The Aviary Transect—a practical welfare assessment tool to improve the management of cage-free laying hens

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ABSTRACT The Aviary Transect (**AT**) is a method for assessing welfare in cage-free laying hen flocks, and comprises standardized walks along each aisle screening the flock for selected welfare indicators: feather loss (**FL**) on head, back, breast, and tail, wounds on head, back, tail, and feet, dirty plumage, enlarged crop, sickness, and birds found dead. The method is quick (20 min for a flock of 7,500 hens), has good interobserver agreement and shows positive correlations with individual bird sampling methods. However, it is less clear whether AT can be used to detect differences in flock health and welfare related to housing and management. The aim of this study was to evaluate how AT findings varied in relation to 23 selected housing, management, environmental, and production factors. The study was conducted on 33 commercial nonbeak-trimmed, white-feathered layer flocks of similar age (70−76 wk) kept in multitiered aviaries in Norway. The most prevalent findings across flocks were feather loss on the back (mean 0.97% of flock) and breast (0.94%) followed by feather loss on the head (0.45%) and tail (0.36%) with differences in feather pecking damage according to the hybrid used ($P < 0.05$). Better litter quality was associated with a lower prevalence of feather loss on the head and breast ($P < 0.05$), and addition of fresh litter during the production cycle resulted in fewer birds with feather loss on the head ($P < 0.05$) and tail ($P < 0.001$). Lower dust levels were linked to a lower prevalence of feather loss on the head, back, and breast ($P < 0.05$), and when access to the floor area underneath the aviary was provided at an earlier stage of production, fewer birds had wounds ($P < 0.001$), but more birds were observed with an enlarged crop ($P < 0.05$) and found dead ($P < 0.05$). In conclusion, findings from AT showed that results of the assessment varied according to housing conditions. These results support the validity of AT as a relevant welfare assessment tool for evaluating cage-free management practices.

Key words: laying hen, aviary housing, animal welfare, welfare assessment methodology

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INTRODUCTION

Noncage housing systems for laying hens, such as multitiered aviaries, are increasingly used across Europe and North-America, with the numbers expected to increase in Europe in the upcoming years as a result of the “End the Cage Age” European Citizens’ Initiative (www.endthecageage.eu). Noncage housing systems offer laying hens opportunities to roam in larger spaces (Rodriguez-Aurrekoetxea and Estevez, 2016) and to perform more natural behavior compared to enriched cages (Widowski et al., 2016). However, aviary systems can also present welfare challenges for the hens, including a higher risk of poor plumage (Heerkens et al., 2015), damaging feather pecking (Lay et al., 2011), high levels of dust and ammonia (David et al., 2015) and mortality (Rodenburg et al., 2008; Weeks et al., 2016), especially in nonbeak-trimmed birds (Sepeur et al., 2015), although increased experience with cage-free systems has been associated with reduced mortality (Schuck-Paim et al., 2021). There are several existing welfare assessment methods used to screen layer flocks, including Welfare Quality (Welfare Quality, 2009; Van Niekerk et al., 2012), LayWel (Tauson et al., 2005; Blokhuis et al., 2007), AssureWel (Main et al., 2012), and NorWel (Vasdal et al., 2022). However, a potential weakness of these methods is high time requirement or limited sample size. Visual assessment of every bird in large flocks is often considered too time

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consuming for practical use. A larger sample size of evaluated birds may, nevertheless, provide a more reliable estimate of the condition of the flock (e.g., plumage condition; Bright et al., 2006).

Previous studies have shown that transect sampling is a practical, time efficient and reliable method for on-farm assessment of animal-based welfare indicators in large flocks of broilers (Marchewka et al., 2013; BenSassi et al., 2019a,b), turkeys (Marchewka et al., 2015, 2019, 2020; Ferrante et al., 2019; Vasdal et al., 2021), and ducks (Abdelfattah et al., 2020). The transect method is based on line transect sampling methodology, where an assessor walks through the house along predetermined paths while counting the number of birds observed within each welfare indicator category. The method requires no animal handling and allows for visual assessment of the entire flock or a representative proportion of it (Marchewka et al., 2013, 2015). It resembles the daily flock checks conducted by farmers and is therefore easy to apply.

In a previous study, Vasdal et al. (2022) presented the Aviary Transect (**AT**) as a method for assessing the welfare of cage-free laying hen flocks. They adapted the methodology used for evaluating broilers, turkeys and ducks to the sampling of laying hens in 3-dimensional aviary housing systems. Twelve welfare indicators were evaluated using this method, including feather loss, wounds, dirtiness, sick birds, and dead birds. These deficiencies were selected as they are known to be relevant for the health and welfare status of laying hens (Blokhuis et al., 2007; Rodenburg et al., 2008), and can have strong interobserver reliability (Decina et al., 2019). All indicators were scored on a binary scale, focusing on the presence or absence of relatively severe rather than mild cases. In this way, interobserver reliability can be improved (D'Eath et al., 2012; Main et al., 2012; Marchewka et al., 2013, 2015), surveillance time is optimized, and the risk of omitting birds is minimized.

