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ENVIRONMENT, WELL-BEING, AND BEHAVIOR

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ABSTRACT The objectives of this study were to 1) identify determinants of poor welfare in commercial broiler chicken flocks by studying the associations between selected resource-based measures (RBM, potential risk factors), such as litter quality and dark period, and animal-based welfare indicators (ABM), such as foot pad dermatitis and lameness, and 2) establish the breadth of effect of a risk factor by determining the range of animal welfare indicators associated with each of the risk factors (i.e., the number of ABM related to a specific RBM). Eighty-nine broiler flocks were inspected in 4 European countries (France, Italy, the United Kingdom, and the Netherlands) in a cross-sectional study. The ABM were contact dermatitis (measured using scores of foot-pad dermatitis and hock burn, respectively), lameness (measured as gait score), fear of humans (measured by the avoidance distance test and the touch test), and negative emotional state (measured using qualitative behavior assessment, QBA). In a first step, risk factors were identified by building a multiple linear regression model for each ABM. Litter quality was identified as a risk factor for contact dermatitis. Length of dark period at 3 wk old (DARK3) was a risk factor for the touch test result. DARK3 and flock age were risk factors for lameness, and the number of different stockmen and DARK3 were risk factors for QBA results. Next, the ABM were grouped according to risk factor and counted. Then, in a second step, associations between the ABM were investigated using common factor analysis. The breadth of a risk factor's effect was judged by combining the number (count) of ABM related to this factor and the strength of association between these ABM. Flock age and DARK3 appeared to affect several weakly correlated ABM, thus indicating a broad range of effects. Our findings suggest that manipulation of the predominant risk factors identified in this study (DARK3, litter quality, and slaughter age) could generate improvements in the related ABM and thereby enhance the birds' overall welfare status.

Key words: animal welfare, broiler chicken, risk factor, leg health, behavior

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INTRODUCTION

European Union Regulation no. 882/2004 "on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules," states that the official animal welfare control in the various member states should be carried out on a risk basis [i.e., the frequency of controls shall be proportionate to the farm's individual risk for poor welfare (EU, 2004)]. This approach optimizes the effective use of the (limited) resources. A prerequisite to the estimation of a poor welfare risk at a specific farm is soundly based knowledge about the risk factors that can harm welfare, that is, farm characteristics (identified in so-called resource-based measures, **RBM**) associated with welfare (Anonymous, 2001). Of course, there is no single gold standard measure for overall

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welfare. Indeed, animal welfare is a multidimensional concept, comprising both physical and mental health and including aspects such as physical comfort, absence of hunger and disease, and the potential to perform motivated behavior (Fraser, 2008). Thus, an adequate assessment of welfare should use a wide range of indicators (SCAHW, 2000; EFSA, 2012a), and consequently, when selecting a set of risk factors, as required by the above EU regulation, factors associated with a broad spectrum of welfare indicators would be preferred.

The European research project Welfare Quality developed a welfare assessment system for farm animals that takes into account the complexity of the welfare concept by systematically breaking it down into 4 principles, 12 related criteria (key welfare questions), and corresponding welfare measures (Botreau et al., 2007; Blokhuis et al., 2010). The present study focuses on broiler chicken production, and all the data used here were collected within the Welfare Quality project (Arnould and Butterworth, 2010). The animal-based measures (**ABM**) used to quantify welfare aspects stem from current broiler chicken welfare issues (Bessei, 2006; De Jong et al., 2012a). The ABM selected herein included contact dermatitis (measured using scores of foot-pad dermatitis, **FPD**, and hock burn, **HB**), lameness (measured as gait score), human-animal relationship and fear responses (measured using the avoidance distance test, **ADT**, touch test, **TT**, and novel object test, **NOT**), and the bird's emotional state (measured by qualitative behavior assessment, **QBA**). We do not regard this set of ABM as complete, but as a useful system that will evolve with time.

Foot-pad dermatitis and HB are characterized by skin lesions on the foot pads and hocks, respectively. Severe lesions (ulcers) may be painful as such (Haslam et al., 2006), and the lesions may become a gateway for bacterial infections (e.g., staphylococci), thereby affecting the bird's health. Contact dermatitis is caused by water and other chemical substances in the litter (Shepherd and Fairchild, 2010).

Lameness is usually thought to be caused by anatomical leg disorders such as long bone deformities, and genetic selection for fast growth is known to be influential (Sørensen et al., 1999; EFSA, 2010). However, the etiology of leg disorders is complex, consisting of infectious, noninfectious, and genetic factors (Bradshaw et al., 2002). Selection for fast growth also influences the incidence of cardiovascular diseases in broilers (Julian, 2004) and may thereby contribute to lameness and impaired gait by causing fatigue and swelling of the extremities. Several studies [e.g., McGeown et al. (1999) or Danbury et al. (2000)] support Mench's (2004) proposal that gait disorders must be painful because of associated clinical manifestations such as inflammation, spinal cord damage, or swelling of the joints. Lame birds may also find it difficult to reach food and water (Butterworth et al., 2002).

For farm animals, encounters with humans seem to be among the potentially most frightening events, despite centuries of domestication (Jones, 1987). Animals may perceive humans as predators, and rough or unpredictable handling of animals can increase their fear. A barren environment and limited contact with humans also contribute to fearfulness (Coleman and Hemsworth, 2010; Jones and Boissy, 2011). On the other hand, regular and gentle handling by a familiar person may promote a positive human-animal relationship, with beneficial effects for welfare and production (Jones and Waddington, 1992; Waiblinger et al., 2006; Jones and Boissy, 2011). For broiler chickens, improvements in first-week mortality (Cransberg et al., 2000), growth rate (Gross and Siegel, 1980), feed conversion ratio (Hemsworth et al., 1994), and immune response (Zulkifli et al., 2002) have been shown.

Qualitative behavior assessment is a whole-animal approach that assesses the expressive quality of animal behavior, using terms such as calm, agitated, confident, or drowsy. Multivariate statistical analysis is used to describe patterns of expression in individual animals or animals in groups, with the aim of generally characterizing the animals' quality of life (Wemelsfelder, 2007). Qualitative behavior assessment has shown good interobserver agreement at farm level in pigs, cattle, and poultry (Wemelsfelder et al., 2009; Wemelsfelder and Millard, 2009; Andreasen et al., 2013). There is not always agreement when terms are analyzed separately (Bokkers et al., 2012), which is why multivariate analysis is generally used to reduce such variability, focusing on underlying patterns of expression (Temple et al., 2011). The biological validity of QBA is supported by studies that found QBA outcomes to be significantly associated with physiological indicators of stress in cattle, sheep, and pigs (Stockman et al., 2011; Rutherford et al., 2012; Wickham et al., 2012; Stockman et al., 2013).

The ABM mentioned above have been used in several previous studies, but to the best of our knowledge, most reports on risk factors for broiler welfare focus on just 1 or 2, often related, ABM: contact dermatitis: Ekstrand and Carpenter (1998), Haslam et al. (2007), Allain et al. (2009); lameness: Knowles et al. (2008); human-animal interactions: Hemsworth et al. (1994); Zulkifli et al. (2002), or on RBM [e.g., stocking density: Dawkins et al. (2004)]. A set of both ABM and RBM that was deliberately diverse, as in Sanotra et al. (2002), has rarely been used.

The main objectives of the present study were 1) to identify potential determinants of poor welfare (risk factors) in commercial flocks of broiler chickens by investigating the associations between selected RBM, such as litter quality, duration of dark period, and ABM such as foot pad dermatitis and lameness, and 2) to establish the likely breadth of effect by determining the range of animal welfare indicators associated with each of the identified resource-based risk factors (i.e., the number of ABM related to a specific RBM). Risk factors are judged to have a broad spectrum if they are associated with several ABM and if these ABM are relatively weakly related.

Rather than defining a prediction model that could be used to estimate the risk for poor welfare on an individual farm, the current sample size limits our analysis to the identification of relationships between the variables. Although this study is not a risk assessment (EFSA, 2012a), its results may contribute to the identification of hazards for broiler welfare in traditional intensive production systems.

MATERIALS AND METHODS

Computations were carried out using the SAS package (version 9.2, SAS Institute Inc., Cary, NC) if not stated otherwise.

Subjects and Data Collection

In a cross-sectional type study, 89 traditional intensive, indoor-reared, broiler chicken flocks were inspected between 2007 and 2009. Of these, 16, 19, 36, and 18 flocks were located in France, the United Kingdom, the Netherlands, and Italy, respectively. Most farms were visited during spring and summer, participation was voluntary, and for each farm one flock was inspected on a single occasion in the week before slaughter. Twelve assessors collected the data, 2 in France and the United Kingdom, and 4 in the Netherlands and Italy, respectively. All assessors were trained within the Welfare Quality project.

Data were collected by means of a farmer questionnaire and an on-farm inspection protocol: the variables recorded are shown in Tables 1 and 2. The ABM were measured in situ using a test order of, first, QBA, and second, NOT/TT/ADT carried out as a set of tests at 3 different locations. After a 30-min pause, FPD/HB/gait scores were recorded, also as a set (of tests) at 10 different locations within the house to ensure that a cross-sectional sample of the flock was assessed. The same birds were scored for FPD and HB, while a new group of birds was penned for gait scoring. Testing or scoring the same bird twice was avoided by systematically moving through the house during the assessment. Data on age, BW, and mortality were obtained from the farmers' records. If necessary, BW on the day of visit was calculated using interpolation from the known weight records. The FPD, HB and gait score were measured as categorical variables at bird level, but they were analyzed as continuous variables at flock level, presented as the sum of birds (in percentage of the flock) with scores regarded to represent compromised welfare. Mortality rate was calculated as the sum of collected dead and culled birds until the day of the visit—expressed as a percentage of the number of chicks placed at d 1. To allow comparison between farms and flocks sampled at slightly different ages, this mortality rate was then linearly transformed (standardized) to percent mortality until 42 d of age.

Assessment of Fear of Humans and Negative Emotional State

In the ADT, an individual bird is approached by the assessor with a speed of 1 s per step, starting at a distance of 1.5 m. At the moment the bird withdraws, the distance between human and bird is measured. Twenty-one birds per farm were tested in this way (Graml et al., 2008).

