

Welfare assessment in broiler farms: Transect walks versus individual scoring

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ABSTRACT Current scientific approaches to welfare assessment in broilers are based on individual sampling that can be time consuming under field conditions. On the other hand, farmers conduct routine checks based on walks through the house to screen birds' health condition. We adapted the walks through following line transect methodology used in wildlife studies to explore their feasibility as a welfare assessment tool. The aim of this study was to compare broiler welfare assessed by individual sampling and transect walks. We evaluated 6 identically managed flocks. For individual sampling, we collected measures on 150 birds, including weight, breast dirtiness, hock and footpad dermatitis, lameness, and immobility. Transect observations were conducted by slowly walking on randomized paths within each house recording: immobility, lameness, back dirtiness, sickness, agony, and dead. Transect walks allowed detection of small variations ($P < 0.003$) in the prevalence of most welfare indicators considered with consistency in interobserver reliability ($P \geq 0.05$). In addition, assessments across transects were highly consistent ($P \geq 0.05$). Individual sampling was also sensi-

tive to differences across houses ($P < 0.01$) with the exception of immobility ($P = 0.783$). No differences were found across sampling locations ($P \geq 0.05$). However, both methods differed greatly in the frequency of the incidence of the parameters considered. For example, immobility varied from $0.2 \pm 0.02\%$ for transect walks to $4 \pm 2.3\%$ for individual sampling, whereas lameness varied between $0.8 \pm 0.07\%$ and $24.2 \pm 4.7\%$ for transect and samplings, respectively. It is possible that the transect approach may have overlooked walking deficiencies because a large number of birds were scored, although if this was the case, the consistency obtained in the scoring across observers and transects would be surprising. Differences may also be related to possibly biased individual sampling procedures, where less mobile and passive individuals may be more likely to be caught. Furthermore the procedure may cause fatigue and fear reactions reducing mobility. Current study provides new insights into constraints and advantages of broiler on-farm assessment methods, which should be considered for designing on-farm welfare assessment protocols.

Key words: welfare indicator, on-farm assessment, broiler, sampling, transect

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INTRODUCTION

Animal welfare has wide-ranging implications for animal-based companies in the global market as it plays an increasingly important role granting competitive advantage for companies presenting better welfare, and sustainability of commercial animal production. A growing number of countries have adopted specific legislation to ensure the welfare of farm species, although often verification of requirements imposed is difficult and expensive (2% of the sector's value in the European Union; EU Commission, 2012). Additionally, other countries such as the United States have certified voluntary wel-

fare programs. The need to develop protocols to evaluate animals on-farm with regard to their welfare status was raised by Rousing et al. (2001) and Webster et al. (2008), and some are already available (Welfare Quality, 2009). These protocols should be characterized by scientific soundness and the possibility of being applied on a commercial farm within a realistic time framework and ultimately becoming a relevant tool to support the decision-making process. In this regard, protocols that are easy to understand to producers, flock supervisors, and farmers would have better possibilities of being adopted. This could be achieved by designing welfare assessment protocols somewhat closer to animal care management procedures conducted by veterinarians and farmers.

Currently, most scientists agree with the need for designing protocols based on the animal (Main et al., 2007). The use of animal-based welfare indicators is

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recognized at international level by organizations such as the World Organization for Animal Health (OIE, 2003). The Welfare Quality assessment protocol (Welfare Quality, 2009) is one of the most recently proposed approaches for on-farm assessment. This protocol has been thoroughly designed, considering all living and welfare requirements of particular species. However, it requires further work with regard to time and labor efficiency as suggested lately by producers (De Jong et al., 2012a). Protocols based on scientifically and practically acceptable methodology become especially challenging when the production systems require keeping large numbers of animals in a common housing, as is the case in broiler production.

The welfare of broilers can be challenged by multiple factors such as by their genetic potential for growth, decline of environmental quality, poor management, or excessive density (Dawkins et al., 2004; Estevez, 2007), which may result in contact dermatitis, metabolic, skeletal and muscle disorders, or behavioral abnormalities (Dawkins et al., 2004; Estevez, 2007; Meluzzi et al., 2009). Besides the great impact of the welfare status on the birds, all these problems have a major economic relevance for industry. For example, in the United States, skeletal problems result in losses to the industry of \$200 million each year (Donoghue, 2012). Therefore, the control of these problems not only would contribute to a better accountability of bird welfare, but also to a higher efficiency of industry.

To ensure proper bird care and welfare, farmers and flock supervisors conduct routine checks based on walks through the broiler production house to screen the health status of the flock. This method distinguishes individuals with visible severe welfare issues, provides a quick estimation of general flock health and welfare status, and usually gives bases for future management decisions. It is generally performed in a way to minimize frightening or interrupting the birds. No direct contact with individuals is included, only visual, which is feasible for evaluation of welfare indicators such as lameness, immobility, back dirtiness, sickness, agonizing, or dead birds. Although this noninvasive method is well accepted by producers, it does not provide them with quantitative data to make reasonable comparisons across the health and welfare status of the birds across farms, or successive flocks of birds within a house.

To date, most scientific assessment methods include bird herding and enclosing, as most of the available studies on broiler welfare evaluation are based on scoring particular welfare deficiencies on the individual level (Welfare Quality, 2009). For welfare assessment, bird samples in diverse numbers are taken usually at random locations of the house, and then scored for the chosen set of welfare indicators (Sanotra et al., 2003; Dawkins et al., 2004; Knowles et al., 2008). This commonly used procedure is time consuming because it requires catching, enclosing, and handling birds, but most importantly, it might be stress inducing (Jones, 1992), influencing birds' performance during gait scor-

ing. Furthermore, slower or unfit individuals might be less likely to escape during catching, similar to passive coopers (Kolhaas et al., 1999), having the potential of influencing randomness of the procedure, therefore increasing the probability of observing unusually high immobility and lameness levels.

