The potential of the transect method for early detection of welfare problems in broiler chickens

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ABSTRACT The potential of the transect method was tested for early detection of welfare problems associated with bird age and genetic line, litter quality, and transect location. On-farm welfare impairment and its consequences on slaughter outcomes were evaluated to test the method's predictive ability. A total of 31 commercial Ross, Cobb, and mixed RC broiler flocks were evaluated at 3, 5, and 6 wk of age. Two observers evaluated 3 transects each, simultaneously and in the same house by detecting welfare indicators including lame, immobile, sick, small, dirty, tail wounds, other wounds (head and back wounds), featherless, terminally ill, and dead birds. Increasing lame, immobile, sick, and terminally ill birds according to bird age ($P < 0.001$) was detected. Higher incidences of small and sick birds were detected in C and RC ($P < 0.001$) as compared to R flocks, whereas more dead and tail wounded were observed in RC compared to R and C flocks at week 5 ($P < 0.001$). Dirty incidence increased as litter quality deteriorated ($P < 0.001$). A higher incidence of immobile, small, sick, dirty, and dead was registered near house walls ($P < 0.001$). Differences across observers were detected for lame, immobile, and terminally ill birds ($P < 0.001$). For the observer by bird age interaction, differences were detected for dirty, tail wounds, and other wounds ($P < 0.05$). Pearson correlations between welfare indicators at week 3 and those at final weeks of age ($P < 0.05$) ranged between r values of -0.2 and 0.654 ($P < 0.05$). Correlations between welfare indicators and slaughter outcomes showed a relationship between flock mortality and dead on arrival, footpad dermatitis, leg problems, and illness ($P < 0.05$). Litter quality positively correlated with downgrades ($P < 0.001$). This study showed the potential of transects to detect differences in welfare indicators according to factors that effects were previously reported. It demonstrated the transect potential for detecting and predicting the consequences of welfare impairment on slaughter outcomes. This would make the transect method a useful tool for notifying and rectifying welfare deterioration as early as at 3 wk of age.

Key words: broiler, welfare, assessment, detection, transect

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INTRODUCTION

Broiler chickens' world production reached 22,705 billion birds in 2016 (FAO, 2018), mostly reared in intensive systems. Housing conditions are designed to maximize performance by providing chickens with the adequate physical environment and resources to fulfill their basic needs. Environmental conditions, however, may deteriorate, compromising birds' health, welfare (Dawkins et al., 2004, Estevez, 2007), and farm profitability (Meluzzi and Sirri, 2009). Fast-growing broiler chickens are prone to develop welfare problems, the consequences of which are high mortality and low body weight due to lameness (Wideman et al., 2012) that ultimately impact the farm economics (Bassler et al., 2013). Thus, assuring birds' welfare is not only an ethical responsibility essential to todays' agribusiness, but it is essential to assure farm revenues and long-term sustainability of the broiler industry.

Assessing animal welfare is not a trivial matter. The Welfare Quality (**WQ**) protocol (Welfare Quality®, 2009) for on-farm assessment of commercial broiler flocks assigns an overall flock score (not classified, acceptable, enhanced, or excellent) based on different health and welfare parameters that were established according to scientific criteria. Nonetheless, there are constraints on the feasible sample size to be analyzed due to time constrains per flock (de Jong et al., 2012), and the protocol requires bird handling that might be stressful in itself. In the past few years, new

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technologies have been proposed for a better livestock management (Wathes et al., 2008; Berckmans, 2014; Ben Sassi et al., 2016). In this context, the transect method (Marchewka et al., 2013, 2015) and associated mobile apps (i-WatchBroiler and i-WatchTurkey; NEIKER-TECNALIA, University of Milan, 2017; Estevez, I., NEIKER-TECNALIA, 2018) were developed as effective, non-invasive tools for the on-farm welfare assessment of commercial broiler and turkey flocks. The transect method is based on walks conducted along predefined paths, or transects, established between drinker and feeder lines. Along these walks data on previously validated broiler welfare parameters (EFSA Panel on Animal Health and Welfare, 2012) are collected. Good interobserver reliability, reduced personnel requirements, and fast implementation on commercial farms were reported in broilers when compared to the WQ protocol (Marchewka et al., 2013). The transect method was validated for turkeys by evaluating the entire flock during loading before their transport to slaughter plant and 2 d after being assessed with the transect method (Marchewka et al., 2015). Due to the large size of broiler flocks (20,000 to 40,000 birds/flock) as compared to turkeys', and the differences in the loading process, the same validation method would be hard to implement in broilers. As an alternative, testing some of the known effects of the broiler rearing cycle may provide insights of the transect method detection ability of welfare impairment caused by such aspects.

Until now, assessment has been carried out by 2 observers visiting flocks once toward the end of the rearing period (Marchewka et al., 2013), but welfare problems start developing earlier. Leg problems start developing between 3 and 5 wk of age causing lameness and immobility (Bradshaw et al., 2002). The incidence of sickness increases with age (Northcutt et al., 2003; Talebi et al., 2005), along with scratches and wounds that seem to occur especially toward the end of rearing when birds are more likely to step on each other (Wideman, 2016). On-farm mortality and dirty feathers are also affected by the deterioration of environmental and management conditions (de Jong et al., 2015). Some of the early signs of lameness, sickness, or any of the above-mentioned welfare problems might be detected, and perhaps controlled, if a reliable and easy to implement assessment method was developed. Therefore, flock assessment at different time points may provide a practical estimation of the transect method for this purpose.