In the TT, the assessor approaches a group of at least 3 birds and then squats. This person then tries to touch 3 individual birds within arm length (one trial). The test is finished after 21 birds have been touched or after 21 trials (Graml et al., 2008). The outcome is recorded as the percentage of birds that could actually be touched. The percentage is used to correct for different stocking densities between flocks.

In the NOT, a novel object (50 × 2.5 cm tube covered with 3.3-cm-wide rings in green, white, red, black, and blue) is placed in the litter area. Scan samples are taken at 30-s intervals over a 2-min period and the numbers of birds within one bird length of the object are recorded. Three tests were conducted per flock (Forkman et al., 2009).

In QBA, the assessor registers the body language of broilers by observation of the expressive quality of the birds' activity at flock level for a duration of 20 min (Welfare Quality, 2009). Twenty-three descriptors (active, relaxed, helpless, comfortable, fearful, agitated, confident, depressed, calm, content, tense, inquisitive, unsure, energetic, frustrated, bored, friendly, positively occupied, scared, drowsy, playful, nervous, stressed) were scored at the end of the observation period using a visual analog scale. The scale was a 12.5-cm-long line, one for each descriptor, with the minimum and maximum expressions at each end, respectively. Each descriptor was scored by placing a cross along the line, and the score was registered by measuring the distance between the minimum end and the cross, in centimeters. The number of descriptors was subsequently reduced by means of principal component analysis. The first 2 principal components were used as the QBA measures for that flock in the subsequent risk factor analysis.

Exclusion of Variables

To avoid collinearity in the regression models, 3 input variables were excluded from the risk factor analysis, due to strong correlations: thinned (i.e., the flock is not removed from the house for slaughter as a whole but in 2 or 3 batches over a period of days or weeks, to allow the remaining birds to become heavier) and BW were excluded because they were both correlated with age. Stocking density, birds per meter^2, was excluded because it was correlated with stocking density, kg/m^2 (**DensKG**). Because age affects BW, but BW does not affect age, we thought it more appropriate to exclude the variable BW than age. This means that the remain-

Table 1. Characteristics of the flocks: bird-based measures

Variable[1]	Unit	n	Mean[2]	Q50[3]	Q5[3]	Q95[3]
Flock age	d	88	38.6	41.0	29.0	47.0
BW (day of visit; estimate, based on farm data)	kg	88	1.93	2.01	1.23	2.51
Mortality (dead and culled birds, transformed to until 42 d of age)	%	89	3.6	3.1	1.4	6.8
Foot pad dermatitis[4,5] (birds with moderate or severe skin lesions)	%	89	37.3	33.0	1.0	91.0
Hock burn[4,6] (birds with moderate or severe skin lesions)	%	89	7.9	2.0	0.0	41.0
Lameness[7] (lame birds, i.e., gait score 3 or above)	%	89	15.6	11.2	0.5	52.0
Avoidance distance test[8] (distance between human and bird)	cm	89	68	71	18	122
Touch test[9] (touched birds), absolute values	n	89	1.4	0.9	0.1	4.3
Touch test[9] (touched birds). 100% = no. of birds that would be within 1/2 circle with a radius of 1 m (arm's reach) if evenly spread in the house, calculated from stocking density	%	88	5.6	3.5	0.2	19.7
Novel object test,[10] birds close to the object	n	89	2.1	1.5	0.1	6.9
Novel object test,[10] birds close to the object. 100% = no. of birds that would be within a circle with a radius of 30 cm (bird's length) if evenly spread in the house, calculated from stocking density	%	88	49.8	31.1	1.6	175.9
Qualitative behavior assessment[11] (weighted sum of values per flock)						
First principal component	Score	88	−0.1	0.4	−4.7	2.7
Second principal component	Score	88	−0.3	−0.1	−4.2	3.0

[1]If not specified, data were gathered at or until the day of assessment.

[2]Unweighted mean (i.e., each flock contributes equally, independent of flock size).

[3]Quantile (percent).

[4]Based on a sample of 100 birds/flock, 10 birds picked at 10 locations.

[5]At bird level: 4 classes. Score 0: no lesion, 1: very small and superficial lesion, 2: mild lesion, 3: moderate or severe lesions (Arnould et al., 2009). At flock level: percent of birds with score 3.

[6]At bird level: 3 classes. Score 0: no lesion, 1: very small or mild lesion, 2: moderate or severe lesions (Arnould et al., 2009). At flock level: percentage of birds with score 2.

[7]At bird level: 6 classes. 0: normal, dextrous, and agile, 1: slight gait abnormality, but difficult to define, 2: definite and identifiable abnormality, 3: obvious abnormality, affects the ability to move, 4: severe abnormality, only takes a few steps, 5: incapable of walking (Kestin et al., 1992). At flock level: percent of birds with score 3 or above. Sample size: 250 birds/flock.

[8]An individual bird is approached with a speed of 1 s per step, starting at a distance of 1.5 m. At the moment when the bird withdraws, the distance between human and bird is measured. Twenty-one birds, mean distance (Graml et al., 2008).

[9]An assessor approaches a group of at least 3 birds and then squats. This stationary, squatting person tries to touch 3 individual birds within arm length (1 trial). The outcome is the number of birds touched. The test is finished after 21 birds have been touched or after 21 trials. Mean no. of birds touched per trial (Graml et al., 2008).

[10]A novel object is placed in the litter area (tube, 50 × 2.5 cm, covered with 3.3-cm rings in green, white, red, black and blue). Birds within one bird-length of the object are scan sampled, at 30-s intervals over a 2-min period. Three tests per flock. Mean no. of birds counted per scan (Forkman et al., 2009).

[11]Assessment of the body language of broilers by observation of the expressive quality of the birds' activity at flock level, for a duration of 15 min (Welfare Quality, 2009). Twenty-three descriptors (active, relaxed, helpless, comfortable, fearful, agitated, confident, depressed, calm, content, tense, inquisitive, unsure, energetic, frustrated, bored, friendly, positively occupied, scared, drowsy, playful, nervous, stressed) were scored at the end of the observation period using a visual analog scale. The number of descriptors was subsequently reduced by means of principal component analysis. The first and second principal component were used as the qualitative behavior assessment measure for that flock in subsequent risk factor analysis.

ing variable age may also represent the effects of BW or thinning in the regression model, and that age, BW, and thinning effects cannot be shown separately in this study.

We decided not to include growth rate (a function of BW and age) as a potential risk factor in the regression models, because one objective was to identify RBM associated with the ABM investigated as welfare indicators in the present study. Although growth rate can be modified by flock management, its inclusion in the regression model would have reduced the magnitude and statistical significance of the regression coefficients for other RBM because statistical associations favor direct over indirect causes (Dohoo et al., 2009).

Variable Preselection

A rule of thumb for this type of study is that at least 10 to 15 flocks are desirable per potential risk factor (Stevens, 2009). Ten RBM were selected for analysis here based on their plausible biological association with the main welfare problems. Atmospheric ammonia and number of different stockmen working with the flock (stockmen, i.e., 1 or >1) were binary variables, whereas flock age (age), DensKG, dark period at 3 wk of age (**DARK3**), light intensity, daily time spent by farmer for flock observation, litter quality (litter), flock size (at day of visit), and number of birds on the farm site (whole farm) were all continuous variables. To limit the number of input variables in the regression models, interactions between RBM (interaction terms) were not selected.

Missing Values

Missing values constituted 2% of the prepared data set. Seventeen missing values were found among the independent variables, 2 among the dependent variables. To use as many observations as possible and to have a comparable number of observations (n) per test during

Table 2. Characteristics of the flocks: resource-based measures

Variable[1]	Unit	n	Mean[2]	Q50[3]	Q5[3]	Q95[3]
Stocking density, birds/m^2	birds/m^2	88	16.5	16.8	9.4	22.5
Stocking density, kg/m^2	kg/m^2	87	31.1	30.7	17.7	48.1
Dark period at 3 wk of age	h/d	88	2.7	2.0	0.0	6.5
Light intensity,[4] 30 cm above the ground (mean of 3 locations)	lux	87	14	8	1	42
Observation time by stockperson(s), per flock	min/d	86	94	70	30	195
Litter quality[5] (mean score from 10 locations; 5 classes, 1–5)	Score	89	2.9	3.0	1.4	4.5
Flock size (no. of birds placed)	kbirds[6] n	89	20.07	20.02	7.28	34.75
Birds on farm site	kbirds, n	86	121.8	97.5	19.9	352.0
Dichotomous variables	0/1	n	Frequency, 0 vs. 1			
Air ammonia, estimated concentration (</>20 mg/kg per m^3)	Low/high	89	66: 23			
Number of different persons taking care of the flock (1 or >1 person)	1/>1	85	39: 46			
Thinned (i.e., a portion of the flock was removed before the visit)[7]	N/Y	89	34: 55			
Categorical variables		n	Frequency, as indicated			
Genotype (Ross 308: Cobb: other or mixed Ross and Cobb)		87	68: 8: 11			
Bedding material (wood shavings: chopped straw: other)		89	53: 21: 15			

[1]If not specified, data were gathered at or until the day of assessment.

[2]Unweighted mean (i.e., each flock contributes equally, independent of flock size).

[3]Q = quantile (percent).

[4]3 measurements per location: sensor facing upward and 90° to the left and right, respectively.

[5]1: free flowing/crumbly, no capping in any area, 2: very slight capping just visible but mostly friable, 3: access to friable litter partially reduced (approximately 50%), 4: most areas capped but litter still friable in small areas, 5: extensive capping/crusting or compaction with access to friable litter negligible (Tucker and Walker, 1992).

[6]kbirds = thousand birds.

[7]The flock is not removed from the house for slaughter as a whole but in 2 or 3 batches over a period of days or weeks, to allow the remaining birds to become heavier.

regression model building, missing values of both continuous and categorical variables were imputed (multiple imputation, m = 5). The imputations were obtained by fitting a sequence of regression models and drawing values from the corresponding predictive distributions, using the software IVEware version 0.2 (Raghunathan et al., 2001; IVEware, 2011). Variables included in the imputation models (i.e., the models used to calculate replacements for the missing values) were as follows: all 10 RBM selected after data set preparation, and all ABM used, plus BW and mortality.

