bodyweight contributes to hen social hierarchy. For instance, heavier hens are more likely to have won a recent fight and perform more double attacks when establishing a social hierarchy (Cloutier & Newberry 2000). Thus, this step enabled us to potentially

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Table 1 Ethogram of behaviours developed to identify posture, behaviour and resource use.

Factor	Description
Posture	
Walk	Walking more than three steps in succession with head up or when walking hen has not been standing, drinking, feeding, or foraging in litter for the previous 5 s
Behaviour	
Feed	Hen pecks at feed in the feeder. Recording starts at first peck
Drink	Head is turned upwards towards water source, and hen uses beak to consume water from nipple drinker
Preen	Hen may be sitting or standing and uses beak to manipulate, rearrange, pull or clean body feathers on self
Dust-bathe	While squatting or lying, hen performs dust-bathing activities, including vertical wing shaking, bill raking, scratching, ground pecking, movements of the feet and wings to raise dust into the ruffled plumage, rubbing of head and sides in the dust, feather-ruffling and shaking dust out of the feathers. Starts with first wing shake
Forage	Hen pecks at substrate while standing or stepping forward with head below rump level. Starts when hen makes > 3 successive pecks at substrate, or when foraging hen has not been standing or walking with head up, or feeding, for the previous 5 s
Rest	Starts when hen lies down (sternum resting on substrate) from an upright position or when lying bird has made no dust-bathing or preening movements for the previous 8 s
Resource	
Perch	Hen is standing, walking, or resting on perch, the rail in front of the next boxes, or black base of slats underneath raised perches
Nest-box	Hen is standing or resting inside a nest box

A hen was considered to be performing a new posture, behaviour, or resource use when she stopped performing the previous behaviour for > 5 s or began performing a new behaviour for > 5 s.

evaluate behaviour of hens from a variety of social ranks. Collection of data prior to the onset of lay as part of a separate experiment was the motivation for fitting the hens with harnesses prior to comb development. Therefore, even though combs are important to hen social hierarchy (O'Connor *et al* 2011), it was not possible to include comb size as a parameter indicative of social status to select hens to wear harnesses. The remaining 125 hens in each room did not receive harnesses. Previous research indicated that wearing the harness did not have any long-lasting effects on hen resource use or agonistic interactions (Daigle *et al* 2012).

Behavioural data collection

The individuals assessed for this study (n = 5 per room; 15 hens in total) were selected retrospectively based upon whether they survived the entire length of the lay cycle as several of the initial hens fitted with harnesses died. Therefore, at the end of the lay cycle, we identified five harness-wearing hens per room that had survived the entire lay cycle and subsequently decoded video data from these individuals for the duration of the lay cycle.

Data were collected when the hens were 19, 28, 48, and 66 weeks across a 48-h time-period with the dark period (2100–0600h) omitted. Therefore, data were collected from the same individuals at four separate time-points throughout the production cycle. Continuous observation of individual hen posture, behaviour, and resource use (Table 1) was performed for a 30-min period every hour and a half (0600–0630h, 0730–0800h, 0900–0930h, 1030–1100h, 1200–1230h, 1330-1400h, 1500–1530h, 1630–1700h, 1800–1830h and 1930–2000h) during the lights-on period (Daigle & Siegford 2014).

Five ceiling-mounted cameras (Sony SSCDC-593, Sony Electronics Inc, San Diego, CA, USA) fitted with a vari-focal wide-angle lens (3.4–12.0 mm) were placed in each room to ensure that hens could be viewed continually regardless of their location in the room. Infra-red cameras were used, which enabled night-time viewing. Movement between perches or areas of the room was not observed, however, hens were observed transitioning between standing and sitting as they readjusted during the night. Behaviour and resource use were recorded using mutually exclusive categories and are reported as duration of time spent (s) in that state across the 48-h period.

Welfare Quality® assessment

All harness-wearing hens were evaluated using the WQ every two weeks throughout the lay cycle. For this analysis, WQ data were analysed for the same five hens per room on which behavioural observations were performed. Parameters measured included bodyweight (kg), claw length (cm), comb-pecking wounds, plumage damage, foot-pad dermatitis, and keel-bone scores (Table 2). WQ parameters were measured once every other week from 16–66 weeks of age. WQ parameters that were measured in the two weeks before, the week of, and in the two weeks after each video recording (at 19, 28, 48, and 66 weeks) were averaged to create a single WQ score for each hen at each data collection time-point. Therefore, the ordinal variables were converted to continuous variables for analyses. This transformation from ordinal to continuous can provide insight as to whether the hen's physical condition changed across the six-week period.

Table 2 Physical parameters measured as part of the Welfare Quality® (2009) assessment programme for poultry.