Differences in welfare and performance sometimes relate to the birds' genetic makeup, such as ascites and sudden death syndrome in the case of fast-growing lines (SCAHAW, 2000; EFSA, 2010). Broiler lines may differ in their immune profiles or immune response (Manzoor et al., 2003), resulting in differences in resistance to necrotic enteritis (Hong et al., 2012; Jang et al., 2013), in their response to heat stress (Azad et al., 2010), or in the prevalence of lameness (Nelson et al., 1992; Dinev et al., 2012). A relationship between genetics and mortality was also established (Kalmar et al., 2013) showing that lines with higher risk of developing welfare problems tend to have higher mortality rates (Rekaya et al., 2013). Thus, broiler chickens' genetic background should be considered when assessing health and welfare of commercial flocks.

Litter quality usually deteriorates along rearing due to the combination of the effect of stocking densities, insufficient environmental control (Petek and Orman, 2013), and excreta accumulation (van der Hoeven-Hangoor et al., 2013). Consequently, poor litter quality increases the risk for leg problems and sickness toward the end of the rearing period (Sorensen et al., 2000; de Jong et al., 2014). Hence, testing the effect of litter quality on welfare indicators is a pivotal aspect in any on-farm welfare assessment. Furthermore, broilers are more likely to crowd near walls when resting (Newberry and Hall, 1990; Cornetto and Estevez, 2001a, Buijs et al., 2010), where dead chickens are more often seen (Tabler et al., 2002). Birds with poor health or deteriorated leg conditions would be more likely to seek the protection of walls (Newberry and Hall, 1990).

Due to the complexity of on-farm welfare assessment, data from slaughter plants have been used to predict on-farm welfare status. For instance, de Jong et al. (2015) predicted on-farm footpad dermatitis (**FPD**), hock burn, cleanliness, and gait scores from slaughterhouse measurements of FPD and hock burns. Dead on arrival (**DOA**) at slaughter can also be used as an indicator of on-farm welfare, as it correlates well with the flock's health status (Jacobs et al., 2017a). Welfare outcomes at slaughter are also affected by the catching and transportation process (Leandro, 2001; Baracho et al., 2006; Jacobs et al., 2017b). Hence, it may be difficult to separate the impact of the rearing conditions from the effects of the catching, transportation, and processing when assessment is only performed at the slaughter plant.

The development of an effective and practical on-farm welfare assessment method with the use of the i-WatchBroiler app, facilitating data collection and analyses, may allow us to the identification of early indicators for welfare risk assessment. Identifying these indicators at early stages would allow the implementation of mitigation strategies increasing bird health and performance. This goal is aligned with the aim of the technological advances developed under the umbrella of precision livestock farming and its application to welfare assessment (Berckmans, 2014; Ben Sassi et al., 2016).

The goal of this study was to test the potential of the transect method for early detection of welfare problems, and to determine its variations according to the effect of birds age and genetic line, litter quality, and transect position (central/wall). We hypothesized that the method would detect differences in the incidence of leg and health problems according to age, genetic line, and litter quality. We predicted higher incidences of welfare problems near walls than in house central locations. We predicted that these outcomes would be associated with slaughter plant results which therefore would suggest the method's potential to predict slaughter outcomes from on-farm welfare impairment.

MATERIALS AND METHODS

Farms and Birds

The study was conducted from April 2015 to July 2016 in 31 commercial broiler flocks located in Northern Spain and all being part of the same integrating company. Because of the distance to our Institute, 3 of the initially assessed farms were replaced with 3 other farms. The initial flock sizes and bird densities ranged from 17,952 to 41,561 birds and 15 to 19 birds/m^2, respectively. Bird lines used were Ross 308 (**R**), Cobb 500 (**C**), or a mix of both (Ross 308/Cobb 500; **RC**), being all mixed gender flocks. All houses were provided with automatic drinkers, feeders, and ventilation systems, although the type of ventilation systems did vary. Bird management was similar across flocks, and followed the integrating company guidelines. This study complied with the Spanish legislation regarding the use of animals for experimental and other scientific purposes (Real Decreto 1201/2005).

Data Collection

Farm Data. Data on welfare indicators were collected at 3, 5, and 6 wk of age. Data collection was based on the transect methodology for welfare assessment for commercial broiler and turkey flocks as previously described (Marchewka et al., 2013, 2015). The method consists on a set of walks (transects) conducted in random order within the areas of the house delimited by the feeder and drinker lines. Marchewka et al. (2013) showed that sampling a minimum of 20% of the house area using the transect method provided a reliable mean of the flock welfare status. Considering this, in our study 2 previously trained observers simultaneously assessed 2 transects each, during each observation day and flock. Observers walked 1 transect starting from the entrance of the house until reaching the opposite wall and returned by a different transect. Transects were randomly chosen, with the precondition that each observer walked 1 central and 1 wall transect. Central transects were delimited by 2 successive feeder and feeder/drinker lines, whereas wall transects were delimited by one of the house walls and the adjacent feeder line. Assessment of 2 transects per observer normally lasted 45 to 60 min. Sequential observation of contiguous transects was avoided to minimize the occurrence of double-counting birds (Marchewka et al., 2013).