Walk-throughs performed by bird caretakers are, to a certain extent, a similar strategy for data collection to line transect methodology, which has been successfully used for years in wildlife studies (Buckland et al., 2010). Some aspects of this approach, such as distance evaluation, were used in a nonintrusive method of plumage condition assessment (Bright et al., 2006). However, the differences in methodology and results between the approach for welfare evaluation closer to the methods used routinely by bird caretakers or flock supervisors and the classical scientific approach of individual sampling have never been compared. The ideal welfare assessment protocol for on-farm conditions should be a method that provides the dynamism of walk-through inspections but is conducted in a way that provides veracity, interobserver reliability, and quantitative results that can be compared across flocks and farms. To our knowledge, none of the available methodologies developed to date would fulfill the requirements of what would be considered a "gold standard."

The goal of this study was to compare the welfare assessment results of broiler flocks evaluated according to 2 different approaches: the transect walks and the individual scoring. The transect walk methodology is based on the idea of walk-through used for broiler care and line transect methodology used in wildlife biology, adding the evaluation of the methodology for interobserver reliability and within- and across-house sensitivity. We compared the results with the individual sampling scoring conducted following the guidelines provided by the Welfare Quality (2009). This is a preliminary study aiming to develop a scientifically sound and practical methodology, combining current scientific findings with the transect approach, for on-farm broiler welfare assessment, with perspective for application in other poultry species.

MATERIALS AND METHODS

Facilities and Birds

The study was conducted from April 30 to May 8, 2012, at 3 farms, located in the same geographical region in Northern Spain belonging to the company Grupo AN from the Navarra region (Spain). Each of the studied farms had paired houses, with flock sizes/house ranging from 13,220 to 27,540 broilers (Cobb 500) reared at a density of 17 birds/m². All houses had identical management, other than for the fact that 4 of the houses used chopped straw as litter substrate, whereas 2 used wood shavings. All houses were provided with automatic drinkers, feeders and ventilation systems, artificial light, and windows allowing natural lighting.

Data Collection

We collected data by using 2 methodologies: the transect walk approach that we developed, and the individual sampling assessment based in the protocols developed by Welfare Quality (2009).

Transect Walks. The transect walk approach is based on the methodology widely and successfully used in wildlife studies for decades (Gates et al., 1968; Buckland et al., 2010). Transect walks for bird welfare assessment in our study consisted of standardized walks divided in randomly set paths covering the full area of the house (Figure 1a).

Broiler houses normally have a rectangular shape, although dimensions may vary across companies and countries. The houses in our study were around 13 m wide (variable length) and were divided in five 2.5-m-wide bands. Transects were numbered from 1 to 5: 1 and 5 being wall and 2, 3, and 4 central transects. Transect widths were limited by the location of feeder and drinker lines (for central transects), or the wall and adjacent drinking line (for wall transects), which appeared to create invisible barriers to birds' movements, caused by a human moving forward along the transect (personal observation). Paired houses at each farm were assessed sequentially by 2 observers within the same day, when birds were 31 to 35 d old (birds' welfare may deteriorate in a day toward the end of rearing). This age range, instead of the end of production cycle, was chosen for assessments because it is a common procedure in Spain to depopulate 25% of the flock at this age. A later evaluation may have provided biased results due to the impact of catching during depopulation, which is considered a major cause of stress, therefore providing misleading information about the welfare status of the birds during the production cycle.

Observers conducted the data collection independently in each house. Transect walks were performed in random order, in both directions, starting at the entrance wall and at the opposite of the entrance wall, alternatively. We avoided sequential observations of contiguous transects to minimize the possibility of double-counting birds that may have moved from adjacent scored transects minutes before. The observers walked slowly through the set transect (Figure 1b) while recording in a spreadsheet (Polaris Office, Infraware, Seoul, South Korea) installed in a handheld tablet (ASUS Eeepad TF 101 Transformer, Taipei, Taiwan). Observations of all occurring incidences of birds within the following categories were recorded: immobile (no attempt to move, even after slight encouragement), limping (visible signs of severe uneven walk), dirty (side and back feathers visibly dirty), sick (bird showing clear signs of impaired health with small and pale comb, red-watery eyes, and occasionally unarranged feathering usually found in resting position), agonizing (the bird lies on the floor with closed eyes, breathing with difficulty), and dead. These are validated welfare indicators, which

are considered critical parameters in terms of broiler welfare (EFSA Panel on Animal Health and Welfare, 2012), which can be clearly described and identified for data collection in broiler flocks, making them ideal for purpose of methodology validation.

Individual Sampling. During individual sampling, a group of 3 trained scientist collected birds in 6 random locations within the house, with at least one of them collected in 1 of the 5 predefined transects. Each sample consisted of 25 randomly collected birds that were gently pushed to a mobile pen and were kept enclosed during sampling. Each bird was handled gently and individually, weighed on an automatic scale (PCE-WS 30, PCE Instruments, Southampton, Hampshire, UK), and evaluated for footpad dermatitis (score 0 to 4), hock burns (0 to 4), and breast dirtiness (0 to 2). Afterward each bird was released away from the scoring area and observed to evaluate gait scoring (scale 0 to 5) when receding. If not showing willingness to move, we used slight encouragement by touching the bird. For each indicator, a lower score meant a higher welfare status of the individual. After scoring all birds in the sample, the procedure was repeated in the next location. Although under ideal circumstances we should have had 2 teams performing dual individual sampling to check for interobserver reliability, this would require a total of 7 people, and unfortunately we did not have sufficient personnel to do this. In addition, because the individual scoring took half a day per house, the 3-person team could only evaluate 2 houses in a day. Therefore, there was not sufficient time to repeat the scoring a second time in each of the paired houses.

Statistical Analysis

Transect Walks. During transect walks, we recorded the number of individuals showing any of the predefined welfare problems. Observed frequencies were transformed into proportions per transect based on the known flock population from each particular house and assuming that birds were randomly distributed through the house.

