



Qualitative behaviour assessment as part of a welfare assessment in flocks of laying hens

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ARTICLE INFO

Keywords:

Behaviour
Emotion
Fear
Mortality
Plumage
Poultry
Laying hen
Qualitative behaviour assessments (QBA)

ABSTRACT

Animal welfare must be considered from a broad perspective, including positive emotional states. Qualitative behaviour assessment (QBA) is one of few scientific methods to assess positive emotional states of animals. QBA for poultry is included in the Welfare Quality® protocol, but has not been validated for laying hens. The aim of this study was to investigate the dimensionality of QBA for laying hens and the associations between the main principal components of QBA and selected animal-based welfare measures. A total of 22 flocks were visited between the age of 70–76 weeks. All flocks consisted of approximately 7500 white-strain hens (Dekalb White, n = 11; Lohman LSL, n = 11) with intact beaks, housed in indoor multi-tiered aviary systems. Each flock was assessed using a fixed list of QBA terms, in addition to scoring of plumage and fear, and collection of mortality data. QBA was analysed using principal component analysis (PCA), revealing three main dimensions that can be recognised from QBA in other species. These were labelled *mood* (PC1), *alertness* (PC2) and *arousal* (PC3), and explained 50.8%, 19.7%, and 12.0% of the variance, respectively (82.5% overall). There were no associations between the QBA scores and plumage score, fearfulness, or mortality in the flocks. The lack of significant associations might be due to a limited sample size, a homogenous study population or the inherent motivation in hens to suppress signs of weakness or sickness. QBA can nevertheless become an important tool for measuring emotional expressions in laying hens. However, the method needs further validation.

1. Introduction

There is an increasing perception among the public and scientists that good animal welfare is not simply about the absence of health problems and negative experiences, but also about the presence of positive experiences (Boissy et al., 2007; Rault et al., 2020). The notion is that farm animals deserve ‘a life worth living’, an expression that incorporates good biological functioning, freedom to perform motivated behaviours, and a positive emotional state (Fraser et al., 1997). Most of the validated animal-based welfare indicators commonly used by the poultry industry today reflect health status, such as mortality, footpad dermatitis, or wounds. These indicators provide reliable and important information about animal health and welfare, however, there is a need to develop reliable methods to measure animal welfare from a broader perspective including behavioural expressions and positive emotions

(Green and Mellor, 2011; Mellor, 2016). Qualitative behavioural assessment (QBA) is currently the only measure in the comprehensive Welfare Quality® protocols to assess positive emotional states (Keeling et al., 2013).

QBA is a “whole-animal approach” used to assess welfare through the observation of the animals’ body language, by scoring a number of behavioural descriptors such as *calm*, *nervous*, *fearful* or *playful*. The behavioural terms, given their emotional connotation, have a direct relevance to animal welfare as they describe the animals’ own experience of their state (Wemelsfelder et al., 2001; Wemelsfelder and Farish, 2004; Wemelsfelder and Lawrence, 2001). In the most widely used approach, the assessors are provided with a fixed list of descriptors that they score on visual analogue scales (VAS) following observations of the animals. Principle components analysis (PCA) is used to consolidate the data from all the VAS scores, resulting in new, uncorrelated variables

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<https://doi.org/10.1016/j.applanim.2021.105535>

Received 4 October 2021; Received in revised form 15 December 2021; Accepted 17 December 2021

Available online 20 December 2021

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(principal components). The behavioural terms that anchor the lower and upper end of each new component are used to interpret and describe the integrated pattern of the animals' behavioural style. The two main components derived from such analyses in the past have often described as mood and arousal (e.g., Minero et al., 2016, Grosso et al., 2016, Diaz-Lundahl et al., 2019, Stubbsjoen et al., 2020).