Vasdal et al. (2022) compared the AT (whole flock, binary scale) with the AssureWel (3 indicators on 50 birds/flock, graded 0−2 scale and 5 whole-flock indicators) and NorWel (8 indicators on 50 birds/flock, graded 0−2 scale) methods in 6 flocks of cage-free laying hens. All 3 methods took approximately 20 min each, and there was better agreement between AT and both AssureWel and NorWel than between AssureWel and NorWel. Furthermore, the AT method was better able to detect less common welfare issues such as wounds, suggesting that it is a more sensitive method. AT also had good interobserver agreement, with dirty birds being the only indicator with inconsistent results between observers. However, there is still a knowledge gap concerning whether transect sampling in cage-free laying hen flocks can reveal quantitative differences in flock welfare according to differences in housing conditions (e.g., air quality, litter quality, provision of environmental enrichments). BenSassi et al. (2019a) found that data from transect sampling varied with environmental inputs and production outcomes in broiler flocks, supporting the validity of transect sampling for practical broiler chicken welfare assessment. Similar investigations are needed to validate the AT method for laying hen flocks.

The aim of this study was to evaluate AT findings in relation to 23 selected housing, management, environmental, and production variables. We hypothesized that factors related to flock management (e.g., provision of multiple types of environmental enrichment, addition of fresh litter, increased time spent in the animal room) and quality of the environment (e.g., better litter and air quality) would be associated with better welfare outcomes as assessed by AT.

MATERIALS AND METHODS

Animals and Housing

The study was conducted between November 2020 and June 2021 on 33 commercial farms located in eastern Norway. The studied flocks (1 flock/farm) were randomly selected from the supplier lists of 2 egg packing companies and were visited once between the ages of 70 and 76 wk. Farmers were contacted a few weeks before the visit, and participation in the study was optional. All flocks comprised white-feathered hens (Dekalb White, $n = 9$; Lohmann LSL, $n = 24$) with intact beaks, housed in indoor multitiered aviary systems. The flocks were managed according to standard practices with regards to feed, water, ventilation, litter, and lighting (KSL, 2020). All farms provided litter material such as saw dust to the floor area at the start of the cycle. The pullets arrived at the farm at around 16 wk of age and were kept until approximately 78 wk.

The flock size ranged from 5,300 to 19,000 birds, and all flocks were housed in fully enclosed houses with automatic feeding, mechanical ventilation, and artificial lighting. None of the houses had windows. The houses were typically around 12 m wide, with concrete floor and wood shavings litter, covering a floor area ranging from 385 m^2 to 1,200 m^2 that extended around and under the tiered aviary structures. There were 5 different aviary systems across the 33 farms: Big Dutchman Natura Step (Big Dutchman, Vechta, Germany; $n = 20$), Landmeco Harmony (Landmeco A/S, Ølgod, Denmark; $n = 6$), Vencomatic Bolegg Terrace (Vencomatic, Krieger AG, Ruswil, Switzerland; $n = 4$), Jansen Comfort 2.0 (VDL Jansen, Barneveld, The Netherlands; $n = 2$), and Fienhage CL (Fienhage Gmbh, Lutten, The Netherlands; $n = 1$). All aviary systems had a similar layout, with 3 tiers above the littered floor, feed and water lines located on tiers 1 and 2, nest boxes on tier 2, and perches on tier 3.

Data Collection

Because the study involved no experimental manipulations or invasive procedures, it was exempt from approval of animal use by the Norwegian Food Safety Authority (Norwegian Regulations on Use of Animals in Research, 2015). Three observers with extensive poultry experience conducted the assessments, 1 observer per flock. Before data collection started, the 3 observers visited 4 laying hen flocks together to practice the methods and they achieved a high level of agreement in scoring, as previously reported (Vasdal et al., 2022). Data collection was then conducted on the 33 farms recruited to the study. Each visit began with a discussion with the farmer about the project goals and data collection procedure. Information on factors related to the farmer's management of the flock was obtained at this time. Upon entering the animal room, the observer made measurements of the housing environment, and then collected bird welfare data using the Aviary Transect method.

Flock Management and Production Factors

As part of the initial conversation with the farmer, the following factors related to the farmer's management and production were recorded per flock: bird hybrid, number of birds placed, number of people managing the flock, average number of minutes spent in the animal room each day, number of previous cage-free flocks kept (as an indicator of farmer experience), whether or not fresh litter was added during the production cycle, how many different types of environmental enrichments were provided, number of roosters present in the flock, bird weeks of age when the floor area underneath the aviary was opened, how many times the manure belts were run each week, and red mite status of the flock (present, absent, or unknown). The feed intake (g/hen) and water intake (mL/hen) on the day before the visit, and mortality until day of the visit (%), were also recorded (Table 1).

Environmental and Housing Factors

The following factors related to the environment were recorded at 4 different locations in the animal room (from 2 locations near the walls and 2 locations near center, with birds from all levels being evaluated in each location) light intensity at bird height (lux—Extech LED meter LT40, FLIR Commercial Systems Inc., Nashua, NH), NH_3 (ppm—Dräger Pac 8000 Single-Gas Ammonia Detector, Dräger, Lübeck, Germany), litter quality score (0—dry/friable, 1—partly wet/crusted, 2—completely wet/crusted/no litter present), and CO_2 (ppm—Extech CO240 Handheld Indoor Air Quality CO_2 Meter, Extech Instruments, Nashua, NH). For each factor, a mean for the 4 locations was calculated. The amount of dust in the room was scored in 1 location from 0 (no dust) to 4 (thick layer of dust), using the dust sheet test as described in Welfare Quality® (2009). The

Table 1. Descriptive data from 33 laying hen flocks on explanatory variables assigned to 2 statistical models.