To test interobserver reliability and sensitivity of transect evaluation, resulting percentages were checked for normality and homogeneity of residual variance. From the whole set of variables, immobility, agony, and death were nonnormally distributed and were subjected to logarithmic transformation, allowing fulfillment of normality requirements. We performed independent mixed-model repeated measures ANOVA for each of the 6 welfare indicators defined above. The model included transect as a repeated measure, with house and observer as fixed factors. We included farm as a random statement because the between-houses comparison was the main point of our interest. We included interactions between observer by transect and observer by house, as well as house by transect. Least squares means differ-

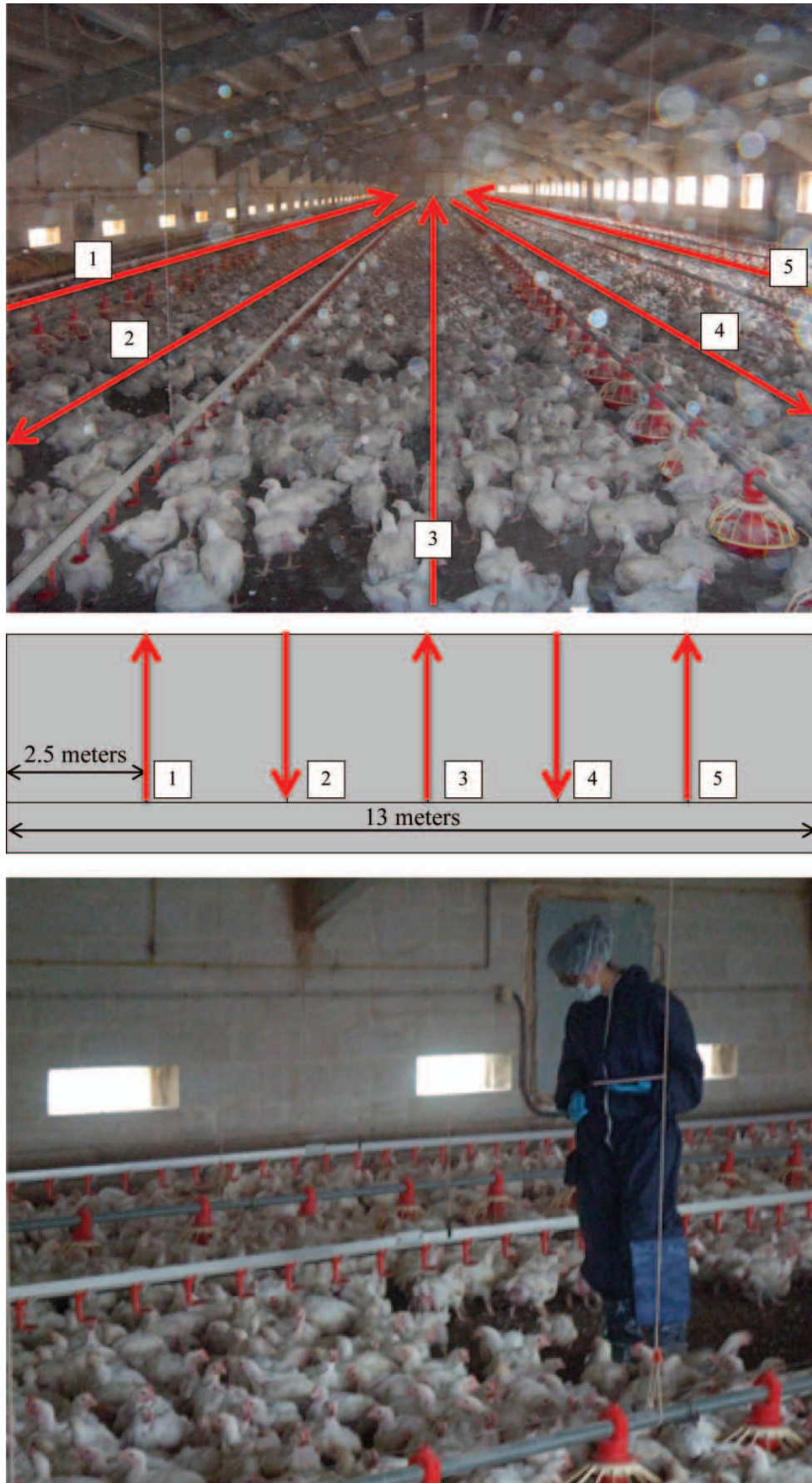


Figure 1. a) Design of the transect walks of 2.5 m within a 13-m-wide production room. The dashed lines (red in color version) show the pathways along which the transect walks were conducted. Arrows show the walking path of the observer between lines of feeders and drinkers. b) Data collection during transects (note the short distance to the observer). Color version available in the online PDF.

Table 1. Effect of house, transect, observer, and the interactions of transect with observer and observer with farm for welfare indicators collected by transects

Welfare indicator	ANOVA component					
	House	Transect	Observer	Transect × observer	House × observer	House × transect
Immobile	<0.00010	0.9033	0.0208	0.1915	0.1235	0.3163
Limping	0.0029	0.7996	0.8496	0.2447	0.0502	0.6451
Dirty	0.0005	0.1003	0.6832	0.1089	<0.0001	0.2046
Sick	0.6293	0.6994	0.6009	0.8107	0.4978	0.9391
Agonizing	<0.0001	0.3656	0.0479	0.7908	0.0604	0.3580
Dead	<0.0001	0.0068	0.0502	0.6666	0.0015	0.0020

ences were adjusted for multiple comparisons by post-hoc Tukey comparison.

We applied bootstrapping techniques to test the precision of the method by taking simulated random samplings combinations from the original data set (Dixon, 1993). Bootstrapping has been used to estimate the accuracy of ecological indices (Stein, 1989; Dixon, 1993) and more recently in a wide range of scientific areas, from genetics (Yang and Rannala, 2012) to economic sciences (Clark and McCracken, 2012). In short, this methodology defines the appropriate model for the observed data, from which it generates n sample data sets using Monte Carlo methods, to finally construct the bootstrap distribution (Efron, 1979, 1987). Expected mean and SE of the data set for each welfare indicator was calculated by taking random samples of one transect (20% of the information), or combinations of 2, 3, and 4 transects (40, 60, and 80% of information, respectively). Simulations were run 10,000 times per house and welfare indicator. All variables, except for immobility, were averaged per house due to lack of differences ($P \geq 0.05$) across observers. Independent bootstrapping was calculated for the indicator immobility for each observer. We used PROC SURVEYSELECT to perform the bootstrap.