Components of QBA have been found to correlate with physiological measures of health and welfare in a range of farm species (sheep; Wickham et al., 2015; pigs; Rutherford et al., 2012 and cattle: Stockman et al., 2011). QBA has been used in a few recently published studies on broiler welfare (Bassler et al., 2013; Buijs et al., 2017; de Jong et al., 2016; Federici et al., 2016; Sans et al., 2021). Muri et al. (2019) investigated associations between QBA and other measures of welfare in 50 broiler flocks and found that flocks with higher scores on both the mood and the arousal components were less likely to allow the assessor to touch them. The authors proposed that higher scores on both components could indicate greater liveliness and a better ability to avoid the assessor. Furthermore, flocks with a higher score on the arousal-component were less likely to be in a high mortality category, suggesting that QBA provided meaningful and useful supplementary information on animal welfare in these flocks. Currently, a chain of supermarkets in the UK has included QBA as part of their commercial welfare assessment scheme, in order to understand the welfare of the farm animals in their value chain (Waitrose.com, 2021). This shows the applicability of the method by advisors and auditors.

When it comes to laying hens, however, associations between QBA and other welfare measures have never been investigated. In fact, very few studies have reported on QBA in laying hens. Niekirk et al. (2012) assessed 122 flocks of laying hens in different housing systems using the Welfare Quality (WQ) protocol (WelfareQuality®, 2009), where QBA was included, and found the lowest (i.e., most negative) QBA score in conventional cages, and the most positive score in organic aviary systems. The authors suggested that the results could be biased against cages, as birds in cages have less room to perform some of the behavioural expressions that QBA is based on, but also due to the subjective perception of the observers. Evidence of expectation bias has been reported in a study by Tuytens et al. (2015), where videos of laying hens were scored as more positive when the observers were told the birds were from an organic farm as compared to cages, when in fact, all birds were from the same farm. This highlights the need to investigate how QBA in laying hens relates to welfare measures that are less based on observer judgement, such as health and environmental factors. Biologically meaningful associations would strengthen the validity of the QBA as a welfare indicator. This is especially important as QBA is part of the Welfare Quality (WQ) protocol for laying hens (Welfare Quality®, 2009), which is currently being used to assess laying hen welfare (e.g., Blatchford et al., 2016). Finally, it is important to note that as suggested by Battini et al. (2018), the holistic approach of QBA may reflect a different aspect of welfare that is valuable even if it is not directly related to other measures included in a welfare assessment protocol.

Therefore, the aim of this study was to investigate the dimensionality of QBA for laying hens and the associations between the main components of QBA and certain other animal-based welfare measures for laying hens in aviary systems. We expected that QBA scores indicating more positive emotional states would be positively related to better plumage condition, reduced fearfulness and reduced mortality in the flocks.

2. Material and methods

2.1. Animals and housing

The study was conducted on 22 commercial laying hen farms located in eastern Norway between August 2020 and April 2021. The studied flocks (1 flock per farm) were randomly selected from the supplier lists of two different egg packing companies and were visited once between

the laying hens' ages of 70–75 weeks (mean 72.1 weeks \pm 1.7). Farmers were contacted one few weeks before the visit, and participation in the study was voluntary. All flocks consisted of approximately 7500 white-strain hens (Dekalb White, $n = 11$; Lohman LSL, $n = 11$) with intact beaks, housed in indoor multi-tiered aviary systems. The flocks were managed according to standardized practices with regards to animal density (9 birds/m² usable area), feed, water, ventilation, litter, and lighting (KSL, 2020). The pullets arrived at the farm at around 16 weeks of age and were kept until 78 weeks when they were depopulated following standard commercial practices for Norway.

All flocks were housed in fully enclosed houses, with automatic mechanical ventilation and artificial lighting. All 22 aviary systems (Big Dutchman $n = 14$, Landmeco $n = 3$, Jansen $n = 2$, Victorsson $n = 2$, Vencomatic $n = 1$), had similar layout, with three tiers above the floor, feed and water lines on tiers 1 and 2, nest boxes on tier 2, and perches on tier 3. All houses had a concrete floor with wood shavings covering a floor area ranging from 385 m² to 1000 m², that extended around and under the tiered aviary structures.

2.2. Farm visits and data collection

One of the authors (GV, ethologist with comprehensive knowledge of poultry behaviour), had been trained in the theory and practice of the Welfare Quality® protocol (2009) by experienced WQ assessors, and performed all the farm visits. The visits started around 0900. Each visit began with an explanation to the farmer of the project goals and the data collection procedure. Flock and house information, including hybrid, rearer, feed intake, production, and mortality was obtained.