Risk Factor Analysis

The risk factor analysis was based on multiple linear regression. One regression model was built independently for each ABM. The initial linear model for each ABM, containing all 10 preselected variables (RBM), was checked for collinearity by calculating the variance inflation factors. The residuals were checked visually for normality, linearity, and equal variances. The FPD, HB, lameness, and NOT scores were log(10)-transformed to better meet the assumption of equal variances. The Pearson product moment correlation coefficient was calculated for RBM that were suspected to be highly correlated.

Following variable preselection, data transformation, and imputation, multiple linear regression models were built for each ABM, using manual backward elimination. The least significant input variable was eliminated first and that process was continued with the least significant variable in the model eliminated in each step until a preliminary model was obtained with only significant input variables. The input variable age was forced into each model because we could not visit all flocks at the same age and we wanted to have any association found between a RBM and an ABM corrected for a possible age effect. The significant input variables were regarded as risk factors. To account for the number of statistical tests performed on the data a α-level of 0.007 was chosen ad hoc for inclusion of original input variables. Confounding was tested by reentering all nonsignificant input variables one by one into the preliminary model. Confounding was regarded present if regression coefficients of any of the risk factors then changed by 20% or more. The effect of litter quality on FPD and HB was confounded by DARK3: a longer DARK3 was associated with poorer litter. The effects of age on ADT and the effect of DARK3 on TT were confounded by litter: increasing age was associated with poorer litter. The effect of age on QBA-pc1 (see Results/QBA Results) was confounded by DensKG, which was positively correlated with age.

Magnitude of the Potential Effects of the Risk Factors

It is not possible to objectively rank the input variables (RBM) according to their importance for the response variable (ABM), because of their differences in nature and the ways they have been measured (e.g., in kilograms, days, or scores). However, to get a feeling for the magnitude of the potential effect of each individual RBM on each ABM, we simulated flocks where one RBM, the one whose magnitude of effect was under investigation, was given specific high and low values that appeared to be realistic for commercial farms. The values for all other RBM in the model were held fixed.

Using the multiple regression models built earlier for risk factor analysis, the response of each ABM was calculated for 3 scenarios (except in the case of binary scoring systems, where only 2 scenarios were possible): 1) The risk factor in question takes the values of its 5% quantile, 2) its 50% quantile, and 3) its 95% quantile, with all other input variables of the regression model held constant at their median values. The difference between the respective responses was then used to judge the magnitude of the risk factor's potential effect.

Associations Between ABM

A common factor analysis with varimax rotation was used to investigate associations between ABM. The results were then compared with those obtained with a promax rotation. Both rotations aim for the original variables (the ABM) to load high on one of the factors and low on the rest (so-called simple structure). Thereby, varimax maintains the orthogonal (uncorrelated) nature of the factors, whereas promax allows correlation. The latter may a) be closer to reality and b) make it easier to obtain simple structure. The first 2 factors with eigenvalues >1 were retained.

Pearson coefficients of correlation were calculated for TT, ADT, lameness, and FPD to check for any potential bias in the risk factor analysis.

Spectrum of Each Risk Factor

After identifying risk factors for each ABM with regression analysis, we then grouped the ABM by risk factor and counted how many ABM were associated with a given risk factor. Simultaneously, the results of the factor analysis revealed any significant associations between the ABM: variables with high positive or high negative coefficients for a particular factor in the factor analysis are statistically associated [e.g., ADT and TT, and hock burn and lameness are associated (see Results)]. Exploring the results of regression analysis and factor analysis together allowed us to characterize each risk factor's range of potential effects with regard to animal welfare measures. Risk factors were judged to have a broad spectrum if they met both of the following criteria: 1) they are associated with several ABM, and 2) these ABM are statistically weakly associated. Thus, a risk factor with a broad spectrum of effects is associated with several ABM that do not otherwise appear to be correlated with each other.

RESULTS

General Observations

Characteristics of the flocks are presented in Tables 1 and 2. Mean flock age on the day of visit was 38.6 d (5% quantile: 29.0–95% quantile: 47.0); mean BW was 1.93 kg (1.23–2.51); mean DensKG was 31.1 kg/m^2 (17.7–48.1) and mean flock size (number of chicks placed) was 20,100 birds (7,300–34,800). Mean and median dark periods at 3 wk of age were 2.7 and 2.0 h, respectively (0.0–6.5). On the day of visit the mean and median light intensities measured 30 cm above the ground were 14 and 8 lx, respectively; mean prevalence of moderate or severe foot pad dermatitis was 37.3% (1–91) and mean prevalence of lameness (gait score 3 and above) was 15.6% (0.5–52). Mean calculated mortality until 42 d of age was 3.6% (1.4–6.8).

QBA Results

Principal component analysis of the 23 QBA variables reveals 2 main principal components, explaining 25.2 and 18.1% of the variation between flocks, respectively. The first component (**QBA-pc1**) is associated with variables that range from calm and relaxed at the positive end, to agitated, unsure, tense, and nervous at the negative end, and also at the negative end, but at a lower level, inquisitive, playful, and energetic. The second component (**QBA-pc2**) is associated with variables ranging from content, positively occupied, energetic, and confident at the positive end to helpless, drowsy, bored, and depressed at the negative end. Table 3 shows the first 2 principal components and their coefficients.

Final Regression Models

Table 4 shows the final linear regression models for FPD, HB, lameness, TT, ADT, and QBA results. The R^2 of the final regression models ranged from 0.11 to 0.66. Litter was identified as a risk factor for FPD and HB; DARK3 was identified for lameness, TT, QBA-pc1, and QBA-pc2; and stockmen was a risk factor for QBA-pc1. Age, the variable forced into each model, had P-values <0.007 (the chosen level for α) only in the preliminary models for lameness and ADT, and adding litter as a confounder to the ADT-model increased the P-value above 0.007. Also the P-values of DARK (TT) and stockmen (QBA-pc1) increased due to confounders. None of the RBM contributed significantly to the model for the results of the NOT.

The sample distributions of DARK3, litter, and BW are presented in Figures 1, 2, and 3, respectively, to illustrate the bases for our identification of the predominant risk factors.

Magnitude of the Potential Effects of the Risk Factors

Table 5 shows the estimated effects on ABM outcomes of specific changes in the risk factors. The scenarios with simulated flocks with high or low values for the risk factor in question resulted in the following estimates: lameness (% birds with a gait score 3 and above) is estimated to increase from 2.0% (95% CI:

Table 3. Principal component analysis of qualitative behavior assessment (QBA) results: the first 2 principal components and their coefficients

	Principal component (pc)	
Item	pc 1	pc 2
Variable (descriptor)		
Active	−0.13	0.22*
Relaxed	0.11	0.08
Helpless	−0.14	−0.29*
Comfortable	0.03	0.18
Fearful	−0.26*	−0.03
Agitated	−0.34*	0.05
Confident	−0.06	0.29*
Depressed	−0.17	−0.25*
Calm	0.17	−0.02
Content	−0.02	0.33*
Tense	−0.32*	−0.05
Inquisitive	−0.25*	0.15
Unsure	−0.33*	−0.03
Energetic	−0.21	0.31*
Frustrated	−0.22*	−0.19
Bored	−0.17	−0.26*
Friendly	0.00	0.19
Positively occupied	−0.17	0.32*
Scared	−0.30*	−0.02
Drowsy	−0.09	−0.27*
Playful	−0.21	0.28*
Nervous	−0.32*	0.01
Stressed	−0.20	−0.23*
Eigenvalues	5.78	4.16
Variance explained (%)	25.2	18.1

*Coefficients beyond 0.21 (±) reflect a considerable positive/negative relationship between the principal component and the original variable (e.g., agitated and unsure have relatively high loadings on pc 1, content and helpless have relatively high loadings on pc 2). The limit for deciding that a variable contributes considerably was that no variable should load positively/negatively on more than 1 principal component.

0.1–11.0) at age 29 d (29 d being the 5% quantile of the sample) to 31.2% (6.6–100) at age 47 d (47 d being the 95% quantile of the sample). The CI given here and below include the variations associated with the scenarios (prediction interval; Dohoo et al., 2009).

Further, an increase of DARK3 from 0.0 h (5% quantile) to 6.5 h (95% quantile) is estimated to be associated with a reduction in the prevalence of lameness from 16.9 to 7.4% or, for the same interval (0.0–6.5 h), twice as many birds are estimated to be touched in a TT. Also for the same increase in DARK3, QBA-pc1 would decrease by 2.03 units (a 20% shift across the sample in relation to the minimum and maximum values, −6 and +4), whereas the value of QBA-pc2 would rise by 1.74 units (a 17% shift).

A deterioration of litter from a mean score of 1.4 (5% quantile) to 4.5 (95% quantile) is estimated to be associated with an increased prevalence of FPD from 10.5 (95% CI 0.4–100) to 56.4% (4.1–100) and of hock burn from 0.2 (0.0–10.6) to 12.2% (0.3–100).

Having more than one stockman working with the flock is estimated to be associated with a decreased value of QBA-pc1 from 0.71 (95% CI −3.54–4.97) to −0.58 (−4.83–3.67), a 13% shift across the population.

Associations Between ABM

Originally, all ABM were included in the factor analysis. The QBA-pc2 was omitted from the final factor analysis because of its low Kaiser's measure of sampling adequacy value (**MSA**, 0.35). After its exclusion, overall MSA was 0.61. Two factors with eigenvalues >1 were retained: factor 1 explained 22.2% of the total variance, factor 2 18.3%. These factors were interpreted as representing the following latent structures in the data: factor 1 as behavior related to fear tests (fear of a human and of a novel object), and factor 2 as health related to legs and feet and to a lesser extent to emotional state (QBA-pc1). Table 6 shows the coefficients of the first 2 factors after varimax rotation. The factor pattern after a promax rotation was similar, indicating that the results of the factor analysis are relatively robust.

Pearson correlation coefficients were found between lameness and TT (n = 88) 0.10 ($P = 0.341$), and between lameness and ADT (n = 89) −0.24 ($P = 0.025$). The correlation coefficients between FPD and TT or ADT were both below 0.2 ($P > 0.14$).