Physical parameter*	Description
Bodyweight	Mass of hen (kg)
Claw length	Length from cuticle to tip of middle right claw (cm)
Comb-pecking wound	0: no evidence of pecking wounds 1: fewer than three pecking wounds 2: more than three pecking wounds
Plumage damage score	Three areas of body (head/neck, back/rump, belly) are given a score. For each body part a score is given on a 3-point scale: A: no or slight wear, (nearly) complete feathering (only single feathers lacking) B: moderate wear, ie damaged feathers (worn, deformed) or ≥ 1 feather-less areas < 5 cm in diameter C: > 1 featherless area ≥ 5 cm in diameter at the largest extent Combine body part scores into single general score per bird 0: all body parts have score 'a' 1: ≥ 1 body parts have a score 'b', but no body part has score 'c' 2: ≥ 1 body parts have score 'c'
Keel -one score	0: no deviations, deformations or thickened sections, keel bone completely straight 2: deviation or deformation of keel bone (including thickened sections) observed
Foot health score	0: feet intact, no or minimal proliferation of epithelium 1: necrosis or proliferation of epithelium or chronic bumble foot with no or moderate swelling 2: swollen (dorsally visible)

*Welfare Quality® assessment programme for poultry (broilers, laying hens).

Table 3 Mean (± SEM) time (s) spent performing behaviour at each age.

Behaviour	Age at data collection (weeks)			
	19	28	48	66
Walk	3,100.27 (± 2,027.82)	3,430 (± 1,614.67)	2,894.40 (± 1,870.43)	3,742.27 (± 1,898.92)
Feed	4,699 (± 3,157.19)*‡	5,902.00 (± 2,845.32)*	7,210.67 (± 5,072.88)‡	5,137.33 (± 3,375.57)*‡
Drink	1,202.67 (± 509.32)	1,448.13 (± 512.07)	1,358.80 (± 990.37)	1,183.73 (± 688.45)
Preen	5,679.47[a] (± 1,292.23)*	4,868.80[ab] (± 1315.81)*‡	4,728.00[ab] (± 1,862.86)*‡	4,574.93[b] (± 2,343.68)‡
Dust-bathe	314.27 (± 482.44)*‡	211.47 (± 333.98)*	494.80 (± 444.12)*‡	506.53 (± 558.29)‡
Forage	6,769.33 (± 4412.24)	6,279.20 (± 3,461.03)	5,392.27 (± 2,935.52)	4,790.67 (± 3,050.85)
Rest	3,548.53[a] (± 2,738.51)*	1,398.93[ab] (± 1,129.3)‡	1,314.00[b] (± 1,499.04)*‡	2,473.60[ab] (± 2,605.91)*‡
Nest box	381.60[a] (± 685.10)*	1,238.67[ab] (± 1,125.51*‡	2,079.60[b] (± 3,411.57)*‡	2337.87[ab] (± 5,759.06)‡
Perch	8,552.80[a] (± 3,509.54)*‡	4,250.93[ab] (± 2,794.14)*	4,912.80[b] (± 4,127.63)*‡	5,181.87[b] (± 4,943.64)‡

Mean differences ($P > 0.05$) are indicated by different superscript letters and differences in variability are indicated by different superscript symbols.

Statistical analysis

All analyses for this paper were conducted using SAS version 9.2 (SAS Institute, Cary, NC, USA). Results were considered statistically significant at a probability of α less than 0.05. The residuals for each variable were tested for normality.

An exploratory correlational analysis (PROC CORR) was performed prior to analysis to identify any relationships among WQ parameters and hen behaviour at the four different ages.

Mean WQ parameters, posture, behaviour and resource use of individual laying hens as they age

Boxplots were created for each WQ parameter and each hen activity (posture, behaviour, and resource use) at the four time-points of interest (19, 28, 48, and 66 weeks). To identify whether there was a difference in either the mean amount of time spent performing each activity; a generalised mixed model analysis (PROC GLIMMIX) with a log link and Poisson distribution was used to identify mean differences across the four age points for each activity. Each behaviour and was assessed independently and each model included a random effect of age with hen nested within room as the subject. To identify whether there was a difference in either the average WQ parameter; a mixed model analysis (PROC MIXED) with an identity link identified mean differences across the four age points for each WQ parameter. Each WQ parameter was assessed independently and each model included a random effect of hen nested within room with hen as the subject and age was a repeated factor. Least squared means without an adjustment are presented, however in total, six separate comparisons were conducted to identify specific differences between the age groups.