Explanatory variable	Categorical variable level	Mean, n[1]	S.E.	Minimum	Maximum
Model 1—Housing and management					
Hybrid (n)	Dekalb White	9 flocks	-	-	-
	Lohmann LSL	24 flocks	-	-	-
Birds placed (n)	-	7985.0	377.2	5300	19004
Aviary structure width (cm)	-	217.3	4.7	170	270
Aviary structure height (cm)	-	258.3	5.6	220	340
Ceiling clearance (cm)	-	144.9	22.9	20	760
People managing flock (n)	1–2	25 flocks	-	-	-
	3	8 flocks	-	-	-
Time in animal room (min/d)	-	54.1	5.6	15	180
Previous cage-free flocks (n)	-	6.4	1.4	0	40
Litter added during production	No	10 flocks	-	-	-
	Yes	23 flocks	-	-	-
Enrichment types provided (n)	-	4.5	0.1	2	5
Roosters (n)	-	1.5	0.3	0	6
Area under aviary opened (wk)	-	23.2	2.6	16	78[3]
Manure belt runs (n/wk)	1	20 flocks	-	-	-
	2–3	13 flocks	-	-	-
Light intensity at bird height (lux)[2]	-	4.7	0.6	0.5	12.4
Model 2—Environment and production					
Dust score (0–4)[5]	0	22 flocks	-	-	-
	1–2	11 flocks	-	-	-
NH_3 (ppm)[2]	-	8.9	2.2	0	57.0
Litter quality score (0–2)[2,4]	-	0.1	0.1	0	1.3
Feed intake on day before visit (g/hen)	-	118.3	1.4	105.0	138.0
Water intake on day before visit (mL/hen)	-	193.7	3.79	151.0	266.2
Mortality up to day of visit (%)	-	3.5	0.3	1.5	9.0
Other factors[6]					
Red mite status	Absent	27 flocks	-	-	-
	Present	2 flocks	-	-	-
CO_2 (ppm)[2]	-	1644.4	160.4	818.7	4666.7

[1]Mean (numerical variables), number of flocks (categorical variables).
[2]Mean from 4 locations/house.
[3]Not opened in 2 flocks so age set to age at depopulation (maximum possible).
[4]0—dry/friable, 1—partly wet/crusted, 2—completely wet/crusted).
[5]0—no dust to 4—thick layer of dust (Welfare Quality®, 2009). Two flocks scored 2, none 3 or 4.
[6]Excluded from Model 2 due to missing flocks (red mites, 4 flocks; CO_2, 5 flocks).

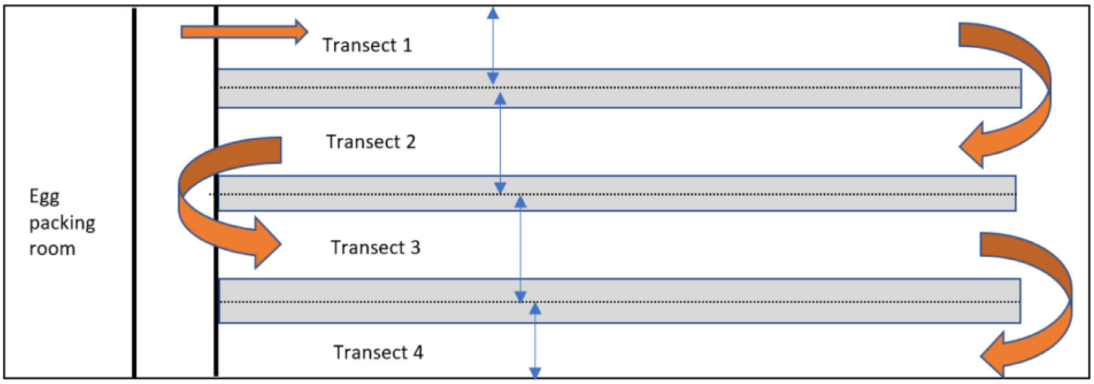

Figure 1. Schematic overview of a hen house (2-dimensional horizontal view, not to scale) showing 3 aviary structures (gray), transect width (blue arrows, dotted lines) and an example of a path taken by observers (orange arrows). Figure first published in Vasdal et al., 2022.

aviary structure width (cm) and height (cm) were determined, and the ceiling clearance was measured with a laser measurer (Bosch Zamo II, Bosch, Gerlingen, Germany) as the distance (cm) between the top of the aviary structure and the lowest point of the ceiling (Table 1).

Aviary Transect Method

There were 2 to 4 rows of tiered structures along the length of the room, with each aisle designating a different transect. Data were collected from the wall transect along each building side, and up to 2 central transects, for a total of 3 to 4 transects in the different houses. Following the method of Vasdal et al. (2022; Figure 1), standardized transect walks were conducted along the full length of the house to record the number of hens observed per transect that were showing each of 12 predefined welfare indicators (Table 2). The aisle width of each transect was measured with a laser measurer (Bosch Zamo II) from the wall to the aviary structure (for wall transects) or between 2 aviary structures (for central transects). The transect area assessed during each transect walk comprised the littered floor area in the aisle as well as half the width of the space under the aviary structure, and on each tier of the structure, on one side of each wall transect, and on both sides of each central transect. The observations always started with the left wall transect. When reaching the other end of the house, the observer returned collecting data in the following transect, and so on. While walking along each transect, stops were made as needed to allow assessment of birds on the floor underneath the aviary structures, and on all 3 tiers. Birds in nest boxes were observed by opening the curtains on around half of them. To observe birds on the top tier, the observer used steps or platforms on the side of the structure. To estimate the number of birds sampled, the total number of birds in the house was divided by the total width of the sampled transects, assuming that birds were homogeneously distributed throughout the length and width of the house. The AT walk took between 16 and 25 min to complete, depending on the flock.