Individual Sampling

Data collected in individual samplings were also checked for normality and homogeneity of residual variance. Hock burns, immobility, and dirtiness were nonnormally distributed, and were subjected to logarithmic transformation. The variables were analyzed by independent mixed-model repeated measures ANOVA. The model included transect as a repeated measure and house as fixed factor. However, for this analysis the interaction among both factors could not be included because of the lack of sufficient degrees of freedom. We included farm as a random statement, as for the transect walk analysis. Least squares means differences were adjusted for multiple comparisons by post-hoc Tukey comparison.

All analyses were conducted with SAS 9.3 statistical package (SAS Institute Inc., Cary, NC).

RESULTS

Transect Walks

Sensitivity. Our results showed that transect walk methodology allowed detection of small variations across the studied flocks on the prevalence of the studied welfare indicators. Differences across houses ($P < 0.003$) were found for the incidence of immobile, limping, dirty back, agonizing, and dead birds (Table 1, Figure 2). Only incidence of sick birds remained invariable across the studied houses (Table 1, Figure 2).

Interobserver Reliability. Welfare assessment across observers, or the interaction of observer and transect, and observer by house remained consistent for most variables as indicated by the lack of differences ($P \geq 0.05$) in the assessment (Tables 1 and 2). The effect of observer was only detected for the incidence of immobile and agonizing birds (Table 1); however, the interobserver difference for both variables was not observed ($P \geq 0.05$) for the interaction between house and observer. On the other hand, the house × observer interaction had an effect ($P < 0.0015$) on dirty and dead birds. Nonetheless, the differences across observers (Table 2) ranged between $0.18 \pm 0.02\%$ and $0.22 \pm 0.03\%$ for the incidence of immobile birds, whereas maximum range of variation across farms and observers for dirty birds was $\pm 0.5\%$.

Transect Effect. No effect ($P \geq 0.05$) of transect location was detected (1 to 5; 1 and 5 being wall transects, and remaining being central transects) for almost all variables (Tables 1 and 2) studied, except for dead for transect ($P = 0.0068$) and transect × house effect ($P = 0.002$). Applying bootstrapping techniques showed that the mean for each house was similar to the observed mean value by using as little as 20% of the information for all the variables (representative example in Table 3 and Figure 3).

Individual Sampling

Sensitivity. By using individual sampling method, we found differences ($P < 0.01$; Table 4) between houses for limping and dirty birds, footpad dermatitis, and BW (Figures 4 and 5).

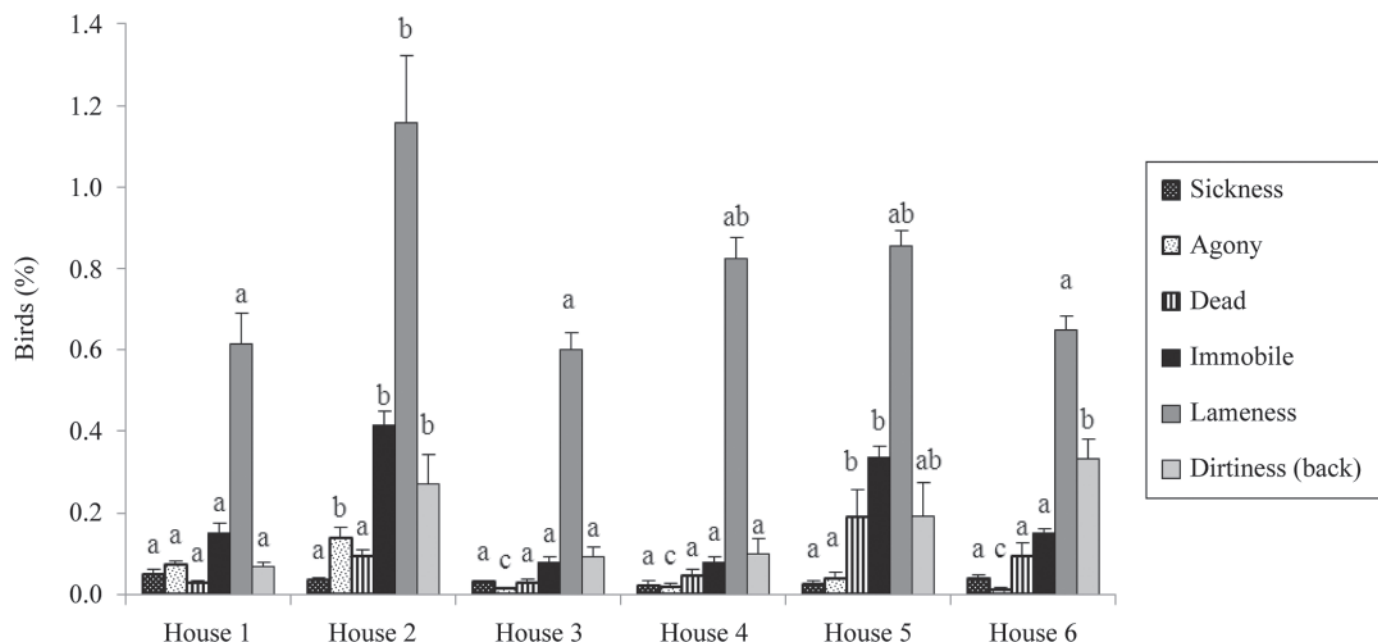


Figure 2. Mean values (\pm SEM) of each welfare indicator expressed as percentages for each house obtained by transect walks. Differences for each specific measure across houses are indicated by letters; means lacking a common letter (a–c) differ ($P \leq 0.05$).

Transect Effect. We did not find any effect of transect ($P \geq 0.05$) on any of the variables collected by individual sampling, nor any effect of interaction between transect and the house on the variables (Table 4).