Spectrum of Each Risk Factor

The ABM associated with the risk factor litter [i.e., FPD and HB (Table 4)] were clearly correlated: loadings on factor 1 are 0.54 and 0.75, respectively (Table 6). The ABM associated with the risk factor DARK3 [i.e., lameness, human-animal relationship (TT), and QBA-pc1 (Table 4)] as well as the ABM associated with the risk factor age [i.e., lameness and human-animal relationship (ADT, Table 4)] were only weakly correlated: loadings on factor 1 and factor 2, respectively, are for lameness 0.26 (0.55), for QBA-pc1 −0.16 (0.32), and for TT and ADT 0.80 (0.05) and −0.78 (−0.09), respectively (Table 6).

DISCUSSION

General Synopsis

Of the selected resource-based measures, litter, DARK3, and age were the numerically predominant risk factors. With decreasing litter quality, the prevalence of contact dermatitis increased. With longer DARK3, lameness and fear of humans (TT) decreased, and with increasing age, the prevalence of lameness increased while fear of humans decreased (ADT). The association between DARK3 and QBA is discussed below.

Some ABM varied considerably across the flocks studied (e.g., the 5 and the 95% quantile for the number of birds with foot pad dermatitis were 1 and 91% in a flock, respectively, and for the number of lame birds 0.5 and 52% in a flock). The estimated effects on the ABM of specific changes in the risk factors identified here (the magnitude of their potential effect) seems sufficiently large to enable manipulation of the risk factors

Table 4. Final linear regression models: 1 model per response variable (ABM)[1]

Model	Intercept[2]	RBM: Flock age (d)	Stocking density (kg/m^2)	Litter quality[3] (score)	Dark period[4] (h)	Stockmen[5] [1 (0) or >1 (1)]
ABM						
Transformed[6]	P.E.[7]	P.E.	P.E.	P.E.	P.E.	P.E.
R^2, mean[8,9]	95% CI	95% CI	95% CI	95% CI	95% CI	95% CI
Pr > F,[9,10] mean	Pr > t[11]	Pr > t	Pr > t	Pr > t	Pr > t	Pr > t
Foot pad dermatitis (% of birds with moderate or severe skin lesions)						
$Log_{10}(x + 0.5)$	1.41	0.01		**0.23**	−0.03	
0.18	1.28 to 1.53	−0.01 to 0.03		**0.08 to 0.38**	−0.08 to 0.02	
0.001	<0.0001	0.240		**0.004**	0.269	
Hock burn (% of birds with moderate or severe skin lesions)						
$Log_{10}(x + 0.5)$	0.49	−0.01		**0.41**	−0.09	
0.25	0.35 to 0.62	−0.03 to 0.02		**0.24 to 0.58**	−0.15 to −0.04	
<0.0001	<0.0001	0.606		**<0.0001**	0.001	
Lameness (% of birds with a gait score indicating obvious abnormality that affects the ability to move, or worse)						
$Log_{10}(x + 0.5)$	1.13	**0.06**		0.12	**−0.05**	
0.66	1.06 to 1.21	**0.05 to 0.07**		0.03 to 0.21	**−0.08 to −0.02**	
<0.0001	<0.0001	**<0.0001**		0.011	**0.0008**	
Avoidance distance test result (the distance to which a human could approach a bird before it turns away, cm)						
Not transformed	65.31	**−1.16**		−5.4837		
0.11	58.68 to 71.94	**−2.27 to −0.04**		−12.66 to 1.69		
0.006	<0.0001	**0.042**		0.132		
Touch test result [% of birds that a squatting human could touch. 100% = no. of birds that would be within 1/2 circle with a radius of 1 m (arm's reach) if birds were evenly spread in the house, calculated from stocking density]						
Not transformed	5.72	0.11		1.03	**0.67**	
0.17	4.35 to 7.09	−0.12 to 0.34		−0.79 to 2.85	**0.14 to 1.20**	
0.001	<0.0001	0.348		0.259	**0.014**	
First principal component of qualitative behavior assessment (behavioral expression of the flock, body language; higher values: flock appeared calm/relaxed, lower: agitated/tense). Flock score, no unit.						
Not transformed	0.72	0.02	0.06		**−0.33**	**−1.36**
0.26	0.02 to 1.42	−0.06 to 0.1	0 to 0.11		**−0.5 to −0.14**	**−2.26 to −0.33**
<0.0001	0.045	0.635	0.048		**0.001**	**0.009**
Second principal component of qualitative behavior assessment (behavioral expression of the flock, body language; higher values: flock appeared positively occupied/content, lower: helpless/drowsy). Flock score, no unit.						
Not transformed	−0.62	−0.04			**0.27**	
0.14	−1.07 to −0.16	−0.11 to 0.03			**0.12 to 0.42**	
0.002	0.008	0.236			**0.0007**	

[1]Input variables (RBM) are listed horizontally.

[2]As all continuous variables were median-centered, the intercept is the value that the response variable takes when all continuous variables take their median value and all dichotomous variables take the value zero.

[3]Mean score, 5 classes: 1 = free flowing/crumbly. Five = extensive capping/crusting or compaction.

[4]At 21 d of age.

[5]Number of different stockmen working with the flock. Two classes: 1 or >1 stockperson.

[6]Transformation of the response variable. The values in this table are not back transformed.

[7]Parameter estimates (P.E.) for the regression equation. Risk factors boldfaced [e.g., for every 1-h increase in the daily period of darkness, across the range of 0 to 6.5 h, the number of birds touched in a touch test increases by 0.67 (0.14–1.20) % units].

[8]Model coefficient of determination.

[9]Mean of 5 models: after multiple imputation (m = 5) of missing values, the final model is based on 5 imputed data sets.

[10]P-value, model F-statistic.

[11]P-value, coefficient t-statistic.

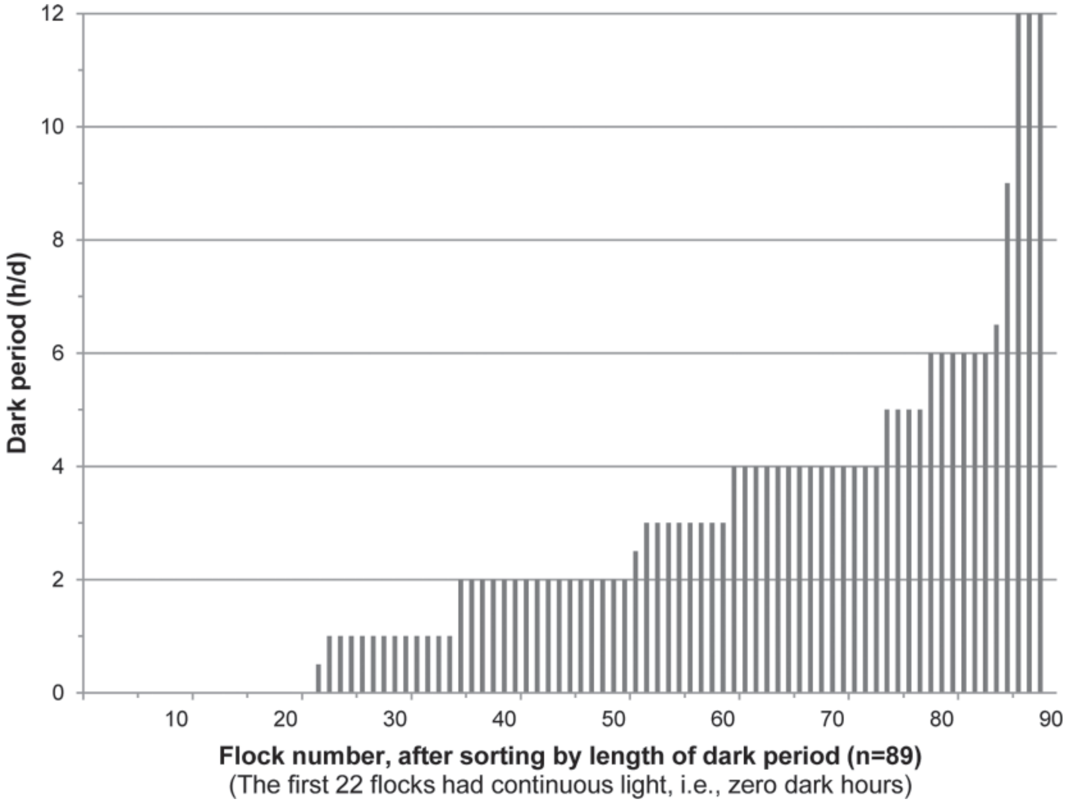

Figure 1. Sample distribution of length of dark period at 3 wk of age.

to improve the outcomes of the ABM and hence the birds' welfare status. But, because we focused only on fast-growing broilers in traditional-intensive production systems, these results do not necessarily challenge the conclusion of EFSA (2012b) that animal husbandry options to prevent the negative side effects of genetic selection for fast BW gain (metabolic and cardiovascular diseases) are limited.