The welfare assessment was performed using the i-WatchBroiler mobile application (Estevez, I., NEIKER-TECNALIA, 2015) installed on an Android tablet. Most relevant broiler welfare indicators (EFSA, 2012) were evaluated, and included lame, immobile, sick, small, dirty, terminally ill, tail, back and head wounded, featherless, and dead birds (see definitions in Marchewka et al., 2013). Assessment was conducted by slowly walking along the transect and clicking on the app screen each time a bird showing one of the above-mentioned indicators was observed. This assessment was conducted similarly to the farmers' daily routine, causing minimal disturbance to birds that slightly moved away as approached. Collected data were transformed to percentage of occurrence of each welfare problem per transect, relative to the estimated total number of birds in each specific transect. The estimation of the number of birds per transect was calculated as follows: flock size on the assessment day × (transect width/house width). The average number of birds per assessed transect was 3572 ± 1553 (mean ± SE).

In addition, litter quality was evaluated along the observed transects in 3 different locations (beginning, middle, and end), based on a 5-point scale (being 0 = dry and loose litter, and 4 = caked litter) according to the WQ protocol for poultry (Welfare Quality, 2009). An average litter quality score per transect was calculated. At 5 and 6 wk of age, a sample of 50 birds was also evaluated for FPD according to the WQ protocol 5-point evaluation scale (Welfare Quality, 2009). This was not implemented during week 3 as FPD incidence is very low at this age (Bilgili et al., 2006). Flock mortality was collected for each flock.

Slaughter Plant Data. Slaughter plant data of each assessed flock were obtained from the integrating company. Carcass quality and production parameters included: DOA, downgraded carcasses, hematomas, broken wings, and average weight gain per day.

Statistical Analysis

Frequencies of occurrence of each welfare indicator per transect, calculated as explained above, were analyzed assuming a binomial distribution. Generalized linear mixed model, repeated measures ANOVAs were carried out using the GLIMMIX procedure in SAS 9.3 (SAS Institute Inc., 2011) software. Due to their low occurrence, back and head wounds were pooled and analyzed together by creating an "Other wounds" variable. The experimental unit was the house, and each flock was uniquely identified. Statistical models included age of the birds when assessed (3, 5, and 6 wk), genetic line (R, C, and RC), transect location (central, wall), observer and the 2-way interactions observer by bird age, transect location by bird age, and genetic line by bird age as fixed factors. All models included the mean litter quality score corresponding to each transect as a covariate. Farm, nested within each data collection round, was included as a random factor in all models, and the week of age at the assessment was included as the repeated measures unit. A first-order autoregressive covariance structure was assumed to account for any linear dependence of measures of each flock over time. For statistically significant effects ($P < 0.05$), least squares means differences were computed, with P-values adjusted for multiple comparisons using Tukey tests. For significant interactions, tests of simple effects (Winer, 1971) were performed to detect differences between levels of each factor at each specific age point ($P < 0.05$).

Pearson partial correlations were calculated using the CORR procedure in SAS to test the relationship between welfare indicators assessed at 3, 5, and 6 wk, and between these and slaughter plant outcomes for thinning (around the end of the fifth week of age) and final transports. The observer effect was taken into consideration in the partial statement. As correlating many variables could lead to false-positive correlations, welfare indicators corresponding to sick, terminally ill, and dead were pooled into an "Illness" variable. Lame and immobile were pooled into a "Leg problems" variable and tail wounds and other wounds into a "Total wounds" variable. In addition to the average value of foot pad dermatitis (**Av. FPD**) calculated for each week (weeks 5 and 6), the percentage of birds with FPD superior to 1 (**%FPD> 1**) for each week was calculated and both variables were used for the correlation analyses.

RESULTS

The effects of the main factors on each welfare indicator are presented in Table 1 and the means (±SE) for these main factors are included in Table 2. Given the low incidence of featherless chickens statistical models did not converge, overall mean value (±SE) for this variable was 0.0067% (±0.001).

Changes in the incidence of lame, immobile, sick, and terminally ill birds were detected with age, with a consistent increment in the frequency for almost all indicators from week 3 to 6. Genetic differences were detected only for small and sick birds (Table 2), whereas the interaction of genetics by bird age was significant for dead ($P = 0.0005$) and tail wounded birds ($P < 0.0001$; Table 3). The incidence of welfare issues was generally higher along wall transects, with differences for immobile ($P = 0.015$), small ($P = 0.013$), sick ($P = 0.01$), dirty ($P = 0.0009$), and dead ($P < 0.0001$) birds as compared to central transects. No effects of location by age interaction for all welfare indicators were detected ($P > 0.05$), and therefore this interaction was removed from the models.

Poorer litter quality caused a higher incidence of dirty birds ($P = 0.0004$) (Table 2). Differences in the incidence of lame, immobile, and terminally ill birds were detected according to observer (Table 1), as well as of the observer by age interaction on small ($P = 0.0041$), dirty ($P = 0.0105$), tail wounds ($P = 0.0001$), and other wounds ($P = 0.0102$). Mean values are presented in Table 4.

Relevant correlations between on-farm welfare indicators collected during the growth period are shown in Table 5 in detail. For example, the incidence of leg problems observed at 3 wk of age positively correlated with results at 5 wk ($P < 0.0001$), and similar positive correlations were observed between incidences of leg problems observed at weeks 5 and 6 ($P < 0.0001$). Positive correlations were also observed between the incidence of leg problems and small birds ($P < 0.0001$), and between small and ill birds ($P < 0.0001$). Litter quality assessed at 3 wk consistently and positively correlated with that of weeks 5 and 6 ($P < 0.0001$).