DISCUSSION

Transect Walks

The aim of our study was to explore the soundness of a new approach to welfare assessment for broiler flocks, considering the scientific validity, time, and personnel requirements. We also considered the potential acceptability by assessors and producers, which might have an interest in self-assessment. The transect walk approach is based on the routine daily checks conducted by farmers and flock supervisors during inspections, combined with line transect methodology commonly used for evaluating wildlife populations (Buckland et al., 2010). The transect walk approach implies surveying birds throughout the entire production house, registering all individuals falling within each welfare indicator category, established in this study within each transect.

In this study, we homogenized field conditions as much as possible by assigning to the study only houses using birds of identical genetic background (Cobb 500) raised under identical standard management practices and within the same geographical region. All houses were sampled when birds were at similar ages (31 to 35 d) and were assessed in less than a month to minimize variations in environmental conditions that may affect the birds' welfare status (Dawkins et al., 2004). Despite the homogeneity in housing conditions, our results showed that the transect walk approach was high-

ly sensitive and allowed detection of small variations in the incidence of the welfare indicators used in this study such as immobility, birds with severe limping, with dirty back, agonizing, or dead (Table 1, Figure 2). These indicators are known to be critical for the welfare status of broilers (Dawkins et al., 2004; Estevez, 2007), but also have a tremendous economic impact. For example, skeletal problems causing immobility in the United Kingdom are responsible for losses estimated in 2 million pounds per year (Walker, 2012). Another indicator used, such as back dirtiness (as used in this study) is considered an important welfare indicator connected to litter quality or stocking density (Berg, 1998; Estevez, 2007).

Welfare assessment with the transect walk approach remained consistent across observers for limping, dirty, sick, and dead birds (Tables 1 and 2, Figure 3). However, minor differences were detected for the incidence of immobility across observers and for the interaction of observer with house for dirty and dead birds (Table 1). The differences across observers ranged between 0.18 ± 0.02 and 0.22 ± 0.03 for the incidence of immobile birds (Table 2). Considering the scope of the sampling (several thousand birds per flock) and the randomized procedures we used for data collection, it is actually quite remarkable that we only found minor effects across observers, whereas house assessment results remained consistent with other studies conducted in broilers under commercial (Sanotra et al., 2003; Dawkins et al., 2004; Knowles et al., 2008) and experimental conditions (Kestin et al., 1992). For example, averages of 0.9% birds unable or with impaired walk were found when using a noninvasive method to evaluate walking ability (Dawkins et al., 2004). These results

Table 2. Mean values (\pm SEM) of incidence of birds within each welfare indicator category expressed as percentages by observer and transect

Welfare indicator	Observer					Transect				
	1	2	1	2	1	2	3	4	5	
Immobile	0.18% \pm 0.02%	0.22% \pm 0.03%	0.20% \pm 0.04%	0.19% \pm 0.05%	0.21% \pm 0.05%	0.21% \pm 0.05%	0.21% \pm 0.05%	0.21% \pm 0.04%	0.19% \pm 0.04%	
Limping	0.79% \pm 0.06%	0.78% \pm 0.07%	0.76% \pm 0.10%	0.75% \pm 0.07%	0.79% \pm 0.09%	0.79% \pm 0.09%	0.79% \pm 0.09%	0.74% \pm 0.09%	0.87% \pm 0.15%	
Dirty	0.18% \pm 0.04%	0.17% \pm 0.04%	0.21% \pm 0.08%	0.21% \pm 0.05%	0.09% \pm 0.03%	0.09% \pm 0.03%	0.09% \pm 0.03%	0.14% \pm 0.04%	0.23% \pm 0.09%	
Sick	0.03% \pm 0.01%	0.04% \pm 0.01%	0.05% \pm 0.01%	0.04% \pm 0.01%	0.03% \pm 0.01%	0.03% \pm 0.01%	0.03% \pm 0.01%	0.03% \pm 0.01%	0.03% \pm 0.01%	
Agonizing	0.04% \pm 0.01%	0.06% \pm 0.01%	0.06% \pm 0.02%	0.03% \pm 0.01%	0.06% \pm 0.02%	0.06% \pm 0.02%	0.06% \pm 0.02%	0.04% \pm 0.01%	0.05% \pm 0.02%	
Dead	0.09% \pm 0.02%	0.07% \pm 0.01%	0.12% \pm 0.05%	0.08% \pm 0.03%	0.05% \pm 0.01%	0.05% \pm 0.01%	0.05% \pm 0.01%	0.05% \pm 0.01%	0.10% \pm 0.03%	

are similar to the values obtained in this study when adding the categories defined as immobile and severe limping. Our results are also comparable with another study (Knowles et al., 2008) in which 0.2% of immobile birds were detected using a method that involved bird handling (Kestin et al., 1992), and using the same methodology (Kestin et al., 1992), averages of 0.3 and 2.7% severely lame birds were noticed for 28- and 42-d-old broilers, respectively (Sørensen et al., 2000). On the other hand, the observer \times house effects detected for dead birds can be explained by the fact that we were working under commercial conditions and in 2 of the houses the farmer removed the mortalities in between the data collection of the 2 observers.

Similarly, an observer \times house interaction was detected for dirtiness, which could have been caused by natural lighting variation occurring over the time in which the walks were performed. The traditional broiler houses in Spain are provided with windows that are automatically regulated according to changes in environmental conditions. Birds in these houses are normally exposed to a wide range of variation in light intensity during the day. Variations might be more drastic during early spring, when wide range of climatic conditions can occur in the course of one day, when this study was conducted.

Interestingly, and contrary to our initial expectations, we found no effect of transect location (1 to 5; 1 and 5 being wall transects, and remaining being central transects) for any of the welfare indicators, except for the incidence of deaths (Tables 1 and 2). This effect could be explained by the farmers' intervention during the data collection period and by the method of the dead birds' removal by collecting them most likely next to the walls. However, overall, the lack of the expected transect location effect obtained in this study suggests that birds varying in welfare status seem to be homogeneously distributed within the house area. Furthermore, these results would at least initially suggest that it would not be necessary to perform all transects to obtain a reliable estimation on the welfare status of the broiler flock.