Measures of hock lesions and legs twisted outward at the intertarsal joint were positively correlated with litter moisture in an earlier study (Dawkins et al., 2004), but the duration of dark period (measured during the

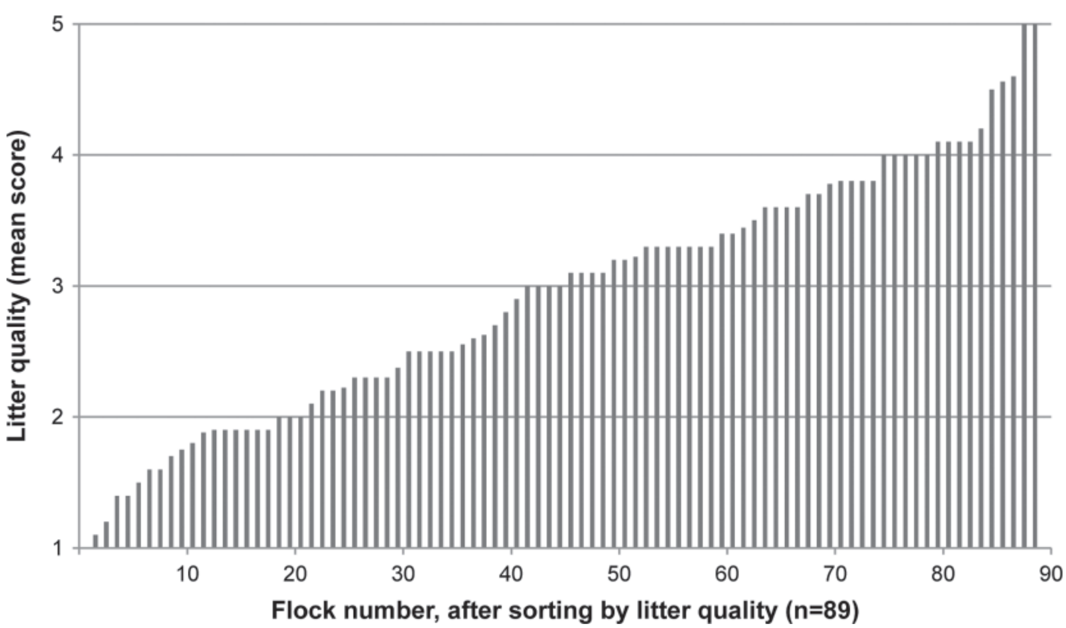

Figure 2. Sample distribution of litter quality at day of visit. The scale for litter quality ranged from 1: free flowing/crumbly, no capping in any area to 5: extensive capping/crusting or compaction with access to friable litter negligible (Tucker and Walker, 1992).

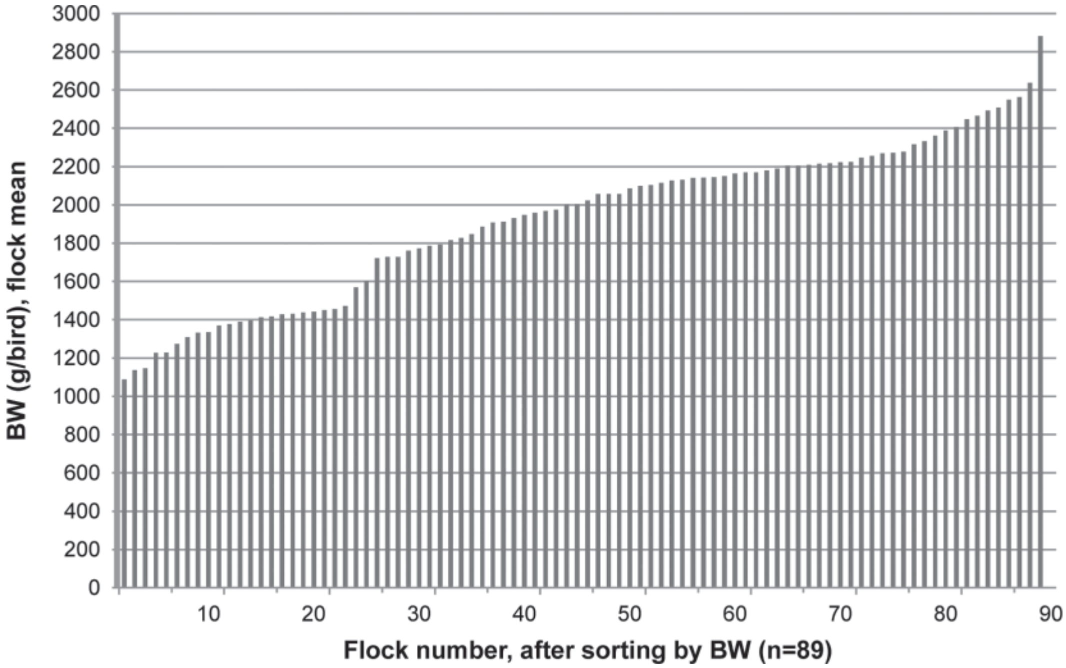

Figure 3. Sample distribution of BW (flock mean) at the day of visit.

last days of production) was not identified as a risk factor. Because the present study recorded dark period at 3 wk and because the lighting pattern may change considerably within a growing cycle (Prescott et al., 2004), it is difficult to compare our findings with those of Dawkins et al. (2004).

Finally, because statistical methods alone cannot establish proof of a causal relationship in any association, the present study cannot provide firm evidence regarding causal effects.

Associations Between ABM, Spectrum of Each Risk Factor

The finding that the RBM DARK3 and age were each associated with several ABM, which in turn were only weakly associated, suggests that the range of effects of these particular RBM is broad. The biological effect(s) of the risk factors may be systemic [i.e., relating to the organism as a whole (as opposed to local health effects)]. Indeed, a light-dark rhythm is one of the major stimuli controlling the birds' diurnal rhythms. It affects, among other things, hormonal cycles, body temperature, and behavior (Takahashi et al., 1968; Cain and Wilson, 1974; Blatchford et al., 2009). Broilers can rest and sleep during light periods, but a dark period offers them a more distinct period of rest as well as probably reducing disturbance by other birds (Coenen et al., 1988; Martrenchar et al., 1997; Alvino et al., 2009). Although the overall function of sleep is still under debate it has been linked to muscle growth, bone mineralization, tissue repair, protein synthesis, and growth hormone release (Blokhuis, 1983; Russell et al., 1984; Pitman and Waddell, 2009). These effects of light-dark rhythm and sleep may explain how the duration of the dark period can affect different ABM that are otherwise not correlated.

Because our data set includes dark periods ranging from near-continuous light (used in several farms) to 6 h darkness, it enabled us to investigate the potential effects of changes within that range. Our results indicate that an increased dark period has relevant advantages for broiler welfare, and thereby support the requirements of the current EU broiler directive 2007/43/EC, instigated in 2010, which specifies a minimum of 6 h dark per day for most of the rearing period.

The ABM associated with litter (i.e., foot-pad dermatitis and hock burn) are clearly correlated. This is to be expected because FPD and HB are manifestations of the same condition, contact dermatitis, and have a common direct cause [i.e., poor litter quality (Shepherd and Fairchild, 2010)].

Dark Period and Litter Quality

We speculate that the negative correlation found between length of dark period and litter quality (see Materials and Methods/Risk Factor Analysis) reflects reduced bird activity. Birds mainly rest during dark periods (Calvet et al., 2009), thereby compacting the litter rather than working it. This effect is likely to be more pronounced with increasing bird age, as they become physically less active and produce more manure (Bokkers and Koene, 2003; Baeza et al., 2012). A decreased prevalence of FPD with increasing day length (Sørensen et al., 1999) supports this hypothesis. On the other hand, we found a trend toward a lower prevalence of FPD and HB with decreasing day length, despite deteriorating litter quality. This raises the question of whether some beneficial effects of a dark period for FPD may compensate for the accompanying deterioration of litter quality.

Dark Period and Lameness

It is generally agreed that a dark period is beneficial for broilers' walking ability (Wilson et al., 1984; Classen and Riddell, 1989; Møller et al., 1999; Knowles et al., 2008) for several likely reasons:

a) Birds given a dark period are physically more active during the light period than are birds kept under near-continuous light (Schwean-Lardner et al., 2012). Physical activity supports bone development (Lanyon, 1992), so increased activity during the light period may help reduce lameness in broiler flocks (Reiter, 2004);
b) Bone mineralization, which peaks during the dark period, is also sensitive to diurnal rhythm (Russell et al., 1984); and
c) A longer daily dark period during the first 2 wk of life reduces feed intake and (muscle) growth rate, thereby allowing the skeletal system more time to develop (SCAHW, 2000; Berg, 2004).

Body weight, growth rate, and age are major determinants for lameness (Kestin et al., 2001; Bokkers and Koene, 2003; Kristensen et al., 2006; Baeza et al., 2012), and as explained earlier, these 3 determinants are correlated (see Materials and Methods/Exclusion of Variables). However, including the variable age in our regression model for lameness corrected the regression coefficient of DARK3 (the regression coefficient quantifies the "effect" of DARK3 on lameness) for any influence of age. Therefore, the estimated effect of DARK3, as quantified in our regression equation, may also be regarded as independent of BW or growth rate.

Dark Period and Human-Animal Relationship

The TT is thought to measure the quality of the human-animal interaction (Graml et al., 2008). In the present study, a longer dark period is associated with a larger number of birds remaining within arm's reach when touched. Longer dark periods are associated with decreased stress responses (Zulkifli et al., 1998) and fear of humans (Jones and Faure, 1981; Jones and Waddington, 1992). This may explain why it was easier to touch broilers in flocks with longer dark periods.

Age was the only risk factor for ADT in our study. Broilers become physically less active with increasing age and BW (Bokkers and Koene, 2003). Age was also a risk factor for lameness and Weeks et al. (2000) showed how lameness affected the broilers' motivation to walk. This suggests that heavier, less agile birds wait longer before turning away from an approaching human because they find it difficult to move. Indeed, some assessors' practical experience during the farm visits raised the question of whether the results of TT and ADT may be biased by lameness, FPD, or crowding, factors that may prevent the birds from moving freely. Furthermore, I. C. De Jong (personal communication) found a positive correlation between the percentage of lame birds and the number of birds that could be touched in a TT. However, our present data do not show any association patterns: the coefficients of correlation between FPD/lameness and TT/ADT are low and DensKG was not identified as a risk factor. We do not doubt that it should be easier to approach lame birds, but our findings that a longer dark period is associated with reduced lameness but also with increased TT counts suggests that in our data set there are other variables than lameness that influenced the results of the approach tests.

Dark Period and QBA

A longer dark period was statistically associated with flocks appearing more content, positively occupied, and energetic (higher values of QBA-pc2). This accords well with reports that a dark period increases the birds' physical activity during light (Schwean-Lardner et al., 2012). Other positive associations herein were that more birds could be touched (TT) and that lameness was reduced when the dark period was longer. However, the association between length of dark period and QBA-pc1 was negative, suggesting that the flocks were more agitated, unsure, tense, and nervous, and even scared and fearful, which certainly does not fit well with the picture drawn above.