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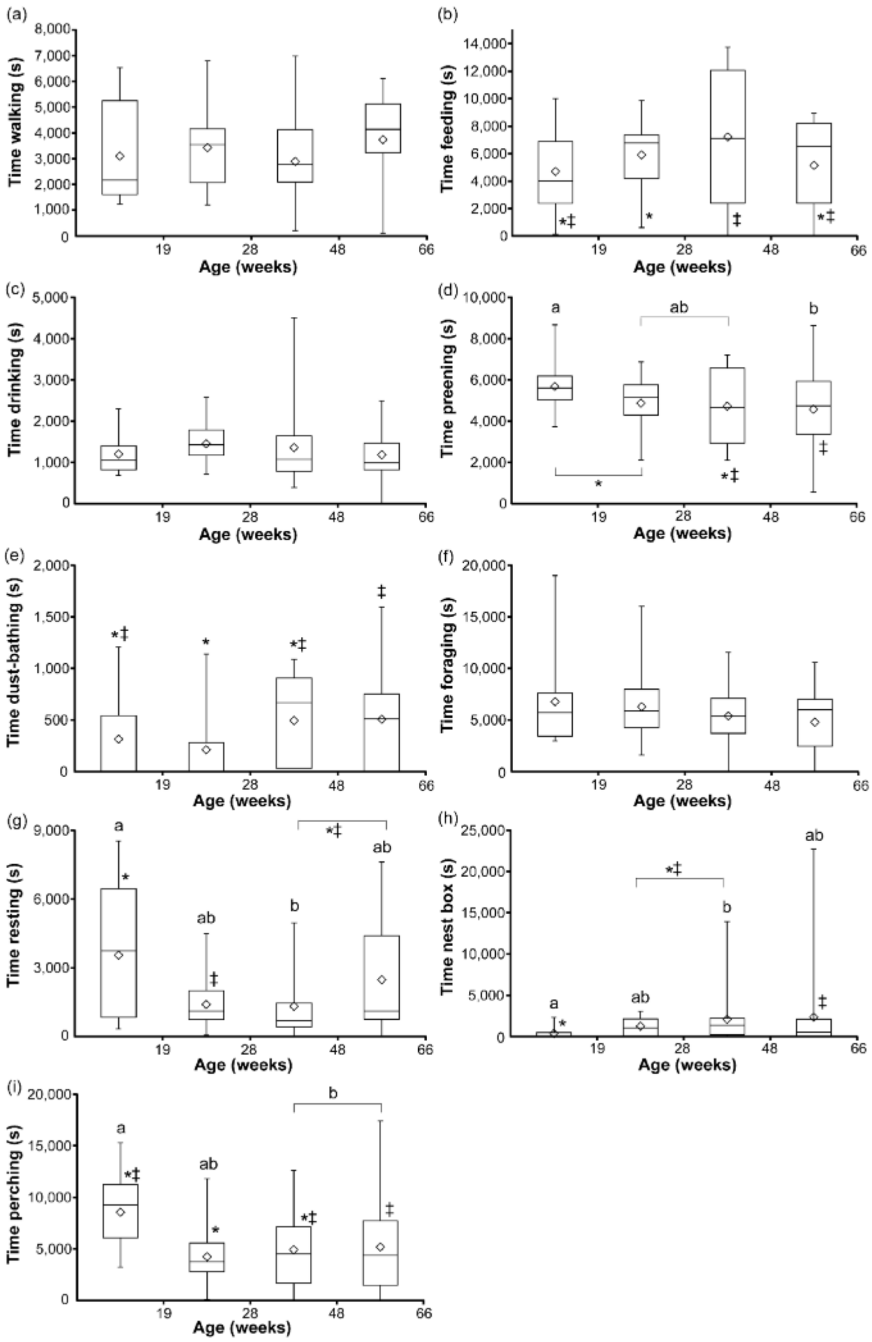
Figure 2

Boxplots of (a) walking, (b) feeding, (c) drinking, (d) preening, (e) dust-bathing, (f) foraging, (g) resting, (h) nest-box use and (i) perch use for non-cage laying hens at four different ages throughout the lay cycle. Mean differences ($P > 0.05$) among the four ages are represented by different letters. Differences in variability ($P > 0.05$) are indicated with different symbols.



Variability of WQ parameters, posture, behaviour, and resource use among individual hens as they age

To examine whether the variability of either the WQ parameters or time spent performing each activity was homogenous across the different age time-points, the COVTEST option was utilised as part of a Generalized Linear Mixed Model (PROC GLIMMIX). The model used age as a fixed effect, hen nested within room as the random effect, and hen as the subject and age as the group. Contrast statements identified specific differences among the standard errors of the different age groups.

Relationship between WQ parameters and hen activity

A cluster analysis (PROC VARCLUS) which analysed the covariance matrix (compared to the standard correlation matrix) was performed on all behavioural and WQ data. The analysis used a centroid approach which clustered according to centroid components instead of principal components and used the default splitting criterion of 0.75. Five separate cluster analyses were conducted: each age was analysed independently, and then parameters from all ages combined were analysed. Prior to cluster analysis, the

Table 4 Mean (± SEM) Welfare Quality® scores from the same hen at four different ages.

WQ® parameter	Age at data collection (weeks)			
	19	28	48	66
Bodyweight	1.68[a] (± 0.17)*	1.92[b] (± 0.19)*	1.99[b] (± 0.25)*‡	1.93[b] (± 0.35)‡
Claw length	1.31[a] (± 0.11)*‡	1.2[b] (± 0.1)*	1.28[ab] (± 0.16)*‡	1.49[c] (± 0.21)‡
Foot health	0[a] (± 0)*	0.71[b] (± 0.31)‡	1.13[bc] (± 0.55)‡	1,11[bc] (± 0.39)‡
Feather damage	0.24[a] (± 0.20)*	1.13[b] (± 0.6)‡	1.47[b] (± 0.59)‡	1.56[b] (± 0.61)‡
Comb score	0.22[a] (± 0.21)*	1.42[b] (± 0.43)‡	1.2[bc] (± 0.53)‡	0.89[c] (± 0.67)‡
Keel bone	0.09[a] (± 0.23)*	0.62[ac] (± 0.64)‡	0.80[bc] (± 0.76)‡	0.89[bc] (± 0.78)‡

Mean differences (P > 0.05) are indicated by different superscript letters, and differences in variability are indicated by different superscript symbols.

variables were standardised (PROC STANDARD) with a mean of zero and a standard deviation of one. Specific relationships between WQ parameters and observed behaviours that were in the same cluster were assessed in a correlational analysis (PROC CORR).