This idea is further supported by the results obtained from applying bootstrapping techniques that allows testing the precision of an estimate by calculating the bias and SE by taking simulated random samplings combinations from the original data set (Dixon, 1993). The resulting expected mean for each house was similar to the observed mean value by using as little as 20% of the information (Table 3, Figure 3). These results indicate that, under the conditions of our study, the assessment of an area covering 20% of the house surface is theoretically sufficient to obtain a reliable mean estimation on welfare status of a broiler flock, based on the parameters we used. If there is an interest in getting the closest to real value of the SEM, then our results suggest that a minimum 60% of the house area should be evaluated. Given that, in this initial study assessing a complete broiler flock by conducting 5 randomly de-

Table 3. Mean value and SEM for limping and dirty birds presented for 20, 40, 60, 80, and 100% of information used in 10,000 simulations using bootstrapping

Variable	House	% of information used									
		20		40		60		80		100	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Limping	1	0.6167	0.1485	0.6171	0.1036	0.6163	0.0850	0.6161	0.0735	0.6160	0.0828
	2	1.1600	0.3300	1.1617	0.2326	1.1605	0.1889	1.1586	0.1645	1.1583	0.1834
	3	0.6009	0.0838	0.6005	0.0595	0.5998	0.0480	0.6003	0.0419	0.6000	0.0469
	4	0.8260	0.1045	0.8255	0.0736	0.8249	0.0597	0.8249	0.0520	0.8250	0.0586
	5	0.8558	0.0755	0.8541	0.0540	0.8546	0.0433	0.8547	0.0376	0.8548	0.0395
Dirty	6	0.6508	0.0668	0.6507	0.0469	0.6508	0.0387	0.6507	0.0337	0.6505	0.0373
	1	0.0676	0.0223	0.0677	0.0158	0.0679	0.0129	0.0680	0.0112	0.0680	0.0124
	2	0.2731	0.1401	0.2736	0.0996	0.2734	0.0816	0.2727	0.0697	0.2723	0.0778
	3	0.0914	0.0476	0.0914	0.0337	0.0916	0.0273	0.0915	0.0237	0.0914	0.0267
	4	0.0983	0.0785	0.0983	0.0557	0.0983	0.0457	0.0982	0.0392	0.0984	0.0440
	5	0.1937	0.1611	0.1928	0.1141	0.1922	0.0924	0.1927	0.0807	0.1929	0.0900
	6	0.0936	0.3337	0.3344	0.0659	0.3341	0.0531	0.3340	0.0462	0.3340	0.0520

terminated transect walks took around 4 h (due to location data collection measuring distances from observer location to the front wall, results of which were not presented in this manuscript), we calculate that if the method is proven for its validity, then farm assessment could be conducted in a time lapse ranging between 30 to 60 min and with minimal interference with the daily farm routines.

Individual Sampling

Individual sampling is the most commonly used procedure for bird welfare assessment in broilers (Welfare Quality, 2009; De Jong et al., 2012c), for which a sample between 100 and 150 birds per flock is recommended, due to time and personnel requirements. We were interested in determining how our transect walk ap-

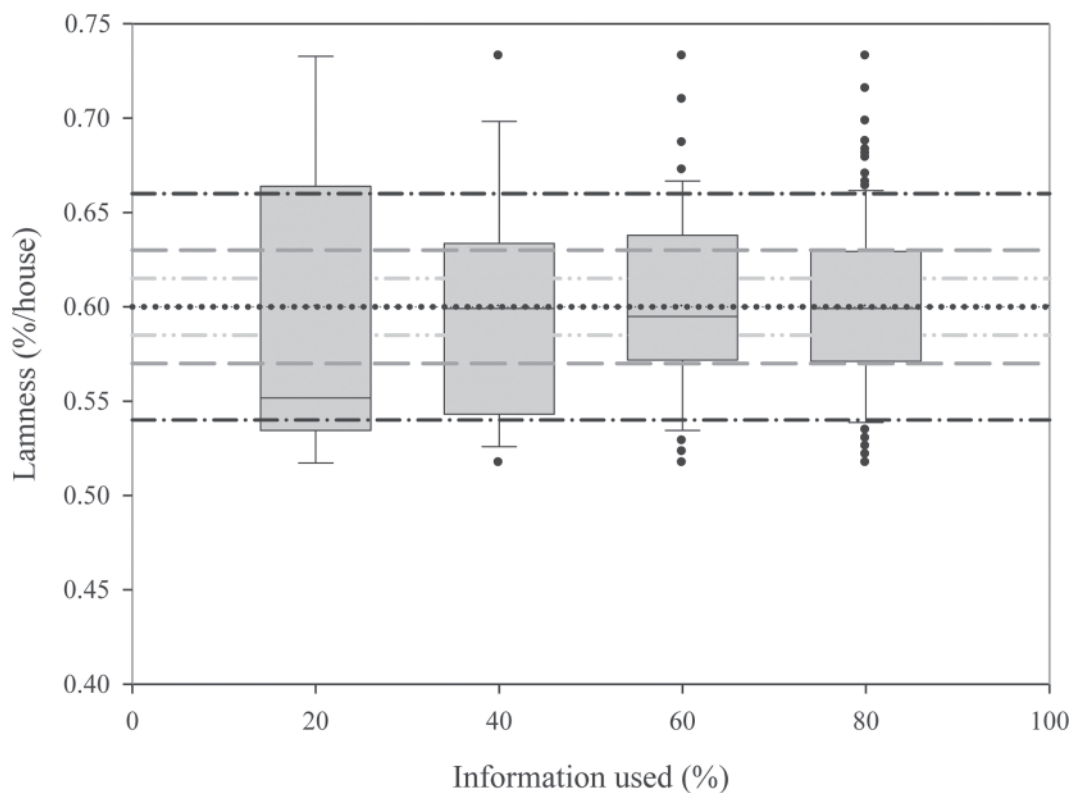


Figure 3. Mean values (\pm SD) expressed as percentages obtained from bootstrapping from 20, 40, 60, and 80% of information for limping birds in comparison with mean ($\cdot\cdot\cdot$), 2.5% ($-\cdot\cdot-$), 5% ($---$), and 10% ($-\cdot-$) of error rates of value obtained from 100% of information for house 3 (example).