Pearson partial correlations between welfare indicators collected from week 3 to 6 and slaughter outcomes are presented in Table 6 for thinned flocks and in Table 7 for final flocks. Correlations were generally low to moderate with some negative values, but there were some interesting results. For example, for both thinning and final transports, moderate positive correlations were found for litter quality with Av.FPD and %FPD>1. Litter quality consistently and positively correlated with downgrades and average slaughter weight (Tables 6 and 7). For final transports, flock mortality positively correlated with Av. FPD and %FPD > 1 ($P < 0.0001$), and with the incidence of leg problems during weeks 5 ($P = 0.0334$) and 6 ($P = 0.0053$) of age. Positive correlations were observed between average slaughter weight and Av. FPD ($P = 0.0003$) and %FPD>1 ($P = 0.0002$). DOA was positively correlated with illness for both thinning ($P = 0.007$) and final

Table 1. Effects of bird age, genetic line, transect location, litter quality, and observer (F and P value) for welfare indicators evaluated by the transect method.

	Bird age		Genetic line		Transect location		Litter quality		Observer	
Welfare indicator	$F_{(2334)}$	P-value	$F_{(2334)}$	P-value	$F_{(1334)}$	P-value	$F_{(1334)}$	P-value	$F_{(1334)}$	P-value
Lame	144.65	<0.0001	0.08	0.9236	0.61	0.4366	0.02	0.8989	114.07	<0.0001
Immobile	151.46	<0.0001	1.96	0.1423	5.97	0.0151	1.46	0.2272	113.79	<0.0001
Small	26.45	<0.0001	6.07	0.0026	6.16	0.0136	3.22	0.0737	2.66	0.1041
Sick	9.9	<0.0001	7.82	0.0005	6.69	0.0101	0.04	0.8425	0.57	0.4495
Dirty	20.35	<0.0001	2.28	0.1036	11.13	0.0009	14.9	0.0001	16.25	<0.0001
Dead	11.07	<0.0001	0.07	0.9297	31.32	<0.0001	0.01	0.9104	0.52	0.4718
Terminally ill	6.46	0.0018	0.19	0.8247	0.87	0.3508	2.88	0.0905	8.67	0.0035
Tail wounds	28.19	<0.0001	5.79	0.0034	1.45	0.2287	1.26	0.2617	2.57	0.1096
Other wounds	2.89	0.0571	2.16	0.1174	0.01	0.9112	0.31	0.5795	2.32	0.1287

Bird age: at 3, 5, and 6 wk; genetic lines: Ross, Cobb, Mixed Ross/Cobb flocks; transect location: central and wall transect.

Table 2. Mean values (SE) of incidence of birds within each welfare indicator expressed as percentage for each main factor.[1]

Welfare indicator	Bird age			Genetic line			Transect position		Litter quality[2]	Observer	
	3 wk	5 wk	6 wk	Cobb	Ross	Cobb/Ross	Central	Wall		1	2
Lame	0.080^{c} (0.010)	0.172^{b} (0.019)	0.422^{a} (0.044)	0.183 (0.024)	0.182 (0.021)	0.175 (0.022)	0.176 (0.019)	0.184 (0.020)	0.006 (0.080)	0.268^{a} (0.027)	0.121^{b} (0.013)
Immobile	0.033^{c} (0.006)	0.124^{b} (0.019)	0.301^{a} (0.044)	0.091 (0.016)	0.113 (0.018)	0.119 (0.019)	0.097^{b} (0.015)	0.118^{a} (0.018)	0.114 (0.092)	0.065^{b} (0.010)	0.175^{a} (0.026)
Small	0.079 (0.018)	0.095 (0.022)	0.167 (0.038)	0.109^{a,b} (0.028)	0.086^{b} (0.020)	0.134^{a} (0.032)	0.097^{b} (0.022)	0.119^{a} (0.027)	– 0.187 (0.101)	0.115 (0.026)	0.101 (0.023)
Sick	0.013^{b} (0.003)	0.017^{b} (0.003)	0.030^{a} (0.006)	0.022^{a} (0.005)	0.011^{b} (0.002)	0.025^{a} (0.005)	0.015^{b} (0.003)	0.022^{a} (0.004)	– 0.017 (0.177)	0.018 (0.003)	0.019 (0.004)
Dirty	0.002 (0.001)	0.0007 (0.0005)	0.012 (0.005)	0.001 (0.0008)	0.003 (0.002)	0.004 (0.002)	0.001^{b} (0.0007)	0.005^{a} (0.002)	1.010 (0.284)	0.001 (0.0007)	0.005 (0.002)
Dead	0.024 (0.004)	0.028 (0.005)	0.048 (0.008)	0.031 (0.006)	0.031 (0.005)	0.033 (0.006)	0.023^{b} (0.004)	0.044^{a} (0.006)	– 0.022 (0.148)	0.031 (0.005)	0.033 (0.005)
Terminally ill	0.004^{a} (0.001)	0.0008^{b} (0.0005)	0.007^{a} (0.002)	0.003 (0.001)	0.003 (0.0009)	0.003 (0.001)	0.003 (0.0009)	0.003 (0.001)	0.518 (0.315)	0.005^{a} (0.002)	0.002^{b} (0.0007)
Tail wounds	0.005 (0.002)	0.039 (0.013)	0.032 (0.011)	0.014 (0.006)	0.040 (0.015)	0.011 (0.005)	0.018 (0.006)	0.019 (0.007)	0.162 (0.164)	0.021 (0.008)	0.016 (0.006)
Other wounds	0.003 (0.001)	0.006 (0.002)	0.003 (0.002)	0.004 (0.002)	0.002 (0.0009)	0.006 (0.003)	0.004 (0.001)	0.004 (0.001)	0.125 (0.340)	0.005 (0.002)	0.003 (0.001)