A possible explanation might lie in the nature of the principal components. They are characterized by descriptors with highest and lowest loadings, and are often labeled by only 1 or 2 terms (e.g., a stress factor). Such labels may make it easier to report observations, but they are at risk of covering up a more subtle and complex picture. For example, the negative side of QBA-pc1 comprises not only descriptors such as agitated, nervous, scared, tense, and fearful, but also, though with somewhat lower loadings, inquisitive, playful, and energetic. Thus, the same flocks seem to have shown both agitated/fearful and inquisitive/playful patterns of expression. A similar concurrence of expressive patterns was found in laboratory rodents in response to environmental enrichment (Carlstead and Shepherdson, 1994; McQuaid et al., 2012). We hypothesize that the seemingly contradictory outcomes (the same flock scoring high on agitated/fearful and inquisitive/playful) are 2 sides of the same coin: both express greater responsivity, or in other words, greater arousal or liveliness in interaction with the environment. What this means for the birds' well-being is not immediately clear. And as the percentage of variation explained by QBA-pc1

Table 5. Responses of animal-based measures (ABM) to specific changes in risk factors[1]

	RBM													
	Flock age (d)			Stocking density (kg/m²)			Litter quality[2] (score)			Dark period[3] (h)			Stockmen[4] (0/1)	
Item	Q5[5]	Q50	Q95	Q5	Q50	Q95	Q5	Q50	Q95	Q5	Q50	Q95	0	1
ABM	29	41	47	17.68	30.74	48.10	1.4	3.0	4.5	0.0	2.0	6.5	1	>1
Foot pad dermatitis (% of birds with moderate or severe skin lesions)														
Response[6]	17.4	25.1	30.2				**10.5**	**25.1**	**56.4**	28.6	25.1	18.8		
LCL95[7]	1.0	1.7	2.0				**0.4**	**1.7**	**4.1**	1.9	1.7	1.1		
UCL95[7]	100	100	100				**100**	**100**	**100**	100	100	100		
Hock burn (% of birds with moderate or severe skin lesions)														
Response	3.1	2.6	2.3				**0.2**	**2.6**	**12.2**	4.2	2.6	0.7		
LCL95[7]	0.0	0.0	0.0				**0.0**	**0.0**	**0.3**	0.0	0.0	0.0		
UCL95[7]	58.3	46.8	44.3				**10.6**	**46.8**	**100.0**	73.1	46.8	18.4		
Lameness (% of birds with a gait score indicating obvious abnormality that affects the ability to move, or worse)														
Response	**2.0**	**13.1**	**31.2**				8.3	13.1	20.2	**16.9**	**13.1**	**7.4**		
LCL95[7]	**0.1**	**2.6**	**6.6**				1.4	2.6	4.0	**3.4**	**2.6**	**1.3**		
UCL95[7]	**11.0**	**59.9**	**100**				39.7	59.9	94.2	**77.1**	**59.9**	**35.4**		
Avoidance distance test result (the distance to which a human could approach a bird before it turns away, cm)														
Response	**79**	**65**	**58**				74	65	57					
LCL95[7]	**20**	**7**	**0**				14	7	0					
UCL95[7]	**139**	**124**	**118**				134	124	117					
Touch test result [% of birds that a squatting human could touch. 100% = no. of birds that would be within 1/2 circle with a radius of 1 m (arm's reach) if birds were evenly spread in the house, calculated from stocking density]														
Response	4.4	5.7	6.4				4.1	5.7	7.3	**4.4**	**5.7**	**8.7**		
LCL95[7]	0.0	0.0	0.0				0.0	0.0	0.0	**0.0**	**0.0**	**0.0**		
UCL95[7]	16.3	17.3	18.1				16.0	17.3	19.2	**16.1**	**17.3**	**20.5**		
First principal component of qualitative behavior assessment (behavioral expression of the flock, body language; higher values: flock appeared calm/relaxed, lower: agitated/tense). Flock score, no unit.														
Response	0.49	0.71	0.83	0.02	0.71	1.65				**1.34**	**0.71**	**−0.69**	**0.71**	**−0.58**
LCL95[7]	−3.83	−3.54	−3.47	−4.32	−3.54	−2.67				**−2.93**	**−3.54**	**−5.01**	**−3.54**	**−4.83**
UCL95[7]	4.81	4.97	5.12	4.36	4.97	5.96				**5.62**	**4.97**	**3.62**	**4.97**	**3.67**
Second principal component of qualitative behavior assessment (behavioral expression of the flock, body language; higher values: flock appeared positively occupied/content, lower: helpless/drowsy). Flock score, no unit.														
Response	−0.12	−0.62	−0.86							**−1.15**	**−0.62**	**0.59**		

Continued

Table 5 (Continued). Responses of animal-based measures (ABM) to specific changes in risk factors[1]

	RBM													
	Flock age (d)			Stocking density (kg/m^2)			Litter quality[2] (score)			Dark period[3] (h)			Stockmen[4] (0/1)	
Item	Q5[5]	Q50	Q95	Q5	Q50	Q95	Q5	Q50	Q95	Q5	Q50	Q95	0	1
LCL95[7]	−4.05	−4.50	−4.78							**−5.05**	**−4.50**	**−3.33**		
UCL95[7]	3.81	3.26	3.05							**2.75**	**3.26**	**4.51**		

[1]Input variables (RBM) listed horizontally; risk factor values are boldfaced. ABM listed vertically.

[2]Mean score, 5 classes: 1 = free flowing/crumbly; 5 = extensive capping/crusting or compaction.

[3]At 21 d of age.

[4]Number of different stockmen working with the flock. Two classes: 1 or >1 stockmen.

[5]Q = quantile (%).

[6]Based on the final linear regression models, the response of each animal based measure is estimated for 3 (binary: 2) scenarios per risk factor (predictor variable): The risk factor takes the values of its 5% (scenario 1), 50% (2), and 95% quantile (3), all other input variables of the model held constant at their medians (e.g., % of birds with foot pad dermatitis is estimated to rise from 10.5 to 56.4 when litter score changes from 1.4 to 4.5, whereas variables of age and dark period are held constant at their medians. Earlier log-transformed data are back transformed).

[7]Lower/upper 95% confidence limit (prediction interval, Dohoo et al., 2009).

(25.2) is relatively low, the results should not be overinterpreted.

In summary, due to the complex nature of the principal components, which reflect the complex nature of animal behavior itself, it is not self-evident that a decrease in the QBA-pc1 score means any deterioration of welfare. Interpretation of the QBA-pc2 in this respect is much more straightforward, which is why it is the only one included in the Welfare Quality assessment protocol. Taking QBA-pc1 into account as we have done here provides additional information on the birds' state and should help inform this discussion.

Sample Size and Quality

According to Cohen (1992), sample sizes required for the sort of multiple regression analysis used here range from n < 50 for models with a coefficient of determination (R^2) around 0.60 (cf lameness model) to n = 100 to 150 for models with $R^2 = 0.13$ (cf QBA-pc1 model). This means that a larger sample size than the 89 flocks used here would be desirable to detect smaller effects and reject any false null hypothesis in the present models.

Our flocks were neither systematically nor randomly selected. Because the flocks could only be assessed with the farmers' permission and most visits were done during spring and summer, our sample may be biased toward flocks with above-average welfare status.

Comparing our gait score results with those of 2 major studies (Dawkins et al., 2004; Knowles et al., 2008) supports this suggestion because they reported 26 and 28% lame birds, respectively, whereas we found an average of 16% at a comparable age. Furthermore, Haslam et al. (2007) and De Jong et al. (2012b) found moderate or severe FPD lesions in 11 and 65% of the birds, respectively, whereas we found 37%. To the best of our knowledge there were no major or systematic differences between our study and those mentioned above in flock age, stocking density, flock size, dark period, litter type, thinning, or number of stockpersons. The considerable variation in scores across flocks in our study and in those mentioned above may imply that our sample is fairly representative even though it might contain relatively few farms with severe welfare problems.

Animal Welfare Implications and Conclusions

It appears likely that there is scope for enhancing welfare in traditional-intensive broiler production and addressing the predominant risk factors identified in this study (length of dark period, litter quality, and slaughter age), may contribute to that effort.

We found that the length of dark period (range of 0 and 6 h herein) as well as age had a broad range of estimated effects on animal-based welfare indicators. The former finding supports the view that a sufficiently

Table 6. Factor analysis of animal-based measures: the first 2 factors and their coefficients after varimax rotation[1]

Variable (animal-based measure)	Factor 1	Factor 2
Foot pad dermatitis (% of birds with moderate or severe skin lesions)	−0.05	**0.54**
Hock burn (% of birds with moderate or severe skin lesions)	0.09	**0.75**
Lameness (% of birds with obvious gait abnormality, affecting the ability to move)	0.26	**0.55**
Avoidance distance test (the distance to which a human could approach a bird)	**−0.78**	−0.09
Touch test (no. of birds that could be touched by the assessor)	**0.80**	0.05
Novel object test (no. of birds close to a novel object)	**0.44**	−0.06
Qualitative behavior assessment, first principal component (pc, no unit; higher values: flock appeared calm/relaxed, lower: agitated/tense)	−0.16	**0.32**

[1]Factor analysis is used to represent associations among the animal-based measures. Variables with high positive or negative coefficients for the same factor are associated (e.g., avoidance distance test and touch test are associated, and hock burn and lameness are associated). Coefficients beyond 0.26 (±) reflect a considerable positive/negative relationship between the factor and the variable (bold typeface). The limit for deciding that a variable contributes considerably was that no variable should load positively/negatively on more than one principal component. No unit.

long scotoperiod is an important basic requirement for the welfare of broiler chickens. Although EU directive 2007/43/EC already requires a minimum of 6 h dark per day, it can still be helpful to measure this factor to assess compliance with the directive. Litter quality and slaughter age could also be considered for estimating the risk of poor welfare, if it can be firmly established that these variables can contribute to a reliable prediction model for broiler welfare.

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REFERENCES

Allain, V., L. Mirabito, C. Arnould, M. Colas, S. Le Bouquin, C. Lupo, and V. Michel. 2009. Skin lesions in broiler chickens measured at the slaughterhouse: Relationships between lesions and between their prevalence and rearing factors. Br. Poult. Sci. 50:407–417. http://dx.doi.org/10.1080/00071660903110901.

Alvino, G. M., R. A. Blatchford, G. S. Archer, and J. A. Mench. 2009. Light intensity during rearing affects the behavioural synchrony and resting patterns of broiler chickens. Br. Poult. Sci. 50:275–283. http://dx.doi.org/10.1080/00071660902942775.