Results

Hen mortality was recorded throughout the lay cycle for both harnessed and non-harnessed hens. Total mortality across the three rooms for the population was 7.96 (± 2.05)% of the population. Within the harnessed hen population, 20 (± 11.5)% of them died, and 7.07 (± 1.93)% of the non-harnessed population died from various causes throughout the study.

Mean change in WQ parameters, behaviour, and resource use of individual laying hens as they age

Duration of time spent performing behaviours across the 48-h observation period is presented in Table 3. No mean differences were observed among the four time-points for amount of time spent walking (Figure 2[a]), feeding (Figure 2[b]), drinking (Figure 2[c]), foraging (Figure 2[f]), or dust-bathing (Figure 2[e]). Hens spent more time preening (Figure 2[d]) at 19 compared to 66 weeks (t_{56} = 2.24, P = 0.03), and more time resting at 19 weeks compared to 48 (t_{56} = 2.35, P = 0.02). The nest box was used more at 48 compared to 19 weeks (t_{56} = –2.33, P = 0.02). More time was spent using the perch (Figure 2[i]) at 19 weeks than was observed at 48 (t_{56} = 2.06, P = 0.04) and 66 weeks (t_{56} = 2.23, P = 0.03).

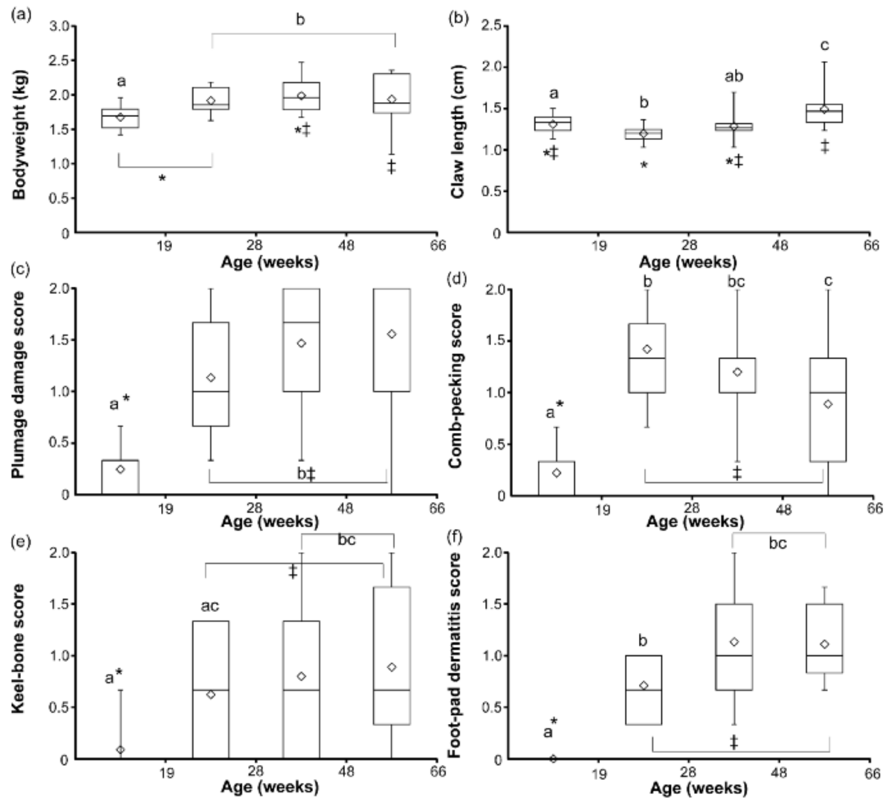
Average physical scores are presented in Table 4. Hens were lighter (Figure 3[a]) at 19 weeks than at 28 (t_{41} = –5.06, P < 0.0001), 48 (t_{41} = –6.55, P < 0.0001) or 66 weeks (t_{41} = –5.46, P < 0.0001). Claws (Figure 3[b]) were shorter at 28 compared to 19 (t_{41} = 3.41, P = 0.002) and 66 weeks (t_{41} = –5.41, P < 0.0001). Subsequently, claws were longer at 66 weeks than at 48 (t_{41} = –6.21, P < 0.0001) and 19 weeks (t_{41} = –8.81, P < 0.0001). Plumage damage scores (Figure 3[c]) were lower at 19 weeks compared to 28 (t_{41} = –5.35, P < 0.0001), 48 (t_{41} = –7.35, P < 0.0001), and 66 weeks (t_{41} = –7.89, P < 0.0001). A similar pattern was observed for comb-pecking scores (Figure 3[d]), which were lower at 19 than at 28 (t_{41} = –7.89, P < 0.0001), 48 (t_{41} = –6.43, P < 0.0001) and 66 weeks (t_{41} = –4.38, P = 0.001). Yet, comb-pecking scores were higher at 28 than at 66 weeks (t_{41} = 3.51, P = 0.007). Keel-bone scores (Figure 3[e]) were higher at 48 (t_{41} = –3.30, P = 0.01) and 66 weeks (t_{41} = –3.71, P = 0.004) compared to 19 weeks. Foot-pad dermatitis scores (Figure 3[f]) were lower at 19 weeks compared to 28 (t_{41} = –5.97, P < 0.0001), 48 (t_{41} = –9.51, P < 0.0001), and 66 weeks (t_{41} = –9.33, P < 0.0001). Furthermore, foot-pad dermatitis scores at 28 weeks were lower than those measured at 48 (t_{41} = –3.54, P = 0.01) and 66 weeks (t_{41} = –3.36, P = 0.01).