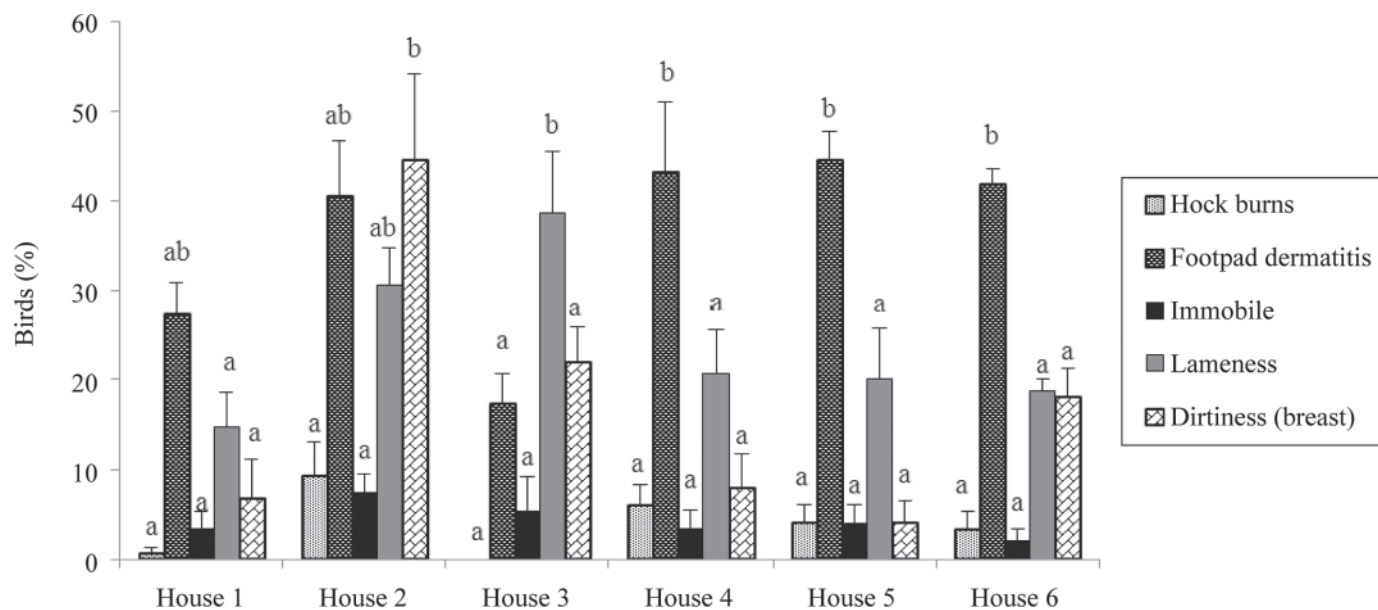


Figure 4. Mean values (\pm SEM) of each welfare indicator expressed as percentages for each house obtained by individual samplings. Means lacking a common letter (a,b) differ ($P \leq 0.05$).

proach would compare with this well-known and widely accepted methodology.

The results of the individual sampling (Table 4) showed differences between houses for limping and dirtiness, but not for the incidence of immobile birds. Differences were also detected for the supplementary variables included in the individual sampling such as footpad dermatitis and BW, but no differences were detected for hock burns. The lack of differences across houses for immobility might have been due to the large variation found, indicated by fairly large SEM values for each house, in relation to the mean value magnitudes. However, it might also be related to the relative small samples that are considered in individual sampling as compared with the size of the flock (usually several thousand birds), which are justified by the personnel requirements of this sampling methodology. In our study, it took 3 people and approximately 4 h

of work to perform the individual scoring as described in the methodology section above. However, a small sample size would imply that differences regarding the incidence of welfare issues with relatively low incidence (compared with limping, for example) might be more difficult to detect.

An important advantage of the individual sampling methodology that cannot be overlooked is that it allows scoring the incidence of footpad dermatitis and hock burns, which are important welfare indicators, in addition to their economic relevance to industry. Our results regarding the values for footpad dermatitis ranging from 18 to 48% for birds with scores 3 and 4 were within the values obtained by a recent study conducted in 386 Dutch flocks, in which 26.1 to 38.4% had mild or severe footpad lesions (De Jong et al., 2012b). With regard to hock burns, an evaluation of more than 2,000 birds at the age of 4 wk showed an incidence of 0.5% (Kjaer et al., 2006), whereas in our study the mean incidence was of 3.6%, with farm values ranging between 0 and 8.43%. The much higher upper range of our results might be caused by the older age of birds

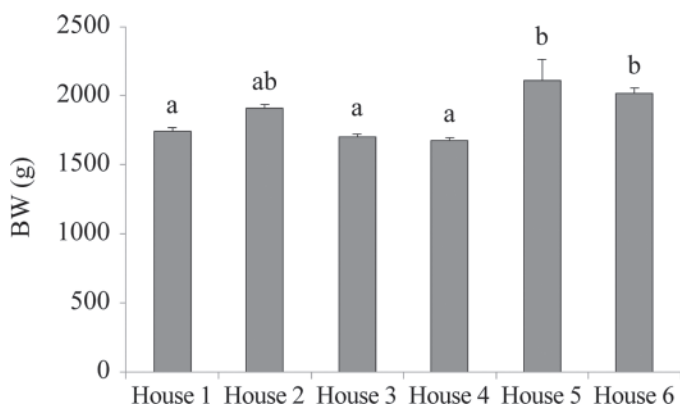


Figure 5. Mean values (\pm SEM) of BW for each house obtained by individual samplings. Means lacking a common letter (a,b) differ ($P \leq 0.05$).