Statistical Analyses

The welfare indicator outcomes were analyzed on a flock basis in SAS 9.4, with the bird count for each variable expressed as a proportion of the total estimated number of birds sampled (i.e., estimated number in area of house sampled assuming uniform distribution). Because wounds to any one body part were rare, these counts were summed to produce a pooled wounds variable prior to analysis. To avoid multicollinearity, 2 models were evaluated using Proc Logistic, 1 focused on the housing and management variables (Model 1) and 1 focused on environmental and production variables (Model 2; Table 1). To avoid loss of flocks from the sample, means imputation was used to address occasional missing explanatory data in a maximum of 2 flocks per variable. Because red mite status was unknown for 4

Table 2. Description of 12 welfare indicator categories assessed by the Aviary Transect (AT) method (as in Vasdal et al., 2022).

Indicator[1]	Description
FL head	Missing feathers on the head, including the neck, ≥5 cm in diameter
FL back	Missing feathers on ≥50% of the back, including the wings
FL breast	Missing feathers on the breast, ≥5 cm in diameter
FL tail	Missing or clearly damaged feathers on the tail, mainly shafts and rachises left
Dirty	Prominent dark staining of the back, wing, or tail feathers, covering at least 25% of the body; not including light discoloration of feathers from dust
Wounds head	Prominent marks on the head and neck, due to fresh or older wounds
Wounds back	Prominent marks on the back, including the wings, due to fresh or older wounds
Wounds tail	Prominent marks on the tail due to fresh or older wounds
Wounds feet	Includes bumblefoot (visible dorsally), and prominent marks on the feet due to fresh or older wounds
Enlarged crop	Pendulous crop hanging in front of the breast
Sick	Clear signs of impaired health, including a small and pale comb, red, watery eyes, disarranged feathers, missing or deformed body parts, and clearly different (pale or yellowish) skin color; often found in a resting position
Dead	Dead bird found when walking along a transect

[1]Hens could be classified as belonging to more than 1 category; FL—feather loss.

flocks, and CO_2 levels were missing for 5 flocks, these variables were excluded from analysis. For 2 flocks in which the area under the aviary structures was not planned to be opened, the age when opened was set to 78 wk (age at depopulation). In addition to bird hybrid (Dekalb White vs. Lohmann LSL) and litter addition (no vs. yes), the number of people managing the flock (1−2 vs. 3) and number of manure belt runs per week (1 vs. 2−3) were categorical explanatory variables in Model 1, and dust level (0 vs. 1−2) was a categorical variable in Model 2. This was due to the limited range of these variables, and sparsity of flocks at 1 or more levels. All other explanatory variables were evaluated as continuous numerical variables (averaged across transects). Based on the number of farms in the sample relative to the number of explanatory variables, we restricted our models to main effects. We used logistic stepwise regression to fit the final models, with a 0.3 significance level for entry into the model and a 0.35 significance level for removal (SAS Institute Inc., 2016). The deviance scaling option was used to address overdispersion.

RESULTS

Descriptive data for the explanatory factors related to housing, management, environment, and production in the 33 assessed flocks are presented in Table 1. The prevalence of each AT welfare indicator in the 33 assessed flocks is presented in Table 3. The most common findings across flocks were feather loss on the back (0.97%) and breast (0.94%) followed by feather loss on the head (0.45%) and tail (0.36%). Few birds with wounds were observed across the flocks: 1 bird with a wound on the head, 3 birds with wounds on the back/wing, 2 birds with a wound on the tail and 12 birds with foot wounds.

Relationships of Housing and Management Factors With AT Outcomes

In Model 1, the factor associated with the largest number of welfare indicators was layer hybrid. Flocks of Dekalb White were observed to have a lower prevalence of feather loss on the head ($P < 0.01$), back ($P < 0.05$), and tail ($P < 0.01$), and enlarged crop ($P < 0.05$), while flocks of Lohmann LSL had fewer dirty birds ($P = 0.01$; Table 4). Smaller flocks (with a lower number of birds placed) were associated with a lower prevalence of enlarged crops ($P < 0.05$) and sick birds ($P < 0.01$). A lower ceiling clearance was associated with a lower prevalence of feather loss on the head ($P < 0.01$) and wounds ($P < 0.01$), while wider aviary structures were related to a lower prevalence of birds found dead ($P < 0.05$). However, more time spent by the farmer in the animal room was associated with a higher prevalence of feather loss on the back ($P < 0.01$) and tail ($P < 0.01$), and more birds with wounds ($P < 0.001$). No significant associations were detected between AT findings and aviary structure height, number of people managing the flock, or number of previous cage-free flocks kept.