Andreasen, S. N., F. Wemelsfelder, P. Sandoe, and B. Forkman. 2013. The correlation of Qualitative Behavior Assessments with Welfare Quality (R) protocol outcomes in on-farm welfare assessment of dairy cattle. Appl. Anim. Behav. Sci. 143:9–17. http://dx.doi.org/10.1016/j.applanim.2012.11.013.

Anonymous, . 2001. Scientists' assessment of the impact of housing and management on animal welfare. J. Appl. Anim. Welf. Sci. 4:3–52. http://dx.doi.org/10.1207/s15327604jaws0401_2.

Arnould, C., and A. Butterworth. 2010. On-farm evaluation of broiler chicken welfare. World's Poult. Sci. J. 66(Suppl.):9.

Arnould, C., A. Butterworth, and U. Knierim. 2009. Standardization of Clinical Scoring in Poultry. Pages 7–30 in Assessment of Animal Welfare Measures for Layers and Broilers. B. Forkman and L. Keeling, ed. Cardiff University, Cardiff, UK.

Baeza, E., C. Arnould, M. Jlali, P. Chartrin, V. Gigaud, F. Mercerand, C. Durand, K. Meteau, E. Le Bihan-Duval, and C. Berri. 2012. Influence of increasing slaughter age of chickens on meat quality, welfare, and technical and economic results. J. Anim. Sci. 90:2003–2013.

Berg, C. 2004. Pododermatitis and hock burn in broiler chickens. Pages 37–49 in Measuring and Auditing Broiler Welfare. C. A. Weeks and A. Butterworth, ed. CABI Publishing, Wallingford, UK.

Bessei, W. 2006. Welfare of broilers: A review. World's Poult. Sci. J. 62:455–466. http://dx.doi.org/10.1079/wps2005108.

Blatchford, R. A., K. C. Klasing, H. L. Shivaprasad, P. S. Wakenell, G. S. Archer, and J. A. Mench. 2009. The effect of light intensity on the behavior, eye and leg health, and immune function of broiler chickens. Poult. Sci. 88:20–28. http://dx.doi.org/10.3382/ps.2008-00177.

Blokhuis, H. J. 1983. The relevance of sleep in poultry. World's Poult. Sci. J. 39:33–37. http://dx.doi.org/10.1079/wps19830003.

Blokhuis, H. J., I. Veissier, M. Miele, and B. Jones. 2010. The Welfare Quality (R) project and beyond: Safeguarding farm animal well-being. Acta Agric. Scand. Sect. Anim. Sci. 60:129–140. http://dx.doi.org/10.1080/09064702.2010.523480.

Bokkers, E. A. M., M. de Vries, I. C. M. A. Antonissen, and I. J. M. de Boer. 2012. Inter- and intra-observer reliability of experienced and inexperienced observers for the Qualitative Behaviour Assessment in dairy cattle. Anim. Welf. 21:307–318. http://dx.doi.org/10.7120/09627286.21.3.307.

Bokkers, E. A. M., and P. Koene. 2003. Behaviour of fast- and slow growing broilers to 12 weeks of age and the physical consequences. Appl. Anim. Behav. Sci. 81:59–72. http://dx.doi.org/10.1016/s0168-1591(02)00251-4.

Botreau, R., I. Veissier, A. Butterworth, M. B. M. Bracke, and L. J. Keeling. 2007. Definition of criteria for overall assessment of animal welfare. Anim. Welf. 16:225–228.

Bradshaw, R. H., R. D. Kirkden, and D. M. Broom. 2002. A review of the aetiology and pathology of leg weakness in broilers in relation to welfare. Avian Poult. Biol. Rev. 13:45–103. http://dx.doi.org/10.3184/147020602783698421.

Butterworth, A., C. A. Weeks, P. R. Crea, and S. C. Kestin. 2002. Dehydration and lameness in a broiler flock. Anim. Welf. 11:89–94.

Cain, J. R., and W. O. Wilson. 1974. Influence of specific environmental parameters on circadian rhythms of chickens. Poult. Sci. 53:1438–1447.

Calvet, S., H. Van den Weghe, R. Kosch, and F. Estelles. 2009. The influence of the lighting program on broiler activity and dust pro-

duction. Poult. Sci. 88:2504–2511. http://dx.doi.org/10.3382/ps.2009-00255.
Carlstead, K., and D. Shepherdson. 1994. Effects of environmental enrichment on reproduction. Zoo Biol. 13:447–458. http://dx.doi.org/10.1002/zoo.1430130507.
Classen, H. L., and C. Riddell. 1989. Photoperiodic effects on performance and leg abnormalities in broiler-chickens. Poult. Sci. 68:873–879.
Coenen, A. M. L., E. Wolters, E. Vanluijtelaar, and H. J. Blokhuis. 1988. Effects of intermittent lighting on sleep and activity in the domestic hen. Appl. Anim. Behav. Sci. 20:309–318. http://dx.doi.org/10.1016/0168-1591(88)90055-x.
Cohen, J. 1992. A power primer. Psychol. Bull. 112:155–159. http://dx.doi.org/10.1037/0033-2909.112.1.155.
Coleman, G. J., and P. H. Hemsworth. 2010. Human-Livestock Interactions: The Stockperson and the Productivity of Intensively Farmed Animals. 2nd ed. CABI Publishing, Wallingford, UK.
Cransberg, P. H., P. H. Hemsworth, and G. J. Coleman. 2000. Human factors affecting the behaviour and productivity of commercial broiler chickens. Br. Poult. Sci. 41:272–279. http://dx.doi.org/10.1080/713654939.
Danbury, T. C., C. A. Weeks, J. P. Chambers, A. E. Waterman-Pearson, and S. C. Kestin. 2000. Self-selection of the analgesic drug carprofen by lame broiler chickens. Vet. Rec. 146:307.
Dawkins, M. S., C. A. Donnelly, and T. A. Jones. 2004. Chicken welfare is influenced more by housing conditions than by stocking density. Nature 427:342–344. http://dx.doi.org/10.1038/nature02226.
De Jong, I. C., C. Berg, A. Butterworth, and I. Estevez. 2012a. Scientific report updating the EFSA opinions on the welfare of broilers and broiler breeders. Supporting Publications EN-295:116.
De Jong, I. C., J. van Harn, H. Gunnink, V. A. Hindle, and A. Lourens. 2012b. Footpad dermatitis in Dutch broiler flocks: Prevalence and factors of influence. Poult. Sci. 91:1569–1574. http://dx.doi.org/10.3382/ps.2012-02156.
Dohoo, I., W. Martin, and H. Stryhn. 2009. Veterinary Epidemiologic Research. VER Inc., Charlottetown, Canada.
EFSA. 2010. Outcome of the Stakeholders and Public consultation on Health and Welfare aspects of genetic selection in broilers. European Food Safety Authority. EFSA Journal 8:1670.
EFSA. 2012a. Guidance on risk assessment for animal welfare. European Food Safety Authority. EFSA Journal 10:2513.
EFSA. 2012b. Scientific opinion on the use of animal-based measures to assess welfare of broilers. EFSA Panel on Animal Health and Welfare. European Food Safety Authority. EFSA Journal 10:2774.
Ekstrand, C., and T. E. Carpenter. 1998. Using a tobit regression model to analyse risk factors for foot-pad dermatitis in commercially grown broilers. Prev. Vet. Med. 37:219–228. http://dx.doi.org/10.1016/s0167-5877(98)00090-7.
EU. 2004. Regulation (EC) No. 882/2004 of the European parliament and of the council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. Official Journal of the European Union L 191: p.1. 28.5.2004. Amended by M1 Commission Regulation (EC) No 776/2006 of 23 May 2006 L 136 3 24.5.2006.
Forkman, B., T. Heiskanen, C. Graml, and S. Waiblinger. 2009. Assessment of general fearfulness. Pages 91–94 in Assessment of Animal Welfare Measures for Layers and Broilers. B. Forkman, and L. Keeling, ed. Cardiff University, Cardiff, UK.
Fraser, D. 2008. Understanding Animal Welfare: The Science in Its Cultural Context. Wiley-Blackwell, Oxford, UK.
Graml, C., S. Waiblinger, and K. Niebuhr. 2008. Validation of tests for on-farm assessment of the hen-human relationship in non-cage systems. Appl. Anim. Behav. Sci. 111:301–310. http://dx.doi.org/10.1016/j.applanim.2007.06.002.
Gross, W. B., and P. B. Siegel. 1980. Effects of early environmental stresses on chicken body-weight, anti-body-response to RBC antigens, feed-efficiency, and response to fasting. Avian Dis. 24:569–579. http://dx.doi.org/10.2307/1589792.
Haslam, S. M., S. N. Brown, L. J. Wilkins, S. C. Kestin, P. D. Warriss, and C. J. Nicol. 2006. Preliminary study to examine the utility of using foot burn or hock burn to assess aspects of housing conditions for broiler chicken. Br. Poult. Sci. 47:13–18. http://dx.doi.org/10.1080/00071660500475046.
Haslam, S. M., T. G. Knowles, S. N. Brown, L. J. Wilkins, S. C. Kestin, P. D. Warriss, and C. J. Nicol. 2007. Factors affecting the prevalence of foot pad dermatitis, hock burn and breast burn in broiler chicken. Br. Poult. Sci. 48:264–275. http://dx.doi.org/10.1080/00071660701371341.
Hemsworth, P. H., G. J. Coleman, J. L. Barnett, and R. B. Jones. 1994. Behavioral responses to humans and the productivity of commercial broiler chickens. Appl. Anim. Behav. Sci. 41:101–114. http://dx.doi.org/10.1016/0168-1591(94)90055-8.
IVEware. 2011. Imputation and variance estimation software. Survey Research Center, Institute for Social Research, University of Michigan, Ann Arbor.
Jones, R. B. 1987. Social and environmental aspects of fear in the domestic fowl. Pages 82–149 in Cognitive Aspects of Social Behaviour in the Domestic Fowl. R. Zayan, and I. J. H. Duncan, ed. Elsevier, Amsterdam, the Netherlands.
Jones, R. B., and A. Boissy. 2011. Fear and other negative emotions. Pages 78–97 in Animal Welfare. M. C. Appleby, J. A. Mench, I. A. S. Olsson, and B. O. Hughes, ed. CABI Publishing, Wallingford, UK.
Jones, R. B., and J. M. Faure. 1981. Tonic immobility (righting time) in laying hens housed in cages and pens. Appl. Anim. Ethol. 7:369–372.
Jones, R. B., and D. Waddington. 1992. Modification of fear in domestic chicks, *Gallus gallus domesticus*, via regular handling and early environmental enrichment. Anim. Behav. 43:1021–1033. http://dx.doi.org/10.1016/0003-3472(92)90015-2.
Julian, R. J. 2004. Evaluating the impact of metabolic disorders on the welfare of broilers. Pages 51–59 in Measuring and Auditing Broiler Welfare. C. A. Weeks and A. Butterworth, ed. CABI Publishing, Wallingford, UK.
Kestin, S. C., S. Gordon, G. Su, and P. Sørensen. 2001. Relationships in broiler chickens between lameness, liveweight, growth rate and age. Vet. Rec. 148:195–197.
Kestin, S. C., T. G. Knowles, A. E. Tinch, and N. G. Gregory. 1992. Prevalence of leg weakness in broiler-chickens and its relationship with genotype. Vet. Rec. 131:190–194.
Knowles, T. G., S. C. Kestin, S. M. Haslam, S. N. Brown, L. E. Green, A. Butterworth, S. J. Pope, D. Pfeiffer, and C. J. Nicol. 2008. Leg disorders in broiler chickens: Prevalence, risk factors and prevention. PLoS ONE 3. http://dx.doi.org/10.1371/journal.pone.0001545.
Kristensen, H. H., G. C. Perry, N. B. Prescott, J. Ladewig, A. K. Ersboll, and C. M. Wathes. 2006. Leg health and performance of broiler chickens reared in different light environments. Br. Poult. Sci. 47:257–263. http://dx.doi.org/10.1080/00071660600753557.
Lanyon, L. E. 1992. Functional load-bearing as a controlling influence for fracture resistance in the skeleton. Proc. Bone Biology and Skeletal Disorders in Poultry, Abingdon, UK.
Martrenchar, A., J. P. Morisse, D. Huonnic, and J. P. Cotte. 1997. Influence of stocking density on some behavioural, physiological and productivity traits of broilers. Vet. Res. 28:473–480.
McGeown, D., T. C. Danbury, A. E. Waterman-Pearson, and S. C. Kestin. 1999. Effect of carprofen on lameness in broiler chickens. Vet. Rec. 144:668–671.
McQuaid, R. J., M.-C. Audet, and H. Anisman. 2012. Environmental enrichment in male CD-1 mice promotes aggressive behaviors and elevated corticosterone and brain norepinephrine activity in response to a mild stressor. Stress 15:354–360. http://dx.doi.org/10.3109/10253890.2011.623249.
Mench, J. 2004. Lameness. Pages 3–17 in Measuring and auditing broiler welfare. C. A. Weeks, and A. Butterworth, ed. CABI Publishing, Wallingford, UK.
Møller, A. P., G. S. Sanotra, and K. S. Vestergaard. 1999. Developmental instability and light regime in chickens (*Gallus gallus*). Appl. Anim. Behav. Sci. 62:57–71. http://dx.doi.org/10.1016/s0168-1591(98)00213-5.
Pitman, J. L., and S. Waddell. 2009. Sleep: What goes up must come down. Curr. Biol. 19:R480–R482. http://dx.doi.org/10.1016/j.cub.2009.04.048.
Prescott, N. B., H. H. Kristensen, and C. M. Wathes. 2004. Light. Pages 101–116 in Measuring and Auditing Broiler Welfare. C. A.