Table 4. Effect of farm, house, observer, transect, and the interactions transect with observer and observer with farm for welfare indicators collected by individual samplings

Welfare indicator	ANOVA component	
	House	Transect
Immobile	0.7839	0.8495
Limping	0.0017	0.2616
Dirty	0.0002	0.7103
Hock burn	0.0941	0.8095
Footpad dermatitis	0.0112	0.4577
BW	0.0010	0.8676

in our study (more than 30 d old) or due to the fact that the observed flocks were placed at the farms in winter, when the incidence of footpad lesion tends to be more important. Regarding BW, relevant differences were found between houses (Figure 5). However, these particular houses did not appear to be the ones with lower incidence of welfare problems such as lameness or dirtiness.

Similar to the results obtained by applying the transect walk methodology, we found no effect of transect location for any of the parameters studied, supporting further our assumption regarding the homogeneous dispersion of birds with welfare issues within the house.

Method Comparison

The results of this study show clear, major discrepancies between both methods of welfare assessment. The results obtained by individual sampling would indicate a substantially reduced welfare status of broiler flocks compared with results obtained by applying the transect walk methodology considering the welfare indicators used in this study. The indicators which could be directly compared across transect walks and individual samplings were severe lameness and immobility. Mean incidence of lameness and immobility was $24.18 \pm 4.68\%$ and $4.22 \pm 2.3\%$, respectively, for individual sampling, whereas for transect walks mean frequency for lameness was $0.78 \pm 0.07\%$ and $0.2 \pm 0.01\%$.

The discrepancies across the 2 methods may be related to the observers failing to detect birds within the immobile or limping (severely lame) category during transect walks. This is a likely possibility and further studies should be conducted for improvement of the accuracy and reliability of this new methodological approach for on-farm welfare assessment. However, it should be also considered that when using 25 birds as the sample size in each location of the house for individual sampling, the effect of scoring just one bird in a given category would already increase the incidence of such category to a 4% incidence for this sample. Therefore, although individual sampling may be ideal for the assessment of large animals in which herd size may be several hundred (Vasseur et al., 2012), it may be more difficult to apply, or at least bring up some methodological questions, when applied to large poultry flocks.

This issue could be easily overcome by increasing sampling size. However, the assessment of 150 birds in our study took between 3 to 4 h for an experienced 3-person team. The speed of assessment could certainly be improved, but still even if doubling the speed it would take around 4 h to sample 300 birds, that would represent 1% of the population for a 30,000-bird flock.

In this respect, the transect approach is proven to be a more agile methodology in terms of time requirements, but certainly validation of the approach should be achieved first. A transect walk in our experience takes trained assessors between 30 min to 1 h depend-

ing on the welfare situation of the flock and house dimensions. According to the evidence supported by the lack of transect effects and bootstrapping methodologies, sampling of only 20% of the area of the house is required to obtain the mean estimated for the house, which could be achieved by conducting one transect in a maximum of 30 to 45 min.

An additional and important concern is also the potential stress effect that the individual sampling may have on the sampled birds. It is known that procedures such as herding, enclosing, and handling of birds causes fear (Newberry and Blair, 1993) and might have a large effect on their behavior, including immobility (Duncan and Kite, 1987; Jones, 1992). This reaction known as tonic immobility is a natural response that provides the bird with an opportunity to escape in an unguarded moment (Thompson and Liebreich, 1987). Tonic immobility reaction has been correlated with fear and stress indicators as proven by serum corticosterone levels (Lin et al., 2006). During herding into the sampling pen, birds are gently pushed into it and are perhaps forced to walk excessively even when the procedure is carefully performed. This can be painful and tiring for the birds before the evaluation starts (Cordeiro et al., 2009). Additionally, birds that are struggling to walk due to leg disorders and pain in everyday conditions are likely to show more severe walking difficulties during evaluation.

It is also possible that the herding procedure requiring several birds to walk into the portable sampling pens might also have compromised the randomness of the sampling. It is obvious that birds with movement difficulties may have less chance to escape and, therefore, be easier to catch, which may have resulted in samples including a disproportionate percentage of birds with high gait scores compared with the population average. Additionally, to be gait scored, birds are usually released to the empty area next to the pen, which might induce increased stress and fear reactions. Available literature has shown that broilers react to touching, handling, holding, to the exposure of acute stressors (Jones, 1992; Newberry and Blair, 1993; Marin et al., 2001), or even to human presence and eye contact, which can cause behavioral changes (Zulkifli and Siti Nor Azah, 2004). Therefore, it seems a likely possibility that the gait scoring evaluation may be affected by the imposed stress of the procedure. On the contrary, because transect walks are conducted slowly, without causing major disturbances to the birds, results obtained should not be affected by these factors. In addition, because the transect method is based on the sampling of the entire population in the house, or within transect, results are less likely to be affected by sample size.

Although all these factors interfering with the sampling procedures appear to be realistic possibilities, the question remains on the adequate or ideal validation method. To our knowledge individual sampling methodology has never been scientifically validated, which was

underlined in a study of foot pad dermatitis as a usual indicator measured with this approach (De Jong et al., 2012c). Clearly, the discrepancy in results depending on the applied methodology raises, until further studies are conducted, questions regarding the adequacy of currently available welfare assessment in broiler flocks.

The outcomes of our study revealed large differences between pictures obtained by the 2 methods analyzed in this study. However, much of the discrepancy can be well explained and justified by the arguments stated above. Certainly the transect methodology still needs much testing to ensure that lameness and immobility are not overlooked and that the methodology provides a realistic quantitative assessment of the most relevant welfare indicators. Indeed, behavioral assessment will also need to be considered if the methodology is validated in future studies.

Conclusion

We provided evidence that transect walks have a large potential as prospective approach to on-farm welfare assessment, showing good interobserver reliability and reduced time and personnel requirements. Because the method is based on daily care farm routine, it may be easier to understand and to accept by prospective assessors and producers. However, this work evidenced major discrepancies between welfare indicator estimates according to the method of assessment. Diversity in results may be caused by a potential reduced sensitivity to detect welfare issues by the transect approach, which would need to be improved. Nevertheless, individual sampling results might also be affected by the reduced sample size, stress effects, and randomization issues. This study provides new insight into constraints and advantages of broiler on-farm welfare evaluation methods, which should be considered in future studies on designing valid and feasible welfare evaluation protocols.

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