Adding fresh litter during the production cycle was associated with a reduced prevalence of feather loss on the head ($P < 0.05$) and tail ($P < 0.001$) but a higher prevalence of dirty birds ($P < 0.05$; Table 4). Farmers in the current study provided up to 5 of the following types of environmental supplements aimed at environmental enrichment: grain scattered in the litter, pecking stones, oyster shells, gravel, and "toys." Providing more enrichment types was linked to fewer dirty birds ($P < 0.05$) as well as fewer birds found dead ($P < 0.05$), although more birds with wounds ($P < 0.001$). The number of roosters in the flock was associated with feather loss on the back ($P < 0.01$) and number of dirty birds ($P < 0.05$), whereas earlier access to the floor area underneath the aviary was associated with fewer birds with wounds ($P < 0.001$) but more birds with an enlarged crop ($P < 0.05$) or found dead ($P < 0.05$). A higher frequency of manure belt runs per week was related to fewer birds observed with enlarged crops ($P < 0.01$) and fewer birds found dead ($P < 0.05$). Further, when the light intensity was set at higher levels, it was related to a higher prevalence of dirty birds ($P < 0.001$), birds with an enlarged crop ($P < 0.05$), and sick birds ($P < 0.05$), but fewer birds with wounds ($P < 0.001$). No significant associations were detected between the explanatory variables in Model 1 and the prevalence of feather loss on the breast.

Table 3. Mean welfare indicator prevalence (% of birds affected/flock) detected by the Aviary Transect method ($n = 33$ flocks).

Welfare indicator[1]	Mean (%)	S.E.	Minimum	Maximum
Feather loss head	0.4539	0.1008	0	2.5532
Feather loss back	0.9672	0.1778	0	4.3721
Feather loss breast	0.9399	0.2071	0	4.9822
Feather loss tail	0.3586	0.0783	0	2.0842
Dirty	0.0106	0.0054	0	0.1336
Wound head	0.0002	0.0002	0	0.0070
Wound back	0.0013	0.0007	0	0.0141
Wound tail	0.0008	0.0006	0	0.0140
Wound feet	0.0052	0.0032	0	0.1026
Wounds (sum)	0.0075	0.0033	0	0.1026
Enlarged crop	0.0405	0.0210	0	0.6205
Sick	0.0102	0.0026	0	0.0688
Found dead	0.0173	0.0095	0	0.3120

[1]Each bird scored according to presence or absence of severe deficiency or found dead; wounds on feet include bumblefoot; wounds (sum) is sum of birds found with wounds to head, back, tail or feet.

Relationships of Environmental and Production Factors With AT Outcomes

In Model 2, less dust was linked with a lower prevalence of feather loss on the head ($P < 0.05$), back ($P < 0.05$), and breast ($P < 0.05$), and fewer birds found dead ($P < 0.05$, Table 5). Lower NH_3 levels were associated with fewer birds found dead ($P < 0.01$) but more birds with feather loss on the head ($P < 0.05$). Better litter quality was associated with a reduced frequency of feather loss on the head ($P < 0.05$) and breast ($P < 0.05$). Across the flocks, a higher feed intake was positively associated with feather loss on the back

Table 4. Logistic regression table showing explanatory variables (df = 1) included in the final "housing and management" model at $P < 0.05$ after stepwise selection, with back-transformed odds ratio estimates.

Response variable[1]	Explanatory variable[2]	Chi-squared	P value	Odds ratio estimate	95% Confidence limits	
Feather loss head	Hybrid (LSL vs. Dekalb)	8.50	0.004	13.893	2.370	81.442
	Ceiling clearance (cm)	9.84	0.002	1.005	1.002	1.008
	Litter added (yes vs. no)	5.93	0.015	0.255	0.085	0.766
Feather loss back	Hybrid (LSL vs. Dekalb)	5.86	0.016	4.335	1.322	14.215
	Time in animal room (min/d)	8.94	0.003	1.014	1.005	1.023
	Roosters (n)	8.24	0.004	1.420	1.118	1.804
Feather loss breast	None $P < 0.05$	-	-	-	-	-
Feather loss tail	Hybrid (LSL vs. Dekalb)	8.57	0.003	17.869	2.593	123.121
	Time in animal room (min/d)	7.23	0.007	1.018	1.005	1.031
	Litter added (yes vs. no)	14.52	<0.001	0.138	0.050	0.382
Dirty	Hybrid (LSL vs. Dekalb)	6.61	0.010	0.075	0.010	0.540
	Litter added (yes vs. no)	5.81	0.016	15.880	1.677	150.357
	Enrichment types (n)	4.74	0.030	0.34	0.13	0.88
	Roosters (n)	5.33	0.021	1.98	1.12	3.50
	Light intensity (lux)	26.84	<0.001	1.54	1.31	1.81
Wounds (sum)	Ceiling clearance (cm)	9.24	0.002	1.005	1.002	1.009
	Time in animal room (min/d)	26.33	<0.001	1.023	1.014	1.033
	Enrichment types (n)	17.16	<0.001	10.971	3.532	34.073
	Area under aviary opened (wk)	18.66	<0.001	1.122	1.065	1.183
	Light intensity (lux)	12.41	<0.001	0.764	0.657	0.887
Enlarged crop	Hybrid (LSL vs. Dekalb)	5.82	0.016	178.090	2.642	>999.999
	Birds placed (n/1,000)	5.95	0.015	1.803	1.123	2.897
	Area under aviary opened (wk)	3.89	0.049	0.569	0.324	0.997
	Manure belt runs (2–3 vs. 1/wk)	9.49	0.002	<0.001	<0.001	0.041
	Light intensity (lux)	6.22	0.013	1.722	1.124	2.639
Sick	Birds placed (n/1,000)	7.50	0.006	1.169	1.045	1.308
	Light intensity (lux)	5.21	0.025	1.146	1.017	1.291
Found dead	Aviary structure width (cm)	4.52	0.034	0.975	0.952	0.998
	Enrichment types (n)	4.02	0.045	0.367	0.138	0.977
	Area under aviary opened (wk)	4.02	0.045	0.946	0.896	0.999
	Manure belt runs (2–3 vs. 1/wk)	6.44	0.011	0.152	0.036	0.651

[1]Each bird scored according to presence or absence of severe deficiency or found dead (see Table 3 for further details).
[2]See Table 2 for further details.