Plumage condition was scored on a 3-point scale from 0 (no loss) to 2 (bare skin visible > 5 cm) for each of four body parts; head/neck, back/wing, chest, and tail, on 50 random birds from a range of locations in the house. Average score per body part was calculated per flock, in accordance with Welfare Quality® descriptions. A novel object test was performed according to the Welfare Quality® Protocol for laying hens: four different objects were placed on different locations in the littered area of the house. Then, the number of hens at a distance of less than 1 bird length from each object were recorded every 10 s for 2 min. The average number of birds approaching the objects was calculated per flock. Thus, a high score indicates a low level of fearfulness in the flock.

2.2.1. QBA

Each flock was observed from different parts of the house for a total of 20 min, followed by scoring of the 22 behavioural expressions on visual analogue scales (VAS), as described in the WQ protocol. Each 125 mm VAS ranged from 'Minimum', indicating that the behavioural expression is entirely absent in any of the animals observed, to 'Maximum', meaning that the expressive quality is dominant across all observed animals. The behavioural expressions used were (in random order): *Active, Relaxed, Helpless, Comfortable, Calm, Content, Tense, Inquisitive, Friendly, Positively occupied, Scared, Drowsy, Fearful, Agitated, Confident, Depressed, Unsure, Energetic, Frustrated, Bored, Playful, Nervous, and Distressed*.

2.3. Data management and statistical analyses

The scores for each individual behavioural term were registered by measuring the distance in millimetres from the "Minimum" anchor of the VAS to the mark made by the assessor, thereby providing a score between 0 and 125. Data were entered into Microsoft Office Excel®, and statistical analyses were conducted in Stata SE/16.1 (StataCorp, College Station, Texas). Principal components analysis (PCA) was conducted using correlation matrix (no rotation). To determine the number of components to retain, we used a combination of the elbow plot criterion and Kaiser's criterion. Component scores were calculated for the components that were retained.

Pearson's correlation coefficients (r) were calculated between all the

variables included in the study. To further investigate associations between the main principal components of QBA and the other animal-based welfare indicators from these flocks, we ran ordinary least squares regression analyses, with the welfare indicator in question as the dependent variable and each of the main principal components as independent variables. The associations were also screened by visually inspecting the regression lines in Lowess smoothing graphs to identify outliers and to assess the possibility of non-linear associations that could be analysed by categorising variables and performing logistic or ordered logistic regression models.

3. Results

3.1. QBA

The principal component analysis of the QBA data revealed three main dimensions with eigenvalues > 1 , and a three-component solution was confirmed by the scree plot. These components explained 50.8%, 19.7%, and 12.0% of the variance respectively (82.5% overall). (Fig. 1).

The first component ranged from descriptors such as *comfortable*, *relaxed*, *calm*, and *friendly*, to *unsure*, *agitated*, *nervous*, *helpless*, and *scared*. This component was labelled *mood*. The second component ranged from *helpless*, *distressed*, *depressed*, and *drowsy*, to *inquisitive*, *confident*, *energetic*, *active*, and *playful*. We labelled this component *alertness*. The third component ranged from *active* and *energetic*, to *relaxed*, *calm*, and *drowsy*. This component was labelled *arousal*.

3.2. Selected animal-based measures

Mean plumage scores for the different body parts and results of the Novel object test can be seen in Table 1. The highest mean plumage score across the flocks (i.e., the poorest plumage condition) was found for back/wing, with a mean score of 0.67, while the lowest mean

Table 1

Mean (\pm SD) and range of scores for the animal-based welfare measures on 50 random birds/flock from the 22 flocks. Plumage flock score could theoretically range between 0 and 2.

Animal-based welfare indicator	Mean (\pm SD)	Range (min-max)
Plumage score head/neck	0.32 (\pm 0.35)	0–1.12
Plumage score back/wing	0.67 (\pm 0.71)	0–2.0
Plumage score breast	0.29 (\pm 0.46)	0–1.69
Plumage score tail	0.55 (\pm 0.58)	0–2.0
Novel object test score	27.1 (\pm 15.31)	1.75–74.25
Mortality rat%	2.95 (\pm 1.32)	0.9–6.6

plumage score across the flocks was breast, with a mean score of 0.29 (Table 1). The novel object test score, where a high score indicates a low level of fearfulness in the flock, ranged from 1.75 to 74.25 between flocks, with a mean of 27.1 (Table 1).