Variability of WQ parameters, behaviour, and resource use among individual hens as they age

No difference in the variability of time spent drinking (Figure 2[c]), walking (Figure 2[a]), or foraging (Figure 2[f]) was observed among the four time-points (Table 3). More variability in feeding behaviour (Figure 2[b]) was observed at 48 when compared to 28 weeks (χ^2_{56} = 4.44, P = 0.04). Hens were more variable in their preening behaviour (Figure 2[d]) at 66 weeks compared to 19 (χ^2_{48} = 5.65, P = 0.02). Dust-bathing behaviour (Figure 2[e]) tended to be less variable at 28 than at 66 weeks (χ^2_{48} = 4.41, P = 0.04), yet resting behaviour (Figure 2[g]) was less variable at 66 weeks compared to resting behaviour at 19 (χ^2_{48} = 6.64, P = 0.01) and 28 weeks (χ^2_{48} = 5.95, P = 0.02). Nest-box use (Figure 2[h]) was less variable at 19 weeks compared to 28 (χ^2_{48} = 6.40, P = 0.01), 48 (χ^2_{48} = 27.63, P < 0.0001) and 66 weeks (χ^2_{48} = 40.16, P < 0.0001). Furthermore, nest-box use at 28 weeks was less variable than nest-box use at 48 (χ^2_{48} = 11.44, P = 0.001) or 66 weeks (χ^2_{48} = 22.59, P < 0.0001). No differences in variability of nest-box use were observed between 48 and 66 weeks (χ^2_{48} = 3.33, P = 0.07). Perch (Figure 2[i]) use was more variable at 66 than at 19 (χ^2_{48} = 4.78, P = 0.03) and 28 weeks (χ^2_{48} = 4.10, P = 0.04).

All physical parameters measured exhibited more variability as the hens aged (Table 4). Bodyweight (Figure 3[a]) was more variable at 66 than at 19 (χ^2_{48} = 5.48, P = 0.02) and 28 weeks (χ^2_{48} = 4.23, P = 0.04). Claw length (Figure 3[b]) was more variable at 66 than 28 weeks (χ^2_{48} = 4.69, P = 0.03). Plumage damage scores (Figure 3[c]) were less variable at 19 compared to 28 (χ^2_{48} = 14.5, P > 0.0001), 48 (χ^2_{48} = 6.73, P = 0.01), and 66 weeks (χ^2_{48} = 14.41,

Figure 3



Boxplots of (a) bodyweight, (b) claw length, (c) plumage damage score, (d) comb-pecking score, (e) keel-bone score, and (f) foot-pad dermatitis score from the Welfare Quality® assessment protocol for non-cage laying hens at four different ages throughout the lay cycle. Mean differences (P > 0.05) among the four ages are represented by different letters. Differences in variability (P > 0.05) are indicated with different symbols.

P = 0.0001). Comb-pecking scores (Figure 3[d]) followed a similar pattern with more variability observed at 28 (χ^2_{48} = 6.46, P = 0.01), 48 (χ^2_{48} = 10.27, P = 0.01), and 66 weeks (χ^2_{48} = 15.92, P < 0.0001) compared to 19 weeks. Keel-bone scores (Figure 3[e]) were less variable at 19 compared to 28 (χ^2_{48} = 11.14, P = 0.0008), 48 (χ^2_{48} = 11.71, P = 0.0006), and 66 weeks (χ^2_{48} = 14.23, P = 0.0002). Foot-pad dermatitis scores (Figure 3[f]) were less variable at 19 compared to 28 (χ^2_{48} = 298.07, P < 0.0001), 48 (χ^2_{48} = 308.98, P < 0.0001), and 66 weeks (χ^2_{48} = 303.93, P < 0.0001).

Relationship between WQ parameters and hen behaviour

Cluster analyses of all ages combined produced five separate clusters, with two of the five clusters containing both WQ and behavioural data (Table 5). One of the clusters contained the behaviours feed and drink along with the WQ parameter of claw length. The other cluster contained the behaviours preening, dust-bathing, and nest-box use as well as the WQ parameter comb score.

When each age was analysed separately, several factors consistently clustered together. Preening and bodyweight were found within the same cluster at all ages and an increasingly positive relationships was observed as the hens aged (Figure 4; 19 weeks [r_{15} = 0.430, P = 0.11], 28 weeks [r_{15} = 0.461, P = 0.08], 48 weeks [r_{15} = 0.520, P = 0.05], and 66 weeks (r_{15} = 0.733, P = 0.002]). However, preening and bodyweight did not cluster when data from all ages were analysed together. Furthermore, a negative relationship was observed between feeding and keel-bone score at 19 (r_{15} = –0.57, P = 0.03), 48 (r_{15} = –0.48, P = 0.07), and 66 weeks (r_{15} = –0.82, P = 0.0002). The duration of time the hens spent feeding and their keel-bone scores did not cluster together at 28 weeks and did not cluster when data from all ages were analysed together.

Discussion

Many of the behaviours did not change with age in their average rate of expression; while the variability of expression did change for most of the behaviours as the hens aged. No behaviours exhibited a change in mean without a change in variability. This suggests that reporting a mean score may omit important information regarding individual hen welfare. By providing both an average score and a range to a producer, we may be able to increase the depth of the information gained from this on-farm assessment. Furthermore, as many welfare concerns manifest at the negative tail end of the spectrum, we may be able to better manage flocks by being aware of and managing for the diversity of hens within the system.