($P < 0.01$) and breast ($P < 0.01$), and both higher feed intake ($P < 0.01$) and higher water intake ($P < 0.001$) were positively associated with enlarged crops. Finally, lower mortality was associated with fewer birds with wounds ($P < 0.05$), while no significant associations were found between the explanatory variables in Model 2 and the prevalence of feather loss on the tail, dirty birds, or sick birds.

DISCUSSION

As hypothesized, there were multiple associations between the AT findings and factors related to the farmers' management and the housing environment. Below, we discuss possible reasons for these associations, bearing in mind that, as in any exploratory correlational study, controlled experiments would be needed to identify which of these associations represent causal

Table 5. Logistic regression table showing explanatory variables (df = 1) included in the final "environment and production" model at $P < 0.05$ after stepwise selection, with back-transformed odds ratio estimates.

Response variable[1]	Explanatory variable[2]	Chi-squared	P value	Odds ratio estimate	95% Confidence limits	
Feather loss head	Dust score (1–2 vs. 0)	3.86	0.049	2.181	1.002	4.747
	NH_3 (ppm)	4.45	0.035	0.923	0.857	0.994
	Litter quality score (0–2)	6.28	0.012	2.929	1.264	6.788
Feather loss back	Dust score (1–2 vs. 0)	4.18	0.041	2.080	1.031	4.199
	Feed intake (g/hen)	7.60	0.006	1.053	1.015	1.092
Feather loss breast	Dust score (1–2 vs. 0)	4.61	0.032	2.524	1.084	5.877
	Litter quality score (0–2)	3.99	0.046	2.826	1.020	7.833
	Feed intake (g/hen)	6.92	0.009	1.061	1.015	1.108
Feather loss tail	None $P < 0.05$	-	-	-	-	-
Dirty	None $P < 0.05$	-	-	-	-	-
Wounds (sum)	Mortality (%)	4.65	0.031	1.304	1.024	1.660
Enlarged crop	Feed intake (g/hen)	7.06	0.008	1.088	1.022	1.159
	Water intake (mL/hen)	45.21	<0.001	1.046	1.032	1.059
Sick	None $P < 0.05$	-	-	-	-	-
Found dead	Dust score (1–2 vs. 0)	5.18	0.023	3.967	1.210	13.001
	NH_3 (ppm)	7.62	0.006	1.053	1.015	1.092

[1]Each bird scored according to presence or absence of severe deficiency or found dead (see Table 3 for further details).
[2]See Table 2 for further details.

relationships. The most common AT findings in the flocks were feather loss on the back and breast, which affected 1% of the birds on average, with some flocks with as high as 4.6% of the birds affected. All the flocks were observed after wk 70, and plumage condition is known to deteriorate with age (Rørvang et al., 2019). However, poor plumage condition where large areas of skin are visible, as scored in the AT method, should be regarded as unwanted even at this age. Given that the hens had intact beaks, it is likely that the majority of feather loss was related to feather pecking (Lambton et al., 2010), although some feather loss on the breast might be related to abrasion.

There were 2 different hybrids used in the assessed flocks and one of them, Lohmann LSL, was more likely to have feather loss but less likely to have dirty birds. This relationship could be caused by fewer feathers to which dirt could adhere. A recent study reports more feather loss and higher mortality in commercial flocks of Lohmann LSL compared to Dekalb White (Kittelsen et al., 2022). Klein et al. (2000) reported a higher level of feather pecking in Lohmann LSL compared to Dekalb flocks, attributing it to genetic differences in foraging behavior. Differences in behavior and levels of feather pecking between hybrids are reported in multiple studies, suggesting that management recommendations should be tailored to the specific hybrid (e.g., Schreiter et al., 2020).

In most flocks ($n = 24$), the number of birds placed was close to 7,500, conforming to current Norwegian legislation limiting flock size to 7,500 hens (Forskrift om regulering av svine- og fjørfeproduksjonen, 2004). One flock was substantially smaller (5,300 birds placed) and a few were substantially larger (maximum 19,004 birds placed) due to a grandfathering agreement. Therefore, the number of flocks of different sizes was unevenly distributed, which may explain why this variable was only associated with the prevalence of enlarged crops and sick birds. These findings may be related to greater difficulty in detecting afflicted birds that should be culled in larger flocks. Flock size was confounded with stocking density as is typical of studies on commercial flocks, but all flocks had ample space (≤9 hens/m^2). Nicol et al. (2006) failed to detect flock size effects when comparing flocks of 2,450 and 4,200 hens kept at a standard high density (12 hens/m^2).