[a–c]For each parameter, the row means followed by different superscript letters are significantly different ($P < 0.05$).
[1]Significant interactions are not shown in this table for indicators where correspondent simple factors are also significant (see Tables 3 and 4).
[2]Values presented are regression coefficients (SE) for this variable estimated with statistical model. A positive coefficient value means that the incidence of each welfare indicator is estimated to increase in the magnitude of the regression coefficient as litter quality value increases (i.e., litter quality decreases) 1 unit.

Table 3. Mean values (SE) of the incidence of birds within each welfare indicator category expressed as percentages for genetic by bird age interaction.

	Week of age	Cobb line (%)	Ross line (%)	Cobb/Ross line (%)
Dead	3	0.028 (0.007)	0.024 (0.005)	0.020 (0.005)
	5	0.017^{b} (0.005)	0.028^{a,b} (0.006)	0.046^{a} (0.009)
	6	0.063 (0.014)	0.044 (0.009)	0.039 (0.009)
Tail wounds	3	0.006^{a,b} (0.003)	0.012^{a} (0.006)	0.002^{b} (0.001)
	5	0.029^{b} (0.012)	0.102^{a} (0.037)	0.020^{b} (0.009)
	6	0.016^{b} (0.007)	0.051^{a} (0.020)	0.042^{a} (0.017)

[a–c]For each parameter, the row means followed by different superscript letters are significantly different ($P < 0.05$).

Table 4. Mean values (SE) of the incidence of birds within each welfare indicator category expressed as percentages for observer by bird age interaction.

	Week of age	Observer 1 (%)	Observer 2 (%)
Small	3	0.099^{a} (0.024)	0.063^{b} (0.016)
	5	0.099 (0.023)	0.091 (0.022)
	6	0.154 (0.037)	0.182 (0.043)
Dirty	3	0.001 (0.0009)	0.004 (0.002)
	5	0.0005 (0.0004)	0.0008 (0.0006)
	6	0.004^{b} (0.002)	0.035^{a} (0.013)
Tail wounds	3	0.003 (0.002)	0.008 (0.003)
	5	0.055^{a} (0.019)	0.028^{b} (0.010)
	6	0.059^{a} (0.021)	0.018^{b} (0.007)
Other wounds	3	0.002 (0.001)	0.003 (0.002)
	5	0.006 (0.002)	0.007 (0.003)
	6	0.008^{a} (0.003)	0.002^{b} (0.0009)

[a–c]For each parameter, the row means followed by different superscript letters are significantly different ($P < 0.05$).

transports ($P = 0.02$), and with on-farm mortality at final transports ($P = 0.02$).

DISCUSSION

Our goal was to test the potential of the transect method for early detection of welfare problems, and to determine the influence of age and genetic line, litter quality, and transect position. We also focused on the potential of the method to predict slaughter outcomes from on-farm welfare impairment. The results of this study indicate that the transect method is effective to detect changes in the welfare status of broiler chickens during the growing period. The results also showed that the flock welfare condition is reflected in the slaughter outcomes.

The incidence of almost all variables and specifically lame, immobile, sick, and terminally ill birds increased with age, as would otherwise be expected in commercial flocks given the fast growth rate of modern broilers and its implications on skeletal, cardiovascular, and immune development (Kestin et al., 1999; SCAHAW, 2000). Although leg problems may be affected by bird weight, deteriorated walking ability was previously reported to increase with age (Sorensen et al., 2000; Cordeiro et al., 2012), which is in agreement with our results. In addition, results of the correlation between welfare indicators and slaughter plant outcomes showed no relationship among the incidence of leg problems and body weight, at least for the data collected in this study. However, contributing factors to the incidence of leg problems include the lack of activity of the birds (Reiter and Bessei, 2009), and the gradual deterioration

Table 5. Pearson correlation between welfare indicators assessed at weeks 3, 5, and 6 using the transect method.[1]