3.3. Associations

3.3.1. Correlation analyses

The Pearson's correlations between the animal-based measures are presented in Table 2. There are some moderate ($r = 0.40$ – 0.69) (Schober et al., 2018) correlations between PC1 and the welfare outcomes of interest, but otherwise the correlations between the components of QBA and other variables are weak ($r = 0.10$ – 0.39) to negligible ($r = 0.00$ – 0.10). These associations were further explored with regression analyses. The different plumage scores are moderately ($r = 0.40$ – 0.69) to strongly ($r = 0.70$ – 0.89) correlated, as might be expected.

3.3.2. Regression analyses with plumage as outcome

In our screening using ordinary least squares regression analyses, PC1 was significantly associated with plumage score for head/neck and

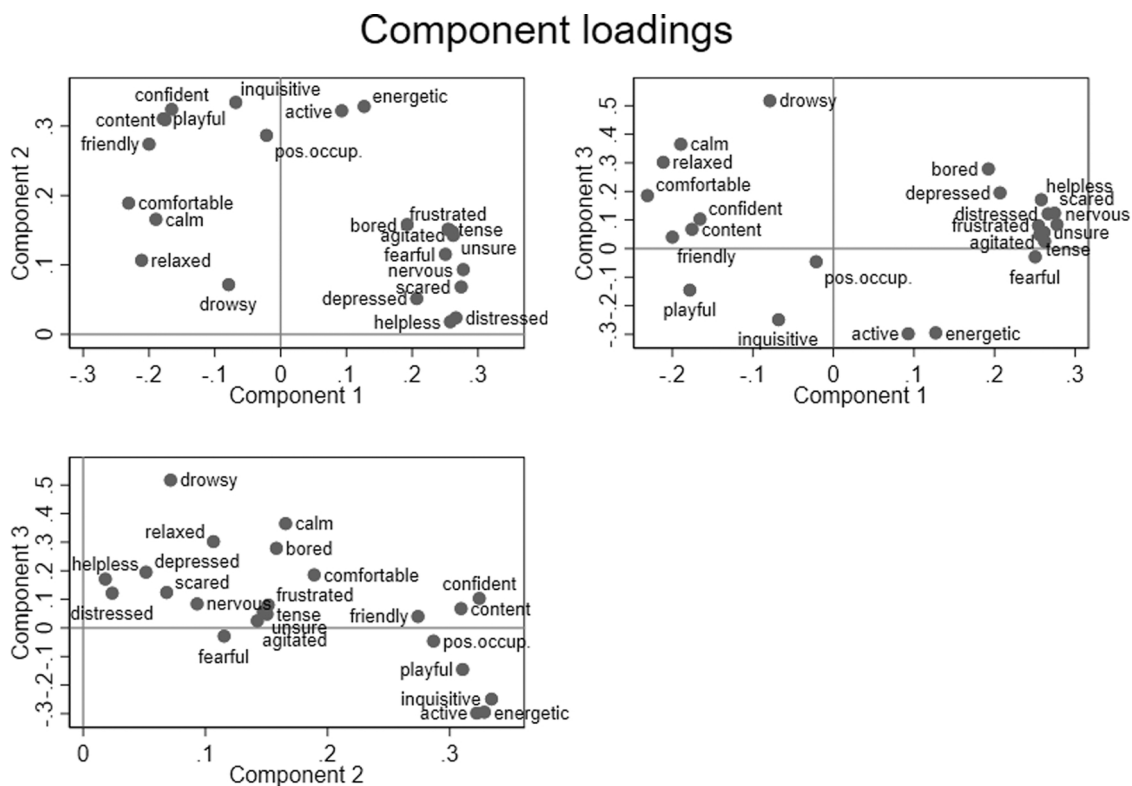


Fig. 1. Loading plot illustrating the component loadings of each behavioural term across the three main components, i.e., *mood* (PC1), *alertness* (PC2) and *arousal* (PC3). The loading matrix for each of the three main components are plotted against the values from each of the other three components. These components account for 82.5% of the variance from the principal component analysis of QBA data from the 22 flocks. (Note different scales on the axes, due to different ranges in values.)

Table 2
Pearson's correlation coefficients for the animal-based measures from the 22 flocks.

Variable	PC1	PC2	PC3	Plumage head/neck	Plumage breast	Plumage back/wing	Plumage tail	Novel object test
PC2	0.00							
PC3	0.00	0.00						
Plumage head/neck	0.48	-0.11	0.09					
Plumage breast	0.50	-0.15	0.05	0.83				
Plumage back/wing	0.33	-0.06	0.04	0.60	0.71			
Plumage tail	0.28	-0.28	0.29	0.54	0.59	0.52		
Novel object test	-0.35	-0.18	-0.13	-0.19	-0.23	-0.37	-0.07	
Mortality	0.46	-0.29	-0.27	0.46	0.43	0.31	0.23	-0.03

for breast (both $p < 0.02$). However, inspection of the Lowess smoothing graphs revealed that these associations were caused by one outlier. Without this outlier, none of the three components of QBA were significantly associated with the plumage score for head/neck or breast. Plumage score on back/wing and on tail were not significantly associated with any of the three components of QBA.

3.3.3. Regression analysis with novel object test score as the outcome

There was a tendency for a positive association between PC1 (*mood*) and novel object test score ($p = 0.1$), however, this association was also caused by an extreme outlier, and with this flock removed from the analyses, there was no longer a tendency for an association. There were no associations between the novel object test score and PC2 (*alertness*) or PC3 (*arousal*).

3.3.4. Regression analysis with mortality as the outcome

The regression analyses revealed that PC1 was significantly associated with mortality ($p < 0.05$). However, this effect was again caused by the same outlier responsible for the significance with plumage scores. When this outlier was removed, none of the three components of QBA were significantly associated with mortality.

None of the Lowess smoothing graphs indicated that categorising variables would give rise to any significant non-linear associations.

4. Discussion

The aim of this study was to investigate the dimensionality of qualitative behavioural assessments of laying hens, as there is a scarcity of publications describing QBA for this species. Moreover, we aimed to investigate associations between QBA and selected animal-based welfare measures in commercial flocks of laying hens in aviary systems.

Principal component analysis (PCA) of data from the qualitative behavioural assessments of the laying hen flocks revealed dimensionalities that partly can be recognised from QBA in other species (Brscic et al., 2009 [veal calves]; Duijvesteijn et al., 2014 [pigs]; Grosso et al., 2016 [goats]; Minero et al., 2016 [donkeys]; Stubbsjoen et al., 2020 [dogs]). In agreement with these studies, we found mood to be a suitable label for the first principal component. However, in our study, we suggest that alertness is a more appropriate description of PC2, whereas arousal, which frequently is identified as the second component, was a more correct label for our third component. Grosso and colleagues (2016) also identified three main QBA components in a study on dairy goats kept in indoor and pasture-based systems. In that study, PC1 was labelled as mood, PC2 as level of arousal, while PC3 ranged from "sociable/playful" to "alert/agitated".

We expected that QBA scores indicating positive emotional states would be associated with good plumage scores. Plumage condition generally deteriorates with age due to abrasion and molting (Rørvang et al., 2019). However, poor plumage may also be due to feather pecking by other hens. Feather pecking is a detrimental behaviour in poultry that causes pain for the victim (Bright, 2008) and increased mortality in the flock (Heerkens et al., 2015). We did observe rather poor plumage in several of the flocks, where all 50 observed birds had larger featherless areas (>5 cm) on head/neck and back/wing. However, we did not find

any associations between poor plumage and the QBA scores. There could be several explanations for this. First, laying hens are prey animals, and hiding pain and weakness is considered a key behavioural response to evade detection and capture by predators (e.g., Dwyer, 2004), possibly making negative emotional expressions more difficult to detect in poultry. It may be easier to score QBA for larger animals, particularly mammals, kept in smaller groups, allowing a better observation of postures, facial expressions and vocalisations, as compared to poultry housed in groups of several thousands (de Jong et al., 2014). Assessment of pain and discomfort in poultry can be recognised, but this requires detailed observations of the birds over a period of time. For instance, subtle behavioural changes such as reduced mobility have been observed in lame broilers (Caplen et al., 2014), and laying hens with keel bone fractures show fewer posture changes compared to hens without these fractures (Casey-Trott and Widowski, 2016). There has been some criticism of the QBA method, since it relies on the observers' ability to perceive and integrate details of behaviour and body language using qualitative descriptors such as *confident*, *unsure* and *content*. However, this does not necessarily mean that the method is more subjective than other methods based on observer judgement, such as scoring of lameness or the severity of skin lesions (Diaz-Lundahl et al., 2019). There may also be differences in the interpretation of qualitative descriptors between different languages, which needs to be taken into account in order to overcome linguistic barriers (Minero et al., 2016).