Furthermore, WQ scores and hen behaviour did not cluster as expected, and they varied in how they clustered depending on the age of the hen. These results suggest that these measurements may not be useful proxies for identifying how hens are behaving, and that the age at which the

Table 5 Cluster analysis for Welfare Quality® (WQ) and behavioural observations conducted when laying hens were 19, 28, 48, and 66 weeks.

Age	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Total variation explained	Proportion
All ages combined	forage walk	rest perch	CL drink (–) feed (–)	CS nest box (+) dust-bathe (–) preen (–)	KB FD FH BW	n/a	8.83	0.59
19 weeks	BW preen (+)	KB feed (–) dust-bathe (+)	CL drink (–)	FD nest box (+)	CS walk (+) forage (+) rest (–) perch (–)	n/a	9.32	0.67
*FH is not assigned to a cluster since all responses were zero								
28 weeks	BW preen (+)	nest box	CL CS perch (+,–) rest (+,–) feed (–,+)	KB FD	forage walk	FH dust-bathe (–) drink (+)	10.53	0.70
48 weeks	BW preen (+) walk (+) forage (+) perch (+)	FD rest (+)	CL FH dust-bathe (–,–)	CS KB feed (–,–) drink (–,–) nest box (+,+)	n/a	n/a	8.50	0.57
66 weeks	BW CS preen (+,–) nest box (–,+) dust-bathe (+,–)	KB feed (–)	FH FD forage (–,+)	CL walk (–)	perch rest drink	n/a	10.53	0.70

Directional relationships between WQ parameters and behaviours within the same cluster are indicated by the '+' or '–' in parentheses. If the cluster contains more than one WQ parameter, then the parentheses contain multiple symbols, separated by a comma, representing the relationship between the behaviour and the first and second WQ parameter, respectively.
CL: claw length (cm); BW: bodyweight (kg); CS: comb score; FD: feather damage; FH: foot-pad dermatitis; KB: keel-bone score.

assessment is conducted could greatly influence the results. Behaviours requiring use of the foot (eg walking) were expected to cluster with foot health scores. However, FH clustered with foraging behaviour at 66 weeks only. Keel-bone scores never clustered with the amount of time spent perching, which suggests that keel-bone scores are not useful indicators for how much the hen is using the perch.

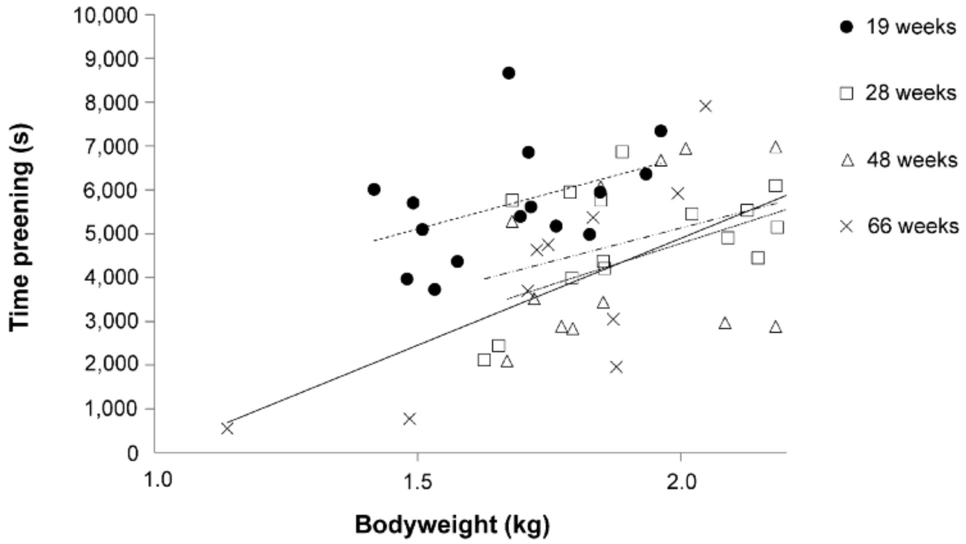
Little is known about how individual laying hens behave and perform in non-cage systems, especially as they age. As far as we know, this is the first time individual behavioural observations and the WQ assessment protocol have been used to examine laying hen behaviour and body condition across a lay cycle in a non-cage system. Furthermore, this is the first study to track the variability in response of individual hens as they age and to observe associations between a hen's body condition and her future and past behavioural profiles. Each physical measurement may provide different degrees of insight into either current or past welfare states of a hen. Each measure, therefore, requires a different interpretation with regard to the hen's welfare based upon the time it takes for the physical condition to manifest or resolve.

Behaviour

The average amount of time performing many of the activities remained unchanged throughout the lay cycle. However, variability in the performance of many of the behaviours observed increased over time among the hens, and this is where welfare concerns might exist. At 19 weeks, all hens were recorded to have spent at least some portion of their time engaged in all behaviours of interest (except dust-bathing and nest-box use). However, by 66 weeks some of the hens were never recorded feeding, drinking, preening, foraging, perching, or resting during the two consecutive, lights-on periods when these observations were made.