Although all flocks were housed in 3-tiered aviaries, there were some design differences between the aviaries coming from 5 different equipment companies, including variation in aviary structure width and height. Only 1 association between these variables and the AT outcomes was detected, whereby wider aviary structures were associated with fewer birds found dead. This result is in the opposite direction to what might be predicted regarding ease of finding dead birds, and may be related to other correlated but unmeasured aspects of aviary design rather than platform width per se. The ceiling clearance between the top tier of the aviary and the lowest point of the ceiling ranged from 20 to 760 cm. Greater clearance was positively associated with the prevalence of feather loss on the head and wounds. Reasons for these findings are unclear but perhaps relate to other potentially correlated variables such as light intensity or perch space on the top tier.

While we expected that a higher number of people caring for the flock might be beneficial for reducing fear of people (Barnett et al., 1993), the number of people taking care of the hens only ranged from 1 to 3 and was not associated with any of the AT measures. We also expected that increased time spent in the animal room would be positive for animal welfare outcomes, as reported by Heerkens et al. (2015). The farmers spent an average of 15 to 180 min/d in the animal room, with substantial differences between farmers. Contrary to expectations, increased time spent in the animal room was associated with an increased prevalence of feather loss on the back and tail, and wounds. This is likely because farmers who observed higher rates of feather loss and wounds in the flock spent more time observing the birds and trying different methods to mitigate these problems. We detected no associations between AT results and the number of previous cage-free flocks kept by the farmer. This was contrary to our expectation, as increased experience with cage-free systems has been reported to have a positive effect in reducing laying hen mortality (e.g., Schuck-Paim et al., 2021). Four farms in our sample had their first flock of cage-free hens, either after converting from cages, or because the house was new. However, the relationship between experience and effort put into managing the flock is not necessarily linear. While farmers inexperienced in keeping cage-free hens may need more time to perform routine tasks, factors such as age of equipment, stocking density, and disease status of the flock also influence time taken to accomplish tasks.

Provision of fresh litter during production was associated with a reduced prevalence of feather loss on the head and tail, but a higher prevalence of dirty birds. Of the 33 flocks in the study, 23 were regularly provided with fresh litter in different areas of the house. Provision of fresh, dry litter is important to prevent feather pecking, both during rearing (Tahamtani et al., 2016), and in adult hens (Lambton et al., 2013; Rodenburg et al., 2013), and the current results are consistent with those findings. Wet litter may result in dirty birds (de Jong et al., 2014), and this could be the reason why fresh litter was added in some cases, resulting in the association between adding litter and dirty birds. We also found that better litter quality was associated with fewer birds with feather loss on the head and breast, further supporting the importance of access to high quality litter for reducing the risk of feather loss. These results emphasize the importance for animal welfare of maintaining good litter quality across the entire production period in order to prevent feather loss, rather than adding litter after plumage condition has deteriorated.

As expected, we found some benefits from providing more types of enrichments, as hens with access to more types were less likely to be dirty or found dead. However, unlike BenSassi et al. (2019a), who observed that provision of more types of enrichments was associated with fewer wounds in broilers, we found the opposite. This result could have occurred if farmers in our study responded to the emergence of cannibalism by adding more enrichments. Consistent with our finding of no association between the number of types of enrichment and feather loss or mortality, Tahamtani et al. (2022), found no association between the quantity of each enrichment type and feather loss or mortality in Norwegian layer flocks. This may have been due to limited variation in provision of enrichments. Environmental enrichment is part of the laying hen welfare scheme for most companies in Norway, resulting in farmers providing enrichment of similar types and quantities. Thus, the results may not fully reflect the benefits of enrichment provision.

Our results show no evidence that the roosters were beneficial in reducing feather loss in contrast to Louton et al. (2017), who reported that the presence of roosters reduced the risk of feather pecking. Pereira et al. (2017) observed that the presence of roosters reduced mortality and altered hen behavior, but their ratio of roosters to hens was far higher (250 roosters in a flock of 4,500 hens) than in the present study. We found that the number of roosters in the flocks was associated with a higher prevalence of feather loss on the back and dirty birds. While some feather loss on the back may have been caused by the roosters, it is unlikely that mating fully explains this result as the number of roosters (0−6) was low compared to the number of hens in the flocks (average 7,985), and 9 of the 11 flocks without roosters also had hens with feather loss on the back. In addition, there was no association between the presence of roosters and feather loss on the head as might be expected if roosters were having a major impact on feather loss on the back. The latter is a commonly observed area of feather damage in laying hen flocks.

We found that earlier access to the floor space under the aviary structures was associated with a lower proportion of birds with wounds, but a higher proportion of birds with enlarged crops, and found dead. Some farmers keep this area closed off from the hens for the first weeks of the production cycle to reduce floor eggs, but a study by Louton et al. (2017) did not find such a relationship. Access to a larger littered area starting as soon as the birds were placed, as practiced in 11 of our study flocks, may have helped to avoid wounds related to cannibalism, considering that all the hens had intact beaks. Regarding enlarged crops, they were only observed in 101 birds across the flocks, with most flocks having no birds affected while only 2 flocks had more than 20 affected birds, so the relationship with age when given access to the floor under the aviaries may be coincidental. Enlarged crops were more common in the Lohmann LSL than the Dekalb White flocks and were associated with higher feed and water intake. Enlarged crops may be cases of sour crop, crop impaction or pendulous crop. Both sour crop and crop impaction are severe conditions that can lead to mortality (Lin, 2022), but we did not detect an association with mortality in the present study. The enlarged crops were more likely pendulous crops resulting from loss of muscle tone (Classen et al., 2016), leading to the crop becoming a large feed and water filled sack (Wood and Willems, 2014).