Variables[2]		Week 3 Litter	Week 3 LP	Week 3 Small	Week 3 Illness	Week 3 Dirty	Week 3 TW	Week 5 Litter	Week 5 LP	Week 5 Small	Week 5 Illness	Week 5 Dirty	Week 5 TW	Week 6 Litter	Week 6 LP	Week 6 Small	Week 6 Illness	Week 6 Dirty	Week 6 TW
Week 3	Litter	1																	
	LP	0.049	1																
	Small	−0.075	0.333***	1															
	Illness	0.2**	0.263***	0.435**	1														
	Dirty	0.076	−0.166*	−0.15*	−0.074	1													
	TW	−0.161*	0.132*	−0.052	−0.095	0.059	1												
Week 5	Litter	0.421***	0.097	0.044	0.07	0.033	−0.027	1											
	LP	−0.028	0.435***	0.254***	0.232**	−0.106	−0.067	0.071	1										
	Small	−0.163*	0.277***	0.618***	0.309***	−0.147*	−0.125	−0.065	0.453***	1									
	Illness	0.039	0.176**	0.634***	0.4***	−0.007	−0.084	0.018	0.215**	0.542***	1								
	Dirty	−0.056	0.051	0.246***	0.168*	0.077	−0.066	0.175**	−0.024	0.127	0.283***	1							
	TW	−0.183**	−0.015	−0.142*	−0.222**	0.108	0.396***	0.012	−0.079	−0.21**	−0.12	0.071	1						
Week 6	Litter	0.365***	0.107	−0.014	0.064	0.075	−0.061	0.495***	0.025	−0.121	0.06	0.056	0.013	1					
	LP	−0.092	0.405***	−0.265***	0.207*	−0.116	−0.129	0.011	0.478***	0.429***	0.196**	0.047	−0.107	0.043	1				
	Small	−0.025	0.297***	0.654***	0.345***	−0.082	−0.101	0.017	0.396***	0.619***	0.599***	0.219***	−0.204**	0.064	0.546***	1			
	Illness	0.095	0.183**	0.437***	0.225**	−0.03	−0.168*	0.003	0.091	0.3***	0.374***	0.054	−0.197**	0.156*	0.424***	0.463***	1		
	Dirty	−0.2**	0.113	0.133*	0.121	0.155*	−0.141*	−0.152*	0.1	0.154*	0.075	0.334***	−0.035	0.068	0.121	0.262***	0.017	1	
	TW	−0.015	−0.123	−0.078	−0.094	0.106	0.111	0.11	−0.203**	−0.094	−0.077	0.035	0.568***	0.128	−0.142*	−0.101	−0.048	0.035	1

[1]Significance of the correlation is indicated as follows: *for $P < 0.05$; **for $P < 0.01$; ***for $P < 0.001$.

[2]Variables presented in this table are as follows: litter: litter quality; LP: leg problems (sum of immobile and lame incidences); illness: sum of sick, terminally ill and dead incidences; TW: total wounds (sum of head, back and tail wounds incidences).

of litter quality throughout the rearing period (Bessei, 2006; Nääs et al., 2010). High bacterial cell counts and bacterial diseases are reported in older birds (Northcutt et al., 2003), which might explain the development of leg problems and sick birds with age, as for the latter, incidence at week 6 was higher than the double compared to the incidence observed during week 3.

Smaller than expected differences among genetic lines were detected for small and sick birds, with C and RC flocks showing slightly higher incidences. Intensive genetic selection is known to predispose modern broiler chickens to cardiovascular disease, sudden death syndrome, and ascites (Julian, 1998; Bessei, 2006; Hocking, 2014), with a heritability value of 0.3 for sudden death syndrome (Moghadam et al., 2005). Some studies have shown that the performance of R lines is worse than that of C lines (Chepete and Mareko, 2008; Marcato et al., 2008), although this might only apply to healthy birds, making the reasons for differences among genetic lines unclear. However, the higher incidence of problems for RC flocks might be easier to explain. Mixed flocks are usually present when no sufficient birds of the same genetic line are available to reach the desired stocking density, which implies filling houses by using 2 lines. It is likely that remaining birds of at least one of the lines corresponded to the very end of the hatching period that are usually smaller than average (Ulmer-Franco et al., 2010). Thus, it is quite possible that mixed flocks are at disadvantage in comparison to flocks composed by 1 genetic line as management requirements are no identical for both lines. Higher frequencies of dead and tail wounded birds were found for RC when compared with R flocks at week 5. The higher incidence of dead birds can also be explained by potential competitive disadvantages of mixed flocks. Indeed, increased mortalities are usually observed during the final weeks of the growing period due to the gradual impairment of flock health and welfare, among other aspects. Besides, farm management could have interfered with the results of small and dead birds, since the decision to cull and remove these birds ultimately depends on the individual farmer. On the other hand, the higher incidence of tail wounds in R compared to C flocks at week 5, which was also numerically higher for R during weeks 3 and 6, is probably related to a higher activity or reactivity of R birds. Given that aggressive interactions are unusual in broiler chickens (Estevez et al., 1997), the higher incidence of tail wounds for R birds could be related to higher activity, resulting in more running and jumping on each other, which might increase the risk of injuries. This is a hypothetical explanation to our results, as bird activity was not measured.

Dirty birds were detected more frequently as litter quality deteriorated. Leaking drinkers (Jones et al., 2005), ventilation problems, higher stocking density, and older age at slaughter (Dawkins et al., 2004; Petek and Orman, 2013) contribute to increased moisture, and to the gradual deterioration of the litter, resulting in increased feather dirtiness. In our study, dirtiness was

Table 6. Pearson correlations between welfare indicators collected with the transect method (at weeks 3 and 5) and slaughter outcomes of the thinning transport.[1]

	Week 3			Week 5			
Variables[2]	Litter	LP	Small	Litter	Illness	Av.FDP	%FDP > 1
Av.Weight	0.348**	–0.008	0.106	0.203	0.263*	0.100	0.179
Downgrades	0.260*	–0.016	0.101	0.035	0.251*	0.341**	0.246*
DOA	0.332**	–0.151	0.148	0.225	0.321**	0.176	0.307*
Hematomas	0.318**	–0.078	0.193	0.144	0.35**	0.331**	0.315**
Brokenwings	0.029	0.324**	0.28*	0.429***	0.442	0.027	0.106
Av.FDP	0.467***	–0.078	–0.013	0.135	–0.01	1	0.960***
%FDP > 1	0.520***	–0.069	0.038	0.170	0.06	0.960***	1

[1]Results are only shown for variables where at least 1 correlation is significant. Significance of the correlation is indicated as follows: *for $P < 0.05$; **for $P < 0.01$; ***for $P < 0.001$.

[2]Variables included are as follows: Av.weight: average slaughter weight; Av.FDP: average value of footpad dermatitis at week 5; %FDP > 1: percentage of birds with footpad dermatitis superior to one at week 5; DOA: birds dead on arrival; litter: litter quality; LP: leg problems (sum of immobile and lame incidences); illness: sum of sick, terminally ill, and dead incidences.

Table 7. Pearson correlations between welfare indicators collected with the transect method (at weeks 3, 5, and 6) and slaughter outcomes of the final transport[1]

	Week 3				Week 5					Week 6						
Variables[2]	Litter	LP	Illness	TW	Litter	LP	Small	Illness	TW	Litter	LP	Illness	TW	Av.FDP	%FDP>1	Mortality
Av.Weight	0.128	0.19*	–0.073	0.253**	0.240*	0.171	–0.238	–0.021	0.075	0.295**	0.048	–0.017	0.011	0.337**	0.350**	0.303***
Downgrades	0.335***	0.245**	0.014	0.221*	0.404***	–0.046	–0.309***	0.107	0.051	0.423***	–0.047	0.18	0.050	0.428***	0.371***	0.214*
DOA	–0.094	–0.118	0.055	–0.058	0.008	0.095	0.036	0.211*	0.064	-0.109	–0.05	–0.021	–0.077	–0.037	– 0.005	0.218*
Hematomas	0.256**	0.122	0.022	0.296**	0.375***	–0.057	–0.211*	0.168	0.058	0.317***	–0.161	0.154	–0.068	0.232*	0.212*	0.033
Brokenwing	0.275**	0.31***	0.052	0.07	0.29**	0.159	–0.193*	0.085	–0.161	0.304**	0.129	0.166	–0.215*	0.486***	0.450***	0.357***
Av.FDP	0.510***	0.17	0.08	–0.149	0.291**	0.095	0.048	0.133	–0.18	0.386***	0.106	0.161	0.216	1	0.970***	0.417***
%FDP > 1	0.552***	0.165	0.097	–0.166	0.3**	0.164	0.078	0.121	–0.196*	0.357***	0.123	0.119	0.033	0.970***	1	0.461***
Mortality	0.359***	0.134	0.299**	–0.251**	0.107	0.201*	–0.024	0.141	–0.144	0.036	0.261**	0.19*	–0.092	0.417***	0.461***	1

[1]Results are shown only for variables where at least 1 correlation is significant. Significance of the correlation is indicated as follows: *for $P < 0.05$; **for $P < 0.01$; ***for $P < 0.001$.

[2]Variables included are as follows: Av.weight: average slaughter weight; Av.FDP: average value of footpad dermatitis at week 6; %FDP > 1: percentage of birds with footpad dermatitis superior to one at week 6; DOA: birds dead on arrival; litter: litter quality; LP: leg problems (sum of immobile and lame incidences); TW: total wounds (sum of head, back and tail wounds incidences).

evaluated on the side and back feathers. Feather dirtiness was previously shown to deteriorate in the breast (de Jong et al., 2014) and back areas (Petek and Orman, 2013). As birds grow, the gradual reduction in activity levels would also increase the duration of contact with the litter (Cornetto and Estevez, 2001a; Alvino et al, 2009; Reiter and Bessei, 2009), increasing the risk of plumage dirtiness. This result supports the relevance of assessing litter quality at the 3 locations of each transect as litter quality can vary considerably among house locations.

A higher incidence of immobile, small, sick, dirty, and dead birds was detected along wall transects as compared to central transects. It is not uncommon to find the worst litter quality around the house periphery where birds tend to sit as they feel more protected against potential predators (Newberry and Hall 1990; Cornetto and Estevez, 2001b; Buijs et al., 2010; Aydin, 2016). Thus, litter quality in this area is likely to deteriorate faster, with negative effects on birds' plumage and health. In addition, although unwell birds are moving in central areas, they will be disturbed resulting in random movement with constant changes in direction until they find the protection of walls. Once close to a wall, unwell birds are less likely to move away, a phenomenon referred to as "wall trapping effect" (Estevez and Christman, 2006). This might explain why broiler chickens with reduced mobility are more likely observed along wall transects. Other studies such as Tabler et al., (2002) reported higher mortalities along sidewalls, which concurs with our results and shows that impaired birds tend to move to the periphery of the house and are later found dead there.

Although Marchewka et al. (2013, 2015) reported almost perfect concordance between observers when scoring broilers and turkeys with the transect method, in this study a significant observer effect was found for lame, immobile, and terminally ill birds, as well as for small, dirty, tail wounds, and other wounds considering the interaction of observer by bird age. Despite this, taking into consideration that over 3000 birds were assessed per transect, the numerical magnitude of the difference between observers was not high although statistical difference was reached. In addition, in many cases differences referred to the interaction with age. When pooling the incidence of lame and immobile into a single "leg problems" variable, the observer effect was no longer detected (results not presented), indicating that statistical differences were due to differences on how to distinguish lame from immobile, which were not uniform among observers, but an overall agreement was reached regarding the detection of total leg problems. In this study, the observers scored 2 different transects each simultaneously and within the same house, whereas in Marchewka et al. (2013, 2015), the entire house was assessed separately by both observers. Thus, differences observed in the individual transects may probably be explained in part by the differences found among observers. However, and without denying the fact that differences reached statistical significance, immobile and lame incidences were comparable with those previously found when testing the transect method at 31 and 35 d of age (Marchewka et al., 2013). Previous studies reported 0.9% of lameness (Dawkins et al., 2004), 0.3 to 3.1% of severe lameness, respectively, at 28 and 42 d of age (Sorensen et al., 2000), and 0.21% of immobility (Knowles et al., 2008). Our results are within the range of values of these studies. Considering dirty chickens, tail, and other wounds, differences between observers might be due to difficulties in detecting these problems. This would be the particular case of tail wounds when stocking densities are high or house lighting is low. We aimed at minimal intervention on the flock during assessments to minimize bird disturbance, and in some cases this might have made visual detection of welfare problems difficult. Preliminary practice of observers and knowledge of species specificities are required before starting data collection.

Correlations between litter scores at different weeks suggest that litter quality evaluation at week 3 can be used as a predictor of litter quality to be expected at 5 and 6 wk. This is critical given the association between poor litter quality, welfare issues, and slaughter results (Dawkins et al., 2004; de Jong et al., 2014). The incidence of leg problems including severe lameness and immobile birds at 3 wk correlated quite well with the incidences observed at 5 and 6 wk. These results suggest a potential of the method for early risk assessment and application of corrective measures that could include improving litter quality, the addition of more bedding material, and adaptations of the ventilation system, or providing better lighting program to promote activity. The frequency of illness and small birds at 3 wk also correlated positively with the frequencies of both problems at 5 and 6 wk. It was previously demonstrated that the incidence of illness and small birds are associated with poor environmental and management conditions, parental flock age, temperature and humidity conditions of the incubator, or to hatching time (Reis et al., 1997; Tona et al., 2004, 2005).

On-farm welfare indicators also correlated with several relevant slaughter outcomes, which could have some implications. For example, positive correlations between litter quality and FPD for thinning and final transports were found. These results are in agreement with those from a number of previous studies demonstrating the effect of poor litter quality on FPD incidence (Dawkins et al., 2004; Bessei, 2006; Haslam et al., 2007; Meluzzi et al., 2008; Bassler et al., 2013; de Jong et al., 2014). Litter quality also positively correlated with the incidence of downgraded carcasses, especially for final transports. A clear relationship was established between litter moisture and the incidence of hock burns, breast blisters, and dirty feathers, which are known to increase the incidence of downgraded carcasses (de Jong et al., 2014; Jacobs et al., 2017b). Higher Av.FPD and %FPD>1 values positively correlated with higher average slaughter weight for final transports, which is in line with studies such as Kristensen et al. (2006), who showed a positive correlation between body weight, gait score, and occurrence of footpad lesions in heavy birds. Our results show a relationship between litter quality, FPD, and downgrades, corroborating once again that on-farm management and birds' welfare conditions have a critical impact on slaughter results.

Dead on arrival is known to be a good indicator of slaughtered flocks' sanitary condition (Chauvin et al., 2011; Kittelsen et al., 2015; Jacobs et al., 2017a). However, DOA can be seriously affected by transportation practices and climatic conditions (Baracho et al., 2006; Chauvin et al., 2011; Jacobs et al., 2017b). Our results showed that illness at week 5 positively correlated with DOA at thinning transports and that flock mortality rates correlated with DOA at final transports, along with a positive correlation between mortality and illness. This indicates that illness might have led to higher on-farm mortality, both leading to higher DOAs at slaughter, which was previously reported (Kittelsen et al., 2015). Furthermore, flock mortality at week 6 positively correlated with Av.FPD and %FPD>1 at final transports, suggesting that on-farm problems leading to increased flock mortality would also influence slaughter outcomes. The positive correlation between flock mortality and the average slaughter weight is reflecting what was already shown in previous studies (Haslam et al., 2007), suggesting that faster growth rates might have negative consequences leading to higher on-farm mortality. The results of our study support the already established relationship between on-farm welfare and slaughter results (Dawkins et al., 2004; Dozier et al., 2005; Thomas et al., 2011; Abudabos et al., 2013; Marchewka et al., 2015), and indicate that the transect method can be used to detect on-farm welfare problems that will later translate into poor slaughter outcomes.

This study, although very intense for the resources at our disposal, only monitored 31 flocks, which is a modest number in order to determine the full potential of this method as a predictive tool. In spite of this, the initial results suggest that transects may be useful to improve bird management by providing farmers with specific quantitative information about the flocks' issues, so precise mitigation strategies could be implemented to correct or minimize on-farm problems. This would translate into better slaughter outcomes, thus permitting a more efficient production system.

CONCLUSION

The transect method, applied with the i-WatchBroiler app, appears to be a practical and effective tool for the on-farm assessment of commercial broiler flocks. In this study, we demonstrated that this method, implemented in about 45 min per flock, allows the quantitative assessment of the potential impact on welfare status caused by factors, such as age and genetic line, litter quality, or the transect location within the house. This method could be considered as a valuable tool to support farmers' decisions and reduce welfare-related problems and their corresponding losses in economic returns. Although discrepancies relative to the observer effect are yet to be improved, our results show that the transect method is a suitable and practical tool for a rapid assessment of on-farm welfare in commercial broiler flocks. If welfare assessments are performed as early as during the third week of age, the transect method could be a valuable tool to anticipate and correct welfare issues at later stages that will result in improving performance at the slaughter house (DOA and downgrades).

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