This would seem to suggest that some hens were either not performing certain vital maintenance behaviours at all, or they were performing them so infrequently that they were not captured with the sampling technique, or that they had shifted performance of such activities, including feeding and drinking to the dark period. However, observation of hen behaviour during the dark period showed that hens were not performing any behaviours, only occasionally transitioning between sitting and standing, and did not use any of

Figure 4



Scatterplot of the relationship between bodyweight (kg) and amount of time spent preening (s) across a 48-h period measured at different ages in the production cycle.

the resources (except the perch) during the dark period (Daigle & Siegford, unpublished data). Understanding the precise factors contributing to these observations is difficult as animals may behave differently based upon information we cannot or do not perceive (Sherwin 2007). These differences in behaviour could be related to physiological changes within the hen due to age or injury, or they could be attributed to social or environmental influences. These hens may also have been what has been anecdotally termed 'social pariahs', where they spend a large amount of their time in a single area of the environment, and venture out only to acquire food and water. While travelling to food and water, these individuals move very quickly through the environment and are repeatedly and regularly aggressively pecked by multiple conspecifics. They may even be targeted while attempting to access feed from the feeder. They make very quick trips periodically throughout the day. Therefore, their feeding behaviour is drastically altered compared to their non-socially ostracised counterparts, and their feeding behaviour may not have been accurately measured with the selected sampling technique. This social phenomenon was observed in our flock of hens, and the specific hens that were observed not feeding or drinking were also observed to be the victim of repeated aggressive attacks while attempting to access food and water (unpublished data). Therefore, hens that may not be well represented by the sampling technique may be experiencing socially or physically influenced welfare concerns. Our results highlight the importance of providing OBM information at the individual level because while the average response of the flock may appear consistent across time or treatment, differences among the hens within the same flock may vary drastically. Many of the assessed activities (eg feeding, drinking, resting) could be considered ultimate needs (Alcock 2009). These ultimate needs must be met to ensure an animal's survival, regardless of environmental condition or social status and could be expected to be performed at a similar rate no matter how different the experiences, preferences, or perceptions were for the individual hen (Dawkins 1983). Brown laying hens housed in a perchery (Channing *et al* 2001) and in conventional cages (Freire *et al* 1999) exhibited similar patterns of feeding and drinking behaviour to what was observed in our study. Therefore, a lack of change in the average amount of time these behaviours were performed was not unexpected since hens, regardless of internal or external pressures, are driven to perform behaviours required for survival.

Other activities important to hen welfare, including preening (Nicol 1989), dust-bathing (Black & Hughes 1974; Vestergaard *et al* 1997), and foraging (Bubier 1996; Browne *et al* 2011), could be considered proximate needs, which are important for hens to perform (again, regardless of social status or environmental condition) though less vital to survival. Because the performance of behaviours linked to proximate needs is driven by internal motivation (Jensen & Toates 1993), and this motivation may be driven by the behaviour's contribution to the individual's overall fitness (Akcay *et al* 2009), they are important to the hen to perform. Variations in their performance may provide insight into individual hen frustration or satisfaction. Yet, since these behaviours are not required for survival, hens may be willing to compromise their ability to perform them or alter how often or when they are performed in exchange for access to necessary resources, to avoid social stress, or to fulfil ultimate needs.

WQ scores

As with individual behaviour, variability in physical condition, as assessed by WQ parameters, increased with age. This illustrates that many hens began the lay cycle in similar physical condition. However, as they aged they were subjected to environmental and social pressures that led to increased variability with regard to behaviour performance and subsequent physical condition. Further, hens may not grow and develop at similar rates (eg slow comb development in some hens), and this inherent physical difference may drive the hen's behaviour. The hen's physical condition could impact a hen's social status thus forcing the hen to perform behaviours differently, in different rates, or develop new behavioural patterns to adapt to her behavioural repertoire to maximise survival.

For all WQ parameters assessed, measurements at 19 weeks differed from those at a later age. This highlights the impact of age on physical condition and is consistent with previous reports of changes in plumage score, claw length, bumble-foot prevalence, comb wounds, and keel-bone deformations as hens age (Wahlstrom *et al* 2001). Physical changes to animals that impact welfare may occur at drastically different rates. Measurements taken today could be reflective of either an immediate welfare concern or may be indicative of behaviour and environmental conditions in previous weeks or months. A keel break may happen instantly, while a keel deviation may take several weeks of repeated perch use and keel remodeling to develop. Foot-pad dermatitis can take up to a week to manifest but may not be immediately apparent during early stages of development. Claws take time to grow and require repeated wear and use to stay short. An aggressive encounter can change a hen's comb-pecking score within minutes, and likewise a hen's feather condition. Thus, each WQ score may be able to provide information on a unique time-scale about a hen's behaviour, and each may have different utility in assessing immediate, past, or potential future welfare concerns.

Keel-bone scores increased with age, in accordance with previous studies investigating keel-bone condition in laying hens (Fleming *et al* 2004). Yet, the interpretation of keel-bone scores can be challenging because the keel bone is cartilaginous and capable of remodeling. However, the WQ scoring system gives hens with keel-bone deviations and hens with broken keels the same score, even though the degree of pain — and therefore impact on welfare — experienced by a hen with a keel-bone deviation compared to a keel-bone break is unknown. Hens that have developed a keel-bone deviation due to perch use may not receive the same score later in life if their perch use changes and their keel bone subsequently remodels. However, as some of the alternative four-point, keel-scoring systems require a histological examination of the keel bone, and identifying differences between moderate and slight deformities can be subjective (Vits *et al* 2005; Scholz *et al* 2008), the WQ palpation method provides an assessment tool that can be done in an on-farm setting without the need for euthanasia and reduces observer bias.

Associations between behaviour and WQ scores

Several behaviours did not cluster with WQ scores as expected. We anticipated that foot-pad dermatitis would have clustered with walking and foraging. Yet, foot-pad dermatitis clustered with foraging behaviour at 66 weeks only. Perch use and keel-bone damage were expected to cluster together, however, these two parameters were never in the same cluster at any age. Clustering of preening, dust-bathing, and comb scoring was also expected, however, comb scores clustered with dust-bathing and preening at 66 weeks only. A relationship between plumage damage score and preening behaviour was expected, however these parameters were never clustered together. Furthermore, plumage damage score and claw length did not exhibit any patterns with regard to which behaviours were in the same cluster.

A consistent negative relationship was observed between feeding behaviour and keel-bone score which could lend support to the claim that chickens can feel pain (Gentle 2001), keel-bone fractures are painful (Nasr *et al* 2012) and the presence of pain, or physical impairment, may result in reduced feed intake or altered behaviour. Lame dairy cows spent less time feeding, fed less often, and ate at a slower rate than non-lame cows (González *et al* 2008). Brown leghorn hens injected with naloxone hydrochloride, an opioid inverse agonist that inhibits pain-lowering endorphins, performed less feeding behaviour than hens injected with saline (Wylie & Gentle 1998). Broken keel bones can cause an inflammatory response, catalysing the release of inflammatory cytokines, which have been associated with a dose dependent decrease in feeding behaviour (Larson & Dunn 2001). An inverse relationship between feeding and keel-bone condition was observed by Nasr and colleagues (2013), where hens with keel-bone fractures ate and drank more than hens without keel-bone fractures even though they were less productive. The productivity of individual hens was not recorded in this study, however, our differing results may be due to differences in coping style to pain and stress among strains (Lohman Brown vs HyLine Brown), or may be indicative of differences in pain sensitivity. However, Morgan's Canon of Interpretation could argue that hens spent less time feeding because they were using the perch more and could not physically feed and perch simultaneously.

The positive association between preening and bodyweight suggests that heavier hens may be more comfortable and experiencing a positive affective state. Preening has been associated with comfort behaviours (Nicol 1989) and is usually performed more when hens are near familiar conspecifics. Furthermore, hens were more likely to choose the environment during a preference test that they had spent more time preening during non-testing periods (Nicol *et al* 2009), supporting the theory that preening can be an indicator of a positive affective state. Increased stress levels have been associated with decreased weight gain, reduced feed efficiency, and reduced feather regrowth. European starlings (*Sturnus vulgaris*) experiencing physical stress (eg feed restriction) had impeded feather regrowth after molt, and birds experiencing both physical (eg feed restriction) and psychological stress (eg one of the following: restraint, cage disturbance, music, or a roller cart and voice for 30 min four times per day) displayed delayed initiation of feather replacement, feather omission, improper calamus development, premature arresting of feather growth and feather dropping (Strochlic & Romero 2008). Heavier hens may have more access to feed and subsequently avoid the combination of a physical (eg the need for nutrients) and psychological (eg hunger and frustration) stressor. Therefore, hens with higher bodyweights may be experiencing a more positive affective state from satiation as well as acquiring the nutrients required for proper feather growth and good feather condition — which could be reflected in higher preening levels.

Even though a predictive statistical model was not used, links were found to exist between certain behaviours and body conditions across time. Identifying whether the body condition caused the behaviour, or the behaviour resulted in the body condition is akin to identifying whether the chicken or the egg came first. Behaviours that are performed and become part of an established behavioural pattern may have physiological consequences that are not immediately identifiable and health issues that arise and resolve (eg keel-bone fracture, foot-pad dermatitis) could impact future behavioural patterns (eg perching and walking behaviour) thus altering how the hen spends its time due to physical limitations.

Conclusion

Welfare assessment protocols must take a group approach due to practical constraints such as the time and resources required to handle each individual. As more research is conducted with the WQ assessment protocols, an epidemiological approach will facilitate our understanding of what should be expected for hens housed in different environments. With this type of information, we may be able to determine an Animal Welfare Potential. If many of the individuals assessed are found to have a high level of welfare, then the potential for animals to have a high level of welfare is present. And, of course, the converse would be true if animals with low levels of welfare are found. Further, resource availability and management practices may provide opportunities for welfare to be high, but we cannot guarantee good welfare for all animals in the environment without assessing all of the individuals housed within. Assessing welfare at the group level gives no insight into the welfare of the individual — and providing individual hen information can help us to make comparisons across groups as to whether the potential for good welfare is higher or lower among different groups. The Animal Welfare Potential, which includes the average and variation of OBM responses, would provide useful information about the welfare state of the animals while using language that would provide more depth of knowledge about how all of the animals are performing, not just how the average animals are performing. These changes could identify facilities where the potential for the animals to experience a high level of welfare is high, even though not all of the hens housed within have a guarantee of good welfare due to factors they impose upon themselves.

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