A higher frequency of manure belt runs per week was previously related to reduce feather loss (Decina et al., 2019) but we did not find any such relationship in the present study. The farmers ran their manure belts between 1 and 3 times per week, with the higher frequencies being associated with fewer birds with enlarged crops and found dead. The reason for these results is unclear but was perhaps related to improved air quality or giving the flock more attention and, thus, finding dead birds and culling birds with enlarged crops sooner.

Brighter light intensity was associated with more dirty and sick birds, and more birds with enlarged crops, but fewer birds with wounds. In general, few dirty and sick birds were observed overall, just 26 and 28 birds respectively. As Vasdal et al. (2022) reported, the dirty bird category was the only AT indicator with a relatively low interobserver agreement, and Marchewka et al. (2013) discussed that assessing dirtiness might be more influenced by the lighting conditions compared to other indicators as brighter lighting could make dirtiness more visible, especially in white hybrids. Wounds were even more rare, with foot wounds being recorded more often than wounds to other body regions. The category of foot wounds included bumblefoot in addition to broken skin, making interpretation of the wounds results challenging. Sickness could also result from multiple ailments making it difficult to interpret. Nevertheless, when accompanied by benchmarking, AT data on elevated levels of any of the indicators can serve to alert farmers to the need for closer investigation.

Air quality is an important environmental factor for laying hen welfare, and dust, NH_3, and CO_2 are common indicators used to assess air quality. Dust is often an issue in cage-free systems, especially when the litter is dry and the birds are active (Zhao et al., 2015). In addition to dust from litter material, dust includes feather and skin particles, feed components, dried fecal matter, molds, fungi, bacteria, and viruses (Sauter et al., 1981), and can be a vector for pathogens entering via the respiratory tract (David et al., 2015). In the present study, flocks with lower dust levels in the house had less feather loss on the head, back, and breast, and fewer birds found dead. A high level of dust may be a stressor that could trigger feather pecking in the hens which could explain the present results. Furthermore, high dust levels are also negative for the farmers' health, and in these cases, efforts should be put in place to safeguard both bird and human health, for instance by using a misting system (Patterson, 2005).

Surprisingly, we found that increased NH_3 levels were associated with reduced feather loss on the head, although also more birds found dead. NH_3 is known to have negative effects on the eyes and respiratory tract at concentrations of 25 ppm and above (David et al., 2015) and could be a source of stress that could further trigger feather pecking and feather loss. Drake et al. (2010)

found increased feather loss with high NH_3 and CO_2 levels in early lay. However, only 5 of our study flocks had NH_3 levels above 20 ppm, implying that high ammonia levels were not a significant issue in most flocks. Due to missing data, we were unable to include CO_2 in the analyses, but casual observation indicated that it covaried with NH_3 levels. As with NH_3, high levels of CO_2 (above 3,000 ppm) are considered aversive for the birds, but most flocks in the present study had CO_2 levels below this limit.

Higher feed intake was associated with a higher prevalence of feather loss on the back and breast. Hens with poor plumage are known to increase their feed intake to compensate for heat loss (Glatz, 2000), and this is likely the cause of the higher feed intake in Lohman LSL flocks, that also had higher levels of feather loss. Wounds were associated with mortality, which would not be surprising if the main reasons for wounds were cannibalism and injuries sustained during navigation up and down the aviary. Red mites were present in 2 of the flocks, while the status of 4 other flocks was unknown, resulting in exclusion of this variable from the analysis. Previous work has indicated an association between red mites and feather loss (e.g., Heerkens et al., 2015), indicating the value of monitoring for them.

The AT method simulates the way a farmer would normally check a flock, and with relatively brief training of those already experienced in detecting hens with health issues, can be performed by farmers, veterinarians, farm advisors, and independent farm auditors. With benchmarking, the numbers obtained for the different welfare indicators can be useful for detecting changes in the welfare status of a flock, prompting closer inspection when an indicator deviates from expected levels. Our choice of welfare indicators emphasized feather loss, but the indicators included in a welfare assessment protocol could be adjusted to focus on welfare conditions of particular interest in different regions or companies, providing that good interobserver reliability can be achieved. Mobile phone applications, such as the i-WatchBroiler (Estevez, 2015) exist for entering data during transect sampling in broiler flocks and these can be modified for use in cage-free egg production facilities. While the flock sizes were modest in our study, the method could be implemented in larger flocks by sampling a representative area of the house and recording the length and width of the sampled area and the number of birds currently in the house. By assuming an even distribution of birds across and along the house, the number present in the sampled area can be estimated and used to calculate the prevalence of each welfare indicator. The sampled area should be determined based on the range in frequencies of the most rare indicators, so that enough birds are sampled to detect the variation across flocks, and should include all tiers, under the aviary and the aisle space within the area. We estimate that sampling approximately 10,000 hens per flock would take around 30 min to complete and would likely suffice in most cases.

In conclusion, the most prevalent findings across flocks were feather loss on head, breast, back and tail, which were associated with management and environmental variables such as addition of fresh litter, dust levels and litter quality. The results show that data collected using the AT method varied with aspects of the housing, management, environment, and production, adding support for the validity of AT as a practical and valuable tool for assessing the welfare of cage-free laying hen flocks and relating the results to farmers' management decisions and environmental factors.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the present study.

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