Weeks, and A. Butterworth, ed. CABI Publishing, Wallingford, UK.

Raghunathan, T. E., J. M. Lepkowski, J. Van Hoewyk, and P. Solenberger. 2001. A multivariate technique for multiply imputing missing values using a sequence of regression models. Surv. Methodol. 27:85–95.

Reiter, K. 2004. Effect of distance between feeder and drinker on exercise and leg disorders in broilers. Arch. Geflugelkd. 68:98–105.

Russell, J. E., B. Grazman, and D. J. Simmons. 1984. Mineralization in rat metaphyseal bone exhibits a circadian stage dependency. Proc. Soc. Exp. Biol. Med. 176:342–345.

Rutherford, K. M. D., R. D. Donald, A. B. Lawrence, and F. Wemelsfelder. 2012. Qualitative behavioural assessment of emotionality in pigs. Appl. Anim. Behav. Sci. 139:218–224. http://dx.doi.org/10.1016/j.applanim.2012.04.004.

Sanotra, G. S., J. D. Lund, and K. S. Vestergaard. 2002. Influence of light-dark schedules and stocking density on behaviour, risk of leg problems and occurrence of chronic fear in broilers. Br. Poult. Sci. 43:344–354. http://dx.doi.org/10.1080/000716601201036023611.

SCAHW. 2000. The welfare of chickens kept for meat production (broilers). Report of the Scientific Committee on Animal Health and Welfare. European Commission Report. B3, R 15, 2000., Brussels, Belgium.

Schwean-Lardner, K., B. I. Fancher, and H. L. Classen. 2012. Impact of daylength on behavioural output in commercial broilers. Appl. Anim. Behav. Sci. 137:43–52. http://dx.doi.org/10.1016/j.applanim.2012.01.015.

Shepherd, E. M., and B. D. Fairchild. 2010. Footpad dermatitis in poultry. Poult. Sci. 89:2043–2051. http://dx.doi.org/10.3382/ps.2010-00770.

Sørensen, P., G. Su, and S. C. Kestin. 1999. The effect of photoperiod: scotoperiod on leg weakness in broiler chickens. Poult. Sci. 78:336–342.

Stevens, J. P. 2009. Applied Multivariate Statistics for the Social Sciences. 5th ed. Routledge, London, UK.

Stockman, C. A., T. Collins, A. L. Barnes, D. Miller, S. L. Wickham, D. T. Beatty, D. Blache, F. Wemelsfelder, and P. A. Fleming. 2011. Qualitative behavioural assessment and quantitative physiological measurement of cattle naive and habituated to road transport. Anim. Prod. Sci. 51:240–249. http://dx.doi.org/10.1071/an10122.

Stockman, C. A., T. Collins, A. L. Barnes, D. Miller, S. L. Wickham, D. T. Beatty, D. Blache, F. Wemelsfelder, and P. A. Fleming. 2013. Flooring and driving conditions during road transport influence the behavioural expression of cattle. Appl. Anim. Behav. Sci. 143:18–30. http://dx.doi.org/10.1016/j.applanim.2012.11.003.

Takahashi, Y., D. M. Kipnis, and W. H. Daughaday. 1968. Growth hormone secretion during sleep. J. Clin. Invest. 47:2079. http://dx.doi.org/10.1172/jci105893.

Temple, D., X. Manteca, A. Velarde, and A. Dalmau. 2011. Assessment of animal welfare through behavioural parameters in Iberian pigs in intensive and extensive conditions. Appl. Anim. Behav. Sci. 131:29–39. http://dx.doi.org/10.1016/j.applanim.2011.01.013.

Tucker, S. A., and A. W. Walker. 1992. Hock burn in broilers. Pages 33–49 in Recent Advances in Animal Nutrition—1992. P. C. Garnsworthy, W. Haresign, and D. J. A. Cole, ed. Butterworth-Heinemann Ltd., Oxford, UK.

Waiblinger, S., X. Boivin, V. Pedersen, M.-V. Tosi, A. M. Janczak, E. K. Visser, and R. B. Jones. 2006. Assessing the human-animal relationship in farmed species: A critical review. Appl. Anim. Behav. Sci. 101:185–242. http://dx.doi.org/10.1016/j.applanim.2006.02.001.

Weeks, C. A., T. D. Danbury, H. C. Davies, P. Hunt, and S. C. Kestin. 2000. The behaviour of broiler chickens and its modification by lameness. Appl. Anim. Behav. Sci. 67:111–125. http://dx.doi.org/10.1016/s0168-1591(99)00102-1.

Welfare Quality. 2009. Welfare Quality assessment protocol for poultry (broilers, laying hens). Welfare Quality, Lelystad, the Netherlands.

Wemelsfelder, F. 2007. How animals communicate quality of life: The qualitative assessment of behaviour. Anim. Welf. 16:25–31.

Wemelsfelder, F., U. Knierim, H. Schulze-Westerath, T. Lentfer, M. Staack, and V. Sandilands. 2009. Qualitative Behaviour Assessment. Pages 113–119 in Assessment of Animal Welfare Measures for Layers and Broilers. B. Forkman and L. Keeling, ed. University of Cardiff, Cardiff, UK.

Wemelsfelder, F., and F. Millard. 2009. Qualitative Behaviour Assessment. Pages 213–219 in Assessment of Animal Welfare Measures for Sows, Piglets and Fattening Pigs. B. Forkman, and L. Keeling, ed. University of Cardiff, Cardiff, UK.

Wickham, S. L., T. Collins, A. L. Barnes, D. W. Miller, D. T. Beatty, C. Stockman, D. Blache, F. Wemelsfelder, and P. A. Fleming. 2012. Qualitative behavioral assessment of transport-naive and transport-habituated sheep. J. Anim. Sci. 90:4523–4535.

Wilson, J. L., W. D. Weaver, W. L. Beane, and J. A. Cherry. 1984. Effects of light and feeding space on leg abnormalities in broilers. Poult. Sci. 63:565–567.

Zulkifli, I., J. Gilbert, P. K. Liew, and J. Ginsos. 2002. The effects of regular visual contact with human beings on fear, stress, antibody and growth responses in broiler chickens. Appl. Anim. Behav. Sci. 79:103–112. http://dx.doi.org/10.1016/s0168-1591(02)00135-1.

Zulkifli, I., A. Rasedee, O. N. Syaadah, and M. T. C. Norma. 1998. Daylength effects on stress and fear responses in broiler chickens. Asian-australas. J. Anim. Sci. 11:751–754.