The QBA-method has not been validated for poultry (de Jong et al., 2014; Wemelsfelder et al., 2009), and observers need to have sufficient knowledge of laying hens and their behaviour for a reliable and valid scoring. In our study, the observer had a comprehensive knowledge of behaviour in laying hens. However, as QBA in laying hens is based on group observations, it is possible that a health or welfare issue must be above a certain prevalence or intensity to influence the QBA scores, which was not the case in the observed flocks in this study.

Another possible explanation for the lack of associations between plumage condition and QBA could be the inclusion of too few flocks. In the study by Muri et al. (2019), 50 broiler flock were included, and significant associations between QBA scores, fearfulness, and mortality was found. Similarly, Niekirk et al. (2012) assessed 122 flocks of laying hens, and found the lowest (i.e., most negative) QBA scores in conventional cages, and the most positive scores in organic aviary systems. Future studies should thus include a higher number of flocks, and attempt to perform the observations without making the birds aware of the observers' presence, to avoid potential anti-predator-related suppression of pain or discomfort. This can be done by installing cameras to survey the animal's behaviour.

There were no significant associations between any of the QBA components and novel object test score. Fearfulness is a negative emotional state that results from the perception of danger and is in itself negative for animal welfare. Novel object tests measure the conflicting motivations between approaching and avoiding a novel object (Miller, 1944) and are extensively used to measure the degree of fearfulness in poultry (Jones, 1996). The novel object test has been used in several studies on laying hens (e.g., Brantsaeter et al., 2016; Uitdehaag et al., 2008), and elevated levels of fear are reported to be associated with increased levels of feather pecking (Rodenburg et al., 2013).

We did not find a relationship between any of the QBA component scores and mortality in the flocks included in the study. Mortality varied across the 22 flocks, from 0.9% to 6.6% thus we have included both low and high mortality flocks (average mortality in Norwegian flocks is 4.54%, *Kjøttets tilstand*, 2021). We expected that high mortality flocks would get QBA scores indicating poorer emotional states. Muri et al. (2019) reported that broiler flocks with higher arousal scores were less likely to be in a high mortality category, suggesting that QBA provided meaningful and useful supplementary information on broiler welfare. A limited sample size may have reduced our possibility of finding statistically significant associations between QBA and the other animal-based welfare indicators. Andreasen et al. (2013) failed to find associations between QBA scores and other Welfare Quality® measures in dairy cattle, and they suggested that the spread between the farms in their study (in terms of results) was too small to detect a statically significant association between QBA and other WQ outcomes. Our data were also quite homogenous (i.e., little variation between the flocks observed), which reduces the possibility for the QBA to detect statistically significant associations.

In conclusion, we identified dimensions of QBA that are in agreement with other species. There were no associations between QBA scores and plumage score, fearfulness, or mortality in the observed flocks of laying hens. The lack of significant associations might be due to a limited sample size, as we only included 22 flocks in the study. Other possible explanations are the inherent motivations in hens to suppress signs of weakness or sickness and a homogenous study population. Nevertheless, QBA can become an important tool for measuring emotional expressions in laying hens in the future, by giving supplemental information about the birds' welfare state.

CRedit authorship contribution statement

Guro Vasdal: Conceptualization, Data sampling, Writing. **Karianne Muri:** Conceptualization, Analyses, Writing. **Solveig Marie Stubsjøen:** Conceptualization, Analyses, Writing. **Randi Oppermann Moe:** Conceptualization, Writing. **Kathe Kittelsen:** Conceptualization, Data Sampling, Writing.

Declaration of Competing Interest

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

Acknowledgment

This work was supported by the Foundation for Research Levy on Agricultural Products (FFL/JA), nr. 309159. The authors also want to thank all the participating farmers for sharing their data and allowing us to observe their birds.

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