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Development and evaluation of an animal health and welfare monitoring system for veterinary supervision of pullet farms

Caroline Mels^{a,b}, Knut Niebuhr^{b,†}, Andreas Futschik^c, Jean-Loup Rault^b, Susanne Waiblinger^{b,*}

^a Veterinary Practice: Tierarzt GmbH Dr. Mitsch, Hauffgasse 24, 1110 Wien, Austria

^b Institute of Animal Welfare Science, Department of Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Veterinärplatz 1, 1210

Wien, Austria

^c Institute of Applied Statistics, Johannes Kepler University, Linz, Altenberger Straße 69, 4040 Linz, Austria

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ABSTRACT

Regular welfare monitoring throughout rearing of pullets may help to identify problems early and take counteractions timely, which helps in guaranteeing good welfare. The aims of our observational study were (i) to establish and test a welfare monitoring system that can be used during (short) routine veterinary and technical staff visits for pullet flocks, (ii) to use the monitoring system to investigate variability between flocks and (iii) to analyse factors that potentially affect pullets' body weight, uniformity in body weight and mortality. The developed monitoring system tries to minimise the time required while not losing important information. Agespecific recording sheets comprise animal-based indicators of welfare and relevant environmental factors (housing, management, care) to allow for identifying causes of problems and targeted action. Finally, the system was implemented in a cross-sectional study and data collected in 100 flocks (67 organic, 33 conventional) on 28 rearing farms in Austria. Linear mixed models were used to identify factors influencing body weight, uniformity and mortality, both including all flocks (A) and only organic flocks (O) and a linear regression model with all flocks to investigate associations within animal-based indicators. High variability was found between flocks in animal-based indicators. Body weight was higher when the pre-rearing period was shorter (p < 0.001, A&O), with higher intensities of light (p = 0.012, O), with only one compared to more stockpersons ($p \le 0.007$, A&O), with a higher number of flock visits per day (p \leq 0.018, A&O), and a lower avoidance distance (p = 0.034, A). Body weight uniformity increased, with age and decreased with the duration of the light period (p = 0.046, A), and, amongst others, was higher on organic farms (farming type; p = 0.041). The latter may reflect a more uniform level of welfare due to a lower stocking density and lowered effects of social competition. Within organic flocks mortality was lower if pullets had access to a covered veranda (p = 0.025) resulting in an overall lower stocking density inside the barn, while in the model including all farms mortality was higher in cases where a disease had been diagnosed. We conclude that our monitoring system can easily be implemented in regular veterinary and technical staff visits, but could also be used by the farmers'. Several easy-to-record animal-based indicators of animal welfare could be analysed more frequently to increase early detection of problems. Implementation of such a routine-based monitoring system with easy-to-assess animal-based parameters and input measures can contribute to better animal health and welfare in pullets.

1. Introduction

The demand for animal products produced under high animal welfare standards is increasing (European Commission, 2016; Alonso et al., 2020) in parallel with the general call of EU citizens for improvements in farm animal welfare (European Commission, 2016). Accordingly, the industry had increased efforts to ensure good animal welfare by establishing welfare monitoring systems either for internal quality management and/or for product labelling (Fraser, 2008; Veissier et al., 2008; Butterworth et al., 2009). In addition, in some countries the animal welfare acts were changed to include an obligatory self-monitoring of welfare for farms (e.g. the German Animal Welfare Act, Tierschutzgesetz

* Corresponding author

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E-mail address: susanne.waiblinger@vetmeduni.ac.at (S. Waiblinger).

[†] Deceased on 1.2.2020.

Deutschland, 2022).

Welfare assessment protocols that allow a standardized, animalbased assessment of animal welfare were developed for different species (Veissier et al., 2008; Butterworth et al., 2009). These protocols can provide feedback for the farmers, information about the current welfare status and information for consumers on animal products (Butterworth et al., 2009). Several welfare assessment protocols were developed for poultry, e.g. the Welfare Quality® protocols Butterworth et al., 2009) and AssureWel protocols (Main et al., 2012) for broilers and laying hens, or the LayWel protocol for laying hens (Blokhuis et al., 2007); the latter two aimed specifically for being used by farmers and extension people. The AssureWel protocol is indeed used by farmers (e.g. in the UK; see Mullan et al., 2016). Self-monitoring tools for farmers based on the above mentioned protocols were also developed for guaranteeing standards of the animal welfare law (e.g. Germany, see Knierim et al., 2020), or private standards, e.g. of organic organisations (Eder et al., 2016). Knierim et al. (2020) also included guidelines specifically for rearing pullets such as the assessment of feather condition on a regular basis.

However, none of these above mentioned protocols have been implemented during routine (veterinary) visits for pullets in Austria so far. Generally, veterinary practices routinely visit rearing flocks multiple times during the whole rearing period for salmonella monitoring, vaccinations and health monitoring. The inclusion of a broader welfare assessment during these veterinary routine visits might enable early detection of problems. It would allow early counteractions and thus improve flock welfare and could also affect later performance and welfare of the adult laying hens (for review see Janczak and Riber, 2015; Nicol, 2019). In order to identify "problem flocks", animal-based parameters should be implemented in such routine based monitoring systems that could not only be used by veterinarians, but also by farmers themselves or the poultry integration company.

However, protocols developed so far are difficult to use under the conditions of a standard veterinary visit or visits by staff of the integration companies, where emphasis is laid on short but frequent visits. For example, the implementation of the full protocol of Welfare Quality® for laying hens is time-consuming with an estimated time of 6–7 h with 100 animals of one flock being individually examined (Butterworth et al., 2009). The AssureWel protocol still requires about 30 min according to Decina et al. (2019) and it only uses visual assessment from distance (Main et al., 2012), thus not allowing to detect small pecking injuries. This also accounts for the transect method, a practical method developed for broilers, turkeys and layers requiring even less time (Marchewka et al., 2013, 2015; Vasdal et al., 2022); therefore this method is suitable mainly to detect more severe cases of welfare issues (Vasdal et al., 2022). In addition, some animal-based parameters such as body weight and flock uniformity are recorded automatically on the farms but are not yet used as part of a welfare monitoring system, while they are relevant for (later) performance and associated with welfare problems: lower body weight and lower flock uniformity are linked to social stress (Costa, 1981), high stocking density (Carey, 1987) or diseases (Olsen et al., 2012; Vasdal et al., 2019). Reaching the recommended body weight at the 16th week of age is beneficial for laying performance later on (Hudson et al., 2001). The initial body weight at the onset of lay further determines egg weight with lighter hens laying lighter eggs and vice versa (Petitte et al., 1982; Lacin et al., 2008). Uniformity in weight indicates the percentage of the animals weighed that falls within a certain range, usually \pm 10%, of the mean bodyweight (Ensminger, 1980). Breeding companies recommend flock uniformity at around 80% or higher at the 16th week of age (e.g. Lohmann Breeders Germany GmbH). Flocks with higher uniformity reach egg production peak earlier and have higher peaks (Abbas et al., 2010). Lower flock uniformity is associated with a higher variability of sexual maturity and therefore a variation in the onset of lay with a delayed onset in lighter hens (Robinson and Robinson, 1991) and an accelerated onset in heavier hens (Yuan et al., 1994). Increased mortality in pullets is known to be caused by bacterial diseases (e.g. yolk sac infections) at a young age

(Chew et al., 2021). Furthermore, higher stocking densities (Carey, 1987; for review see Meseret, 2016) and the occurrence of cannibalism and feather pecking (Kjaer and Vestergaard, 1999) may lead to higher mortality (Savory, 1995; McAdie and Keeling, 2000; De Haas et al., 2013). Mortality was higher in organic layer production compared to conventional production in some studies (Fossum et al., 2009; Stokholm et al., 2010; Bestman and Wagenaar, 2014). Nevertheless very little is published regarding these animal-based parameters for pullets and little is known about the variability in commercial flocks or the relation between flock body weight, body weight uniformity or mortality and possible influencing factors on farm. Monitoring these parameters on a regular basis, together with indicators of feather pecking and fear of humans, could enable early detection of potential welfare problems and allow for counteractions by adapting management or by early nutritional supplementation which might prevent the necessity of antibiotic treatment. Consequently, the use of a regular monitoring protocol could contribute to better flock health and animal welfare. Such a monitoring system could also be used for benchmarking of farms and help to identify farms that constantly perform below others.

Therefore, the main aim of our study was to establish and test a welfare monitoring system for pullets that can be used in the framework of (short) routine veterinary visits and integration staff visits and can be used for above-mentioned purposes. Further, by using the monitoring system we wanted to investigate variability between flocks regarding the occurrence of feather pecking, body weight, body weight uniformity and mortality and, finally, to analyse factors that potentially affect these variables. However, the detailed assessment methods for feather pecking, the identified risk factors of feather pecking and easy-to-apply animal-related measures valuable for its early detection are described in a separate paper (Mels et al., 2022), while in this paper we focus on the automatically recorded measures of body weight and uniformity as well as mortality and the evaluation of the full monitoring system.

2. Material and method

The study was reviewed and approved by the ethics and animal welfare committee of the University of Veterinary Medicine, Vienna, in accordance with the Good Scientific Practices guidelines and national legislation (ETK-09/11/2018). The farmers were informed about the aim of the study and signed an informed consent form including the agreement for analysing and publishing the results of the study including interviews data in anonymized form.

2.1. Development of the monitoring system

The monitoring system was compiled in 2018 and was based on previous protocols and studies in laying hens, mainly Welfare Quality® (Butterworth et al., 2009), AssureWel (Main et al., 2012; http://www. assurewel.org/layinghens.html) and (Niebuhr et al., 2007). It was adapted to be feasible for routine veterinary visits performed several times during rearing, by reducing the time needed for evaluation by partly omitting and partly including additional animal-based indicators of welfare and especially a wide set of environmental factors. Not all animal-based and environmental variables were relevant throughout the rearing period. Therefore, age-specific sheets were developed for data recording compiling animal-based and environmental variables (overview about all assessed variables in Table 1).

Animal-based indicators of animal health and welfare thus comprised the following (for more details regarding recording see Section 2.2.3): Uniformity and weight were included as new indicators, because both parameters were recorded automatically on the pullet rearing farms and thus easily available. Further, sample size of animals that were caught and inspected individually for feather score and bloody lesions (fresh and encrusted pecking wounds) was limited to 15, respectively, instead of 100 as in Welfare Quality®, thus reducing time needed for assessment largely while still being able to identify problems

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Table 1

Overview of animal-based indicators of welfare and potential influencing factors (breed, environmental conditions) included in the monitoring system. The number of levels / values or units, sample size or sampling strategy in case of animal based indicators, source of data and the week when they were assessed are shown.

Variable		Source of data	Week				
Animal-based indicators (unit/levels)	Sampling strategy (in relevant week)		1	6	10	16	
body temperature (°C)	20 animals	veterinarian ^a	х				
feather score (3 levels)	10, 15, 10 animals	veterinarian		х	х	х	
bloody lesions (yes / no)	10, 15, 10 animals	veterinarian		х	х	х	
avoidance distance (m)	6 sampling points/flock	veterinarian			х		
reactivity score (3 levels)	flock	veterinarian		х	х	х	
down feather present ^b (3 levels)	floor area	veterinarian		х	x	x	
uniformity (%)	number of animals (500–3000)	automatic scale	х	x	x	x	
weight (g)	number of animals (500–3000)	automatic scale		х	х	x	
mortality (%)	flock	farmer's record	х	х	х	х	
diseases in the period (yes / no)	flock	farmer's record	x	x	x	х	
Flock and environmental characteristics							
Breed(s) ^c		farmer's record	х				
farming type (organic / conventional)		veterinary data base ^d					
stocking density (animals per m ²)		farm documents	x				
barn temperature (avg. °C)		farmer's record	х	х	х	х	
pre-rearing (days)		farmer's record		х			
early rearing (floor / aviary)		veterinarian	х				
aviary opened (weeks)		farmer's records		х			
antibiotic treatment (yes / no)		veterinary data base	x	х	х	х	
use of supplements (yes / no)		veterinary data base	х	х	х	х	
feed changes (number / period)		farmer's record		х	х	x	
feed mill changes (number / period)		farmer's record		х	х	x	
litter quantity (high/ little / no)		veterinarian		х	х	х	
light hours per day (hours)		farmer's record		х	х	х	
light intensity (bright / average / dark)		veterinarian		х	х	х	
daylight provided (yes / no)		veterinarian		х	х	х	
technical problems (yes / no)		farmer's record			х	х	
pecking stones (yes / no)		veterinarian			х	x	
access to free covered veranda (yes / no)		veterinarian			x	х	
access to free range (yes / no)		veterinarian			x	х	

^a assessed directly by the veterinarian during the routine visit

^b though assessed in the environment, presence of down feathers is related to animal behaviour and feather change and animal welfare and no environmental factor

^c breeds that were found in the project: Lohmann Sandy (LS), Lohmann Braun (LB), Lohmann Silver Line (LSL), mix of LS + LB, mix of LSL + LB

^d assessed once per farm (farming type did not change throughout the study)

with feather pecking at an early stage where animals have only small bloody lesions which are not visible from a distance, i.e. without catching birds (for more details see Mels et al. (2022). We developed a simplified avoidance distance test for assessing pullets' relationship with humans that was based on tests previously developed and validated for laying hens (Graml et al., 2008) for the Welfare Quality® protocol, but was easier to perform, less time consuming, reliable and still valid (Mels et al., 2022). The monitoring system also included subjective scores, which are easy and quick to assess, to test their potential to identify problems by analysing associations with quantitative parameters (thus testing indicating convergent validity): a reactivity score (similar to AssureWel; Main et al., 2012) and the presence of down feathers on the floor were included (see Mels et al. (2022) for their associations to bloody lesions). Finally, cloacal temperature of 20 chicks was recorded by the veterinarians during the visit in the first week of life, and occurrence of diseases and mortality were used from farm records.

Breed was included as a flock characteristic that may affect health and welfare but is no indicator of welfare. Environmental factors that can vary between flocks on the same farm were included in the agespecific recording sheets.

All recordings were noted down on paper sheets during farm visits and were then included in an online-data base with the aim to provide access to all concerned parties (veterinarians, poultry integration and the respective farmer) and building the basis for benchmarking after a successful test period.

2.2. Evaluating the monitoring system and assessing risk factors for uniformity, weight and mortality

2.2.1. Study design, farms and animals

In a cross-sectional approach, data were collected on 100 rearing pullet flocks in the period August 2018 to October 2019. Flocks were raised on 28 different farms (11 conventional and 17 organic) located in Austria, thus 3-4 flocks per farm were included. Flock size ranged from 14,251 to 52,400 pullets (mean \pm SD: 29,427 \pm 11,496.6) on conventional and from 4659 to 10,783 pullets (mean \pm SD: 9360 \pm 952.5) on organic farms. All birds were hatched in a commercial hatchery that is part of the integration company and were vaccinated in the hatchery or during rearing against the most common diseases. All flocks were fed ad libitum in the first week of life, from the 2nd week until the pullets were nine weeks old the feeding intervals increased continuously from 3 times to eight times per day in case they had access to a feeding chain in the aviary; if pullets were reared on the floor in the first weeks, pullets were fed ad libitum (see 2.2.4. for description of rearing conditions in the first weeks of life). In general, rearing conditions on organic farms differ from conventional farm in terms of: mandatory provision of daylight and litter on the ground, lower stocking density (maximum organic according to the EU organic regulation 12 animals/m² usable space, maximum conventional, according to the 1. Tierhaltungsverordnung (2022) 20 animals/m²), and mandatory access to a covered veranda and free range at a certain age and organic feed. All animals had intact beaks. All farms of one single poultry integration company in service of one single veterinary poultry practice were included, thus constituting a convenience sample. This veterinary practice visited each flock routinely four times (1st, 6th, 10th and 16th week) per rearing period as part of the flock health management program. Six veterinarians were involved in data acquisition (for more details see Mels et al., 2022).

2.2.2. General information on data collection

The monitoring system outlined in Section 2.1. and Table 1 was applied during the routine veterinary visits of the 100 flocks using the procedure described below in Section 2.3. and 2.4. Additional data on housing and management factors, that did not vary on the same farm and are not included in the monitoring system, were gathered by a structured interview performed once on every farm.

2.2.3. Animal-based indicators

Body Weight (g) and body weight uniformity (%) were recorded from one automatic scale (Veit Electronics – BAT 2) per barn in the 6th, 10th and 16th week of age, but not in the 1st week of life, because scales were not available on pre-rearing farms or inside the aviaries. For correct evaluation of the body weight the automatic scale periodically recorded the weight on the weighing platform and checked for birds that entered and left the weighing platform. The scale was programmed for an uniformity range of \pm 10% (user manual available on https://www. veit.cz/downloads-and-materials-poultry-scales) and calculated body weight uniformity (%) by numbers of animals that deviate from the average weight by \pm 10% divided by the total number of weighed animals (formula based on Knierim et al., 2016). At the day of the visit, body weight and uniformity were recorded from the day before (ranging from 500 to 3000 animals weighed per day) to always cover a 24 h measurement and thus increase standardization. Mortality (%; percentage of all dead or culled animals until the day of the visit relative to the number of animals placed in the barn at day 1) was calculated at every visit using farmers' records in their electronic data base. Thus mortality in the respective week equals to the cumulative mortality.

For assessing feather condition and bloody lesions, a scoring system was developed based on previous ones (e.g. Welfare Quality®) and 10 (6th and 16th week) or 15 (16th week) animals that were caught randomly from different areas of the barn (from the front to the back of the whole barn, from the floor and from both sides of the aviary) were assessed by the veterinarian. To assess the animal-human relationship, the animals' distance kept towards the veterinarian (avoidance distance) at several points was assessed in the 10th week using a test with a moving and stationary phase of the human (Waiblinger et al., 2006) modified from earlier tests (Barnett et al., 1992; Graml et al., 2008), see Mels et al. (2022) for a detailed description.

General animal reactivity (reactivity score) was evaluated by assessing the behaviour of the animals when walking through the barn together with the farmer on a three-point scale as calm (animals are curiously watching the unfamiliar person, some even come closer and show explorative pecking behaviour), medium (some animals are curiously watching the unfamiliar person, some try to escape and flee) or nervous (the whole flock shows flight reactions or even panic and tries to escape; Mels et al., 2022).

At every visit, it was assessed, if diseases occurred until the day of the visit; from this it was calculated how often diseases occurred during the whole rearing period.

2.2.4. Flock-specific factors

Flock characteristics and flock-specific management factors were assessed at the different visits or only once per flock (e.g. breed, stocking density). Rearing conditions differed in the first weeks of life regarding being reared on one farm throughout the whole period or on two farms; that is, pullets were either reared from day 1 on the rearing farm where pullets stayed until the end of rearing at week 16–18 (no pre-rearing, 54 flocks; 53 flocks with aviary rearing, i.e. pullets were confined in the

aviary for 28-49 days, and 1 flock with early floor-rearing) or there was pre-rearing on the floor on another, specialised pre-rearing farm (46 flocks) for some weeks. After the 24-51 days pre-rearing period the pullets were transported to the actual rearing facility, where they were confined in the aviary for three days to three weeks. Further details about the rearing conditions can be found in Mels et al. (2022). Light intensity was evaluated subjectively by the veterinarian by walking through the barn on a three point scale as dark, average or bright light and light hours per day were recorded from computer records. In organic farms the provision of daylight was assessed in the 6th, 10th and 16th week, the access to a covered veranda was checked in 10th and 16th week of life (pullets must have access from the beginning of the 10th week according to BioAustria 2019) and access to free range was assessed in the 16th week only (pullets must have access to free range from the 12th week on according to BioAustria 2019). Further flock-specific factors included assessment of barn temperature in the first week of life, litter quantity (no litter, small amounts, high amounts) in the 6th, 10th and 16th week of life and if any antibiotics were used during the whole rearing period. Further environmental factors that were assessed but not considered in the models are described in Mels et al. (2022).

2.2.5. Farm and barn characteristics and farm-specific management

Detailed information about farm characteristics, barn characteristics, farmers' management and their handling practices was either measured in the barn (barn size), noted down from farm records, (aviary size) or gathered by a structured interview of the farmer (decision-maker), performed once on all 28 farms during the course of the study. A detailed description can be found in Mels et al. (2022). The interview comprised different questions about housing conditions (e.g. construction year, farming type organic/conventional, barn size, and size of covered free area and free range), hygiene management (e.g. separate barn clothing, use of a hygiene barrier or disinfection mats and routine of cleaning and disinfection), farm characteristics (e.g. number of stockperson, years of farmers' experience, farmers' educational background), daily work organization (e.g. frequency of flock visits per day or if the farmer spreads additional grain on the floor) and animal care and handling (e.g. how much time the farmer spent watching the animals' behaviour). The farmers were also asked if they regularly caught animals to assess their feather condition, or for other reasons and if so, how often and for what reason.

2.2.6. Statistical analysis

The statistical evaluation was done with the program SPSS 28. Data were first evaluated descriptively. For identifying factors associated with the dependent variables uniformity (%), weight (g) and mortality (%), multivariable analyses were performed by calculating linear mixed models (LMM). For pre-selecting independent variables for the models, i.e. potentially influencing factors, bi-variable associations were investigated by use of Spearman rank correlation coefficients and Mann-Whitney-U tests. Descriptive data of the potential influencing factors that were analysed for bi-variable associations are shown in the supplementary material of Mels et al. (2022). To be included in model analysis, a science-based hypothesis was a precondition and variables had to be associated with the dependent variable significantly or by trend (i.e. $p \le 0.1$) in bi-variable analysis. LMM were calculated with age (6th, 10th and 16th week) as well as the pre-selected variables of potentially influencing factors as fixed effects and flock nested in farm as random effects, including random slopes. Some of the pre-selected independent variables showed bivariate correlations with each other of $r_{s} \ge 0.5$. To test for potential effects on the models these variables were excluded one by one and effects on model results and models' BIC were evaluated; predictors largely stayed the same and BIC of the full models were always lowest, except for mortality in organic only models: there, a model where avoidance distance and stocking density were excluded had the lowest BIC and thus was selected. All models were calculated

both for all flocks (conventional and organic farms) and for organic flocks only. All models using all flocks were calculated with farming type and breed included as well as without, since strains used on organic farms were largely different from those in conventional farms (all LS flocks were organic) and thus farming type and breed were clearly confounded. There was only an effect in the model of uniformity which will be reported in the results section. For the model calculated for uniformity for organic only, we found neither significant associations nor tendencies except for age. To exclude that this result was caused by a too high number of (inter-linked) variables included in the model, a stepwise forward inclusion procedure using all pre-selected potential influencing factors was performed as well. No model with significant predictors could be identified, there was one model with a tendency, but there BIC was higher compared to the full model, which is therefore presented.

For identifying possible associations within our target variables uniformity, weight and mortality in different weeks (6th, 10th and 16th) as well as with other animal-based indicators (reactivity score, down feather present on the floor, avoidance distance, occurrence of bloody lesions and plumage damage) linear regression models were calculated with a stepwise inclusion of the animal-based indicators as well as farm as independent variables and the dependent variables uniformity, weight and mortality in the 6th or 10th week. For these six models a Benjamini-Hochberg False Discovery Rate (FDR) correction was calculated to account for multiple testing in these models (n = 51 hypotheses in total) and thus to control the proportion of falsely rejected null hypothesis.

For all models, assumptions of normality and homoscedasticity were checked graphically. Two farms with only one flock each had to be excluded in model analysis to be able to include farm as random effect into the model thus reducing sample size to 98 flocks. One flock was prereared on a farm in Germany which was supervised by another veterinary practice. There were no data available for this specific flock until the 6th week of age, thus sample size was further reduced to 97 flocks, if variables depending on data from the first week were included in the model. For models calculated with mortality, two flocks with mortality higher than 10% were excluded, since those high mortality rates were caused by smothering events. The model for mortality for both conventional and organic was therefore calculated with only 95 flocks, and for the organic only model with 65 flocks.

3. Results

3.1. Variability between flocks

3.1.1. Animal-based indicators

There was large variability between flocks in all animal-based indicators and all ages. Descriptive statistics for the three main variables in focus of the present paper are depicted in Table 2. For example, **uniformity** ranged from 61% to 99% in week 16, and **weight** ranged from 380 g to 542 g in week 6 and from 1.2 kg to 1.5 kg in week 16 (Table 2). Mortality varied largely as well; over the first 16th week of life **mortality** ranged from 0.39% to 15.72% (mean \pm SD: 2.09 \pm 2.22%, Table 2). In total 35 flocks were diagnosed with some kind of **disease** throughout the rearing period, 22 (32.8%) organic and 13 (39.4%) conventional. The **mean avoidance distance** ranged from 0.0 m to 6.0 m (mean \pm SD: 2.4 \pm 1.20 m). Regarding the **reactivity score** flocks were less often assessed as nervous: in the 16th week of age (four flocks) than in the 6th week of age (20 flocks).

3.1.2. Flock and environment characteristics

Three different **breeds** of pullets were raised; distribution differed according to farming type organic or conventional: Lohmann Sandy (LS, 53 organic flocks, 0 conventional flocks). Lohmann Braun (LB, 1 organic and 25 conventional flocks) and Lohmann Selected Leghorn (LSL, 4 conventional flocks), mixed of LS and LB hens (13 organic flocks) and mixed of LSL and LB hens (4 conventional flocks).

The average duration of **pre-rearing** was 36 ± 6.273 days, ranging

Table 2

Descriptive statistics of the target variables uniformity (%), weight (g) and mortality^a (%).

variable	farming type	Ν	week of age	mean	SD	median	Min	Max
uniformity (%)	conventional & organic	100	6	69.48	5.607	70.00	57.00	93.00
			10	76.24	6.965	75.50	62.00	90.00
			16	81.82	8.146	83.50	61.00	99.00
	conventional	33	6	66.85	3.874	65.00	59.00	76.00
			10	70.85	4.597	72.00	63.00	80.00
			16	74.45	6.563	75.00	61.00	88.00
	organic	67	6	70.78	5.893	72.00	57.00	93.00
			10	78.90	6.389	80.00	62.00	90.00
			16	85.45	6.177	86.00	70.00	99.00
weight (g)	conventional & organic	100	6	458.81	42.242	451.50	380.00	542.00
			10	884.72	53.964	878.50	765.00	1100.00
			16	1360.61	62.235	1358.50	1230.00	1512.00
	conventional	33	6	467.24	41.073	470.00	388.00	540.00
			10	896.91	53.699	903.00	788.00	992.00
			16	1352.15	56.702	1352.00	1230.00	1440.00
	organic	67	6	454.66	42.492	443.00	380.00	542.00
			10	878.72	53.472	873.00	765.00	1100.00
			16	1364.78	64.788	1359.00	1230.00	1512.00
mortality ^a (%)	conventional & organic	99 ^b	0	0.21	0.222	0.15	0.00	1.40
			6	1.39	1.853	0.98	0.20	14.43
			10	1.77	2.093	1.23	0.24	15.61
			16	2.09	2.218	1.48	0.39	15.72
	conventional	32 ^b	0	0.16	0.226	0.10	0.00	1.24
			6	1.16	0.649	1.10	0.20	14.43
			10	1.51	0.944	1.31	0.36	4.92
			16	1.83	1.078	1.58	0.43	5.52
	organic	67	0	0.23	0.163	0.17	0.00	1.40
			6	1.51	2.205	0.89	0.28	3.80
			10	1.89	2.457	1.11	0.24	15.61
			16	2.21	2.591	1.32	0.39	15.72

^a cumulative mortality (percentage of all dead and culled birds until the day of visit)

^b one flock was pre-reared in Germany under supervision of a different veterinary practice. There was no information about mortality in the first weeks of life.

between 24 and 51 days (n = 46 flocks). Organic flocks were pre-reared for 37 ± 6.353 days (mean \pm SD, range 24–51 days, n = 37 flocks), conventional flocks for 33 ± 5.085 days (mean \pm SD, range 28–42 days, n = 9 flocks). Out of 67 organic flocks 57 had access to a covered free area in the 10th week of age. In the 16th week 61 flocks had pecking stones in the barn, all 67 flocks had access to a covered free area and two organic flocks had access to free range already in the 10th week of age. In the 16th week, there were 51 flocks with access to free range. Organic flocks generally had more light hours per day (6th week: 14.2 h; 10th week: 12.3 h; 16th week: 11.69 h) than conventional flocks (6th week: 11.94 h; 10th week: 10.12 h; 16th week: 9.97 h). There was a large variation regarding light intensities throughout the different weeks of age also within farming type, the percentage of organic flocks that were assessed bright was higher than in conventional flocks in week 10 and 16. Daylight was provided at 40 (59.7%) organic farms in the 6th week of age, 60 (89.6%) in the 10th week and 66 (98.5%) in the 16th week of age.

In total 60 flocks only had one stockperson, 40 flocks had two. 13 flocks were visited one or sometimes, depending on the farmers' work load, two times per day, while 87 flocks were visited at least two or more times per day. The mean observation score was 2.74 ± 1.637 ranging from 0 to 6. One farmer checked the pullets' behaviour additionally to the daily routine inspections multiple times per day. The mean farmers' experience in working with pullets was 12.8 ± 10.972 years ranging from one to 50 years. Overall organic farmers had less experience (mean \pm SD: 8.96 \pm 4.653years) with the most experienced organic farmer working with pullets for 15 years.

Conventional barns were generally older (0–40 years) than organic barns (0–20 years). The mean cleaning and disinfection score was 0.72 \pm 0.109 ranging from 0.5 to 0.83. Organic farms had a higher score (mean±SD: 0.75 \pm 0.114) than conventional farms (mean±SD: 0.67 \pm 0.072). Detailed descriptive data of further environmental factors can be found in the supplementary material of Mels et al. (2022).

Table 3

Estimated means of both conventional and organic and organic only models for uniformity (%), body weight (g) and mortality^a (%) are shown. Only significant associations and tendencies are shown.

				conv	entional an	d organic		organic				
Target						CI					CI	
variable	influencing factor	unit	Ν	estimate	SD	min.	max.	Ν	estimate	SD	min.	max.
uniformity (%)	age of pullets	6 weeks	97	69.84	1.561	66.74	72.94	67	71.60	2.389	66.86	76.34
		10 weeks	97	75.35	1.476	72.42	78.28	67	77.20	2.134	72.96	81.44
		16 weeks	97	80.74	1.529	77.70	83.77	67	82.58	2.353	77.91	87.25
	age of barn	0-20 years	249	73.39	1.337	70.72	76.05					
		over 40 years	42	77.23	2.192	72.86	81.60					
	farming type	organic	201	81.62	3.396	74.84	88.39					
		conv.	90	69.00	3.281	62.46	75.54					
	light intensity	dark	65	73.61	1.604	70.43	76.79					
		average	172	75.28	1.477	72.34	78.22					
		bright	54	77.03	1.619	73.82	80.24					
	light hours	hours	291	-0.72	0.359	-1.43	-0.01					
	farmer's experience	years	291	-0.18	0.081	-0.34	-0.02					
body weight (g)	age of pullets	6 weeks	97	449.72	9.575	430.69	468.76	67	428.08	16.788	394.84	461.32
		10 weeks	97	869.96	9.095	851.92	888.01	67	834.06	14.627	805.08	863.05
		16 weeks	97	1343.00	10.111	1323.00	1363.01	67	1333.10	16.611	1300.24	1365.95
	farming type	organic	201	921.29	22.516	876.39	966.20					
	0 91	conv.	90	853.83	18.117	817.73	889.94					
	days of pre-rearing	days	291	-1.01	0.200	-1.41	-0.61	201	-0.86	0.253	-1.37	-0.349
	light intensity	dark	65	874.96	10.136	854.86	895.06	52	849.13	14.058	821.12	877.14
	0	average	172	889.50	9.153	871.31	907.70	112	857.77	13.449	831.00	884.53
		bright	54	898.23	10.617	877.18	919.28	37	888.35	14.485	859.52	917.18
	number of stockperson	1	177	899.64	9.028	881.65	917.63	141	885.89	12.324	861.30	910.49
	1	2	114	875.49	10.023	855.50	895.47	60	844.27	15.793	812.62	875.93
	flock visits per day	1–1.5x	39	870.24	13.324	843.67	896.81	30	843.32	19.555	804.09	882.55
	1 9	2–3x	252	904.89	6.729	891.48	918.30	171	886.84	9.043	868.84	904.85
	observation score ^b	0	33	904.31	12.890	878.63	929.99	12	891.89	24.357	843.03	940.74
		1	39	869.38	16.957	835.58	903.18	0				
		2	57	917.33	12.233	892.95	941.71	57	888.17	13.296	861.56	914.77
		3	42	903.66	12.649	878.42	928.89	24	856.23	19.902	816.31	896.14
		4	87	896.61	11.182	874.32	918.91	75	879.18	13.583	852.02	906.33
		5	24	863.86	15.463	833.02	894.70	24	846.91	18.868	809.01	884.81
		6	9	857.80	21.875	814.18	901.42	9	828.12	25.475	776.94	879.30
	mean avoidance distance	metres	291	-8.68	4.019	-16.70	-0.66					
	access to free range	no						148	878.14	13.369	851.50	904.77
	Ũ	yes						53	852.03	15.424	821.49	882.56
mortality ^a (%)	age of pullets	6 weeks	95	1.24	0.264	0.719	1.770	65	0.89	0.278	0.34	1.44
• • •	- *	10 weeks	95	1.58	0.255	1.070	2.089	65	1.61	0.255	1.10	2.12
		16 weeks	95	1.91	0.266	1.378	2.436	65	2.12	0.276	1.58	2.67
	diseases occurred	no	183	1.36	0.276	0.813	1.911					
		yes	102	1.79	0.273	1.248	2.336					
	cleaning & disinfection score ^c	score	95	2.31	1.330	-0.346	4.957	65	2.60	1.533	-0.49	5.68
	access to covered veranda	no						74	1.75	0.264	1.23	2.28
		yes						121	1.33	0.244	0.84	1.81

^a cumulative mortality

^b observation score: How often are animals observed besides the daily tasks? 0, never; 1, if problems, multiple times per week; 2, if problems, daily; 3, if problems, multiple times daily; 4, routinely, multiple times per week; 5, routinely, daily; 6, routinely, multiple times per day.

^c cleaning & disinfection score: average score of dry cleaning yes (1) or no (0), high pressure cleaning yes (1) or no (0), warm (1) or cold (0) water used for cleaning, chloride used for drinking line disinfection yes (1) or no (0), use of silicate dust yes (1) or no (0).

Table 4

Final stepwise reduced regression models for associations within animal-based parameters within and between weeks (N = 98 flocks). A Benjamini-Hochberg False Discovery rate calculation confirmed the P-values in bold for rejection of the null-hypothesis, while P-values in italics fell below the corrected level.

animal- based parameter	variable	reg. coeff.	SD	Beta	Т	Р
uniformity 10th week	$R^2 = 0.199 P < 0.0001$					
	constant	70.241	9.190		7.643	0.000
	uniformity 6th week	0.214	0.119	0.172	1.803	0.075
	reactivity score 6th week ^a	-1.994	0.893	-0.214	-2.234	0.028
	farm	-0.288	0.080	-0.344	-3.609	0.000
uniformity 16th week	$R^2 = 0.457 P < 0.0001$					
	constant	20.158	10.314		1.955	0.054
	uniformity 6th week	0.228	0.118	0.158	1.944	0.055
	uniformity 10th week	0.601	0.095	0.517	6.341	0.000
	reactivity score 6th week ^a	-1.326	0.913	-0.123	-1.453	0.150
	avoidance distance 10th week	1.231	0.585	0.177	2.105	0.038
body weight	$R^2 = 0.174 P < 0.0001$					
10th week	constant	677.748	55.110		12.298	0.000
	weight 6th week	0.471	0.120	0.369	3.910	0.000
	mortality 6th week	-6.976	2.728	-0.241	-2.557	0.012
body weight	$R^2 = 0.354 P < 0.0001$					
16th week	constant	727.690	98.365		7.398	0.000
	uniformity 10th week	1.661	0.751	0.187	2.212	0.029
	weight 10th week	0.579	0.100	0.500	5.801	0.000
	mortality 10th week	-3.714	2.529	-0.125	-1.469	0.145
mortality ^b 10th week	$R^2 = 0.080 P = 0.005$					
-	constant	3.520	0.637		5.528	0.000
	reactivity score 6th week ^a	-0.787	0.275	-0.282	-2.865	0.005
mortality ^b 16th week	$R^2 = 0.169 P = 0.004$					
-	constant	7.498	3.819		1.964	0.053
	weight 6th week	0.008	0.005	0.158	1.521	0.132
	weight 10th week	-0.009	0.004	-0.214	-2.053	0.043
	reactivity score 6th week ^a	-0.914	0.309	-0.309	-2.954	0.004
	avoidance distance 10th week	-0.263	0.198	-0.139	-1.326	0.188
	down feathers present on the floor 10th week	0.548	0.258	0.209	2.123	0.036

^a 1, nervous; 2, medium; 3, calm

^b cumulative mortality

3.2. Risk factor analysis

Uniformity increased with age in all farms (model with all flocks: $F_{2170} = 45.113$, p < 0.001; model with organic flocks only: $F_{2119} =$ 7.809, p = 0.001, for estimated means see Table 3). No further predictors were identified in the model including organic flocks only (Table 3). In the model for uniformity including all flocks, i.e. both conventional and organic, uniformity was higher in organic flocks compared to conventional flocks ($F_{1,68} = 4.336$, p = 0.041, Table 3). Uniformity was higher with fewer light hours per day ($F_{1211} = 4.042$, p = 0.046, Table 3) and tended to be higher with brighter light intensities $(F_{2234} = 2.791, p = 0.063, Table 3)$. Uniformity was negatively associated with farmers' experience ($F_{1.68} = 4.847$, p = 0.031, Table 3). There was a tendency that flocks had higher uniformity in older barns ($F_{1,70} =$ 2.783, p = 0.1, Table 3). The complete models including all independent variables are depicted in the supplementary material Table S1. When excluding farming type from the model, breed got highly significant $(F_{4,73} = 4.683, p = 0.002)$, with LS or mixed LS/LB herds having higher uniformity), while probability values of other independent variables linked to farming type increased, i.e. for light duration (p = 0.08), experience of the farmer (p = 0.09) and age of the barn (p = 0.24), while it slightly decreased for light intensity (p = 0.052).

In the model for bodyweight for all flocks, organic flocks tended to have a higher weight than conventional ($F_{1,71} = 3.291$, p = 0.074, Table 3). In both models for bodyweight, weight was higher when the pre-rearing period was shorter (model with all flocks: $F_{1,69} = 25.284$, p < 0.001; model with organic flocks only: $F_{1,45} = 11.504$, p = 0.001, Table 3) and with brighter light intensities (all flocks: $F_{2220} = 2.433$, p = 0.09; organic flocks only: $F_{2154} = 4.585$, p = 0.012, Table 3). Weight was higher with only one person taking care of the pullets as compared to two (all flocks: $F_{1,73} = 7.651$, p = 0.007; organic flocks only: $F_{1,49} = 9.094$, p = 0.004, Table 3). Weight was also higher when stock people visited flocks more frequently per day (all flocks: $F_{1,69} = 7.598$, p = 0.004, Table 3).

0.007; organic flocks only: $F_{1,46} = 5.991$, p = 0.018, Table 3). The observation score was a significant predictor when all flocks were included ($F_{6,70} = 2.987$, p = 0.012, Table 3) and tended to be associated in the model with organic flocks only ($F_{5,47} = 2.132$, p = 0.078, Table 3), but associations were more complex (Table 3). Further, weight was associated negatively with avoidance distance in the model for all flocks of $(F_{1,69} = 4.662, p = 0.034, Table 3)$. In the model for organic flocks only, weight tended to be higher when pullets did not have access to free range until the day of the farm visit in week 16 ($F_{1,79} = 3.081$, p = 0.083, Table 3). As to be expected in growing pullets, age was highly positively related with weight. The full model results including all independent variables are shown in the supplementary material Table S2. When excluding farming type from the model with all flocks, breed stayed insignificant, while when excluding breed, farming type's probability value was no longer significant (p = 0.49).

Mortality tended to be higher when the cleaning and disinfection score was higher (all flocks: $F_{1,71} = 3.007$, p = 0.087; model with organic flocks only: $F_{1,48} = 2.867$, p = 0.097, Table 3). In the model including all flocks, mortality was higher when pullets were diagnosed with some kind of disease ($F_{1215} = 4.944$, p = 0.027, Table 3). In the model for organic only, mortality was higher when pullets did not have access to a covered veranda at the day of the farm visit ($F_{1,54} = 5.287$, p = 0.025, Table 3). For full model results including all independent variables see supplementary material Table S3.

3.3. Associations within animal-based parameters

Results of the regression models for associations within animal-based indicators across different weeks of age are depicted in Table 4. The model for uniformity in the 10th week explained 19.9% of the variations and 45.7% were explained by the model for uniformity in the 16th week. Uniformity in week 16 or 10 were associated positively with uniformity in the previous weeks, however this was confirmed after FDR correction

only for the association of week 10 with uniformity in week 16 (Table 4). The model for body weight in the 10th week explained 17.4% of the variation and the model for bodyweight in the 16th week explained 35.4% of the variation. Body weight was associated positively with body weight at the previous visit, while the positive associations of uniformity or mortality in the previous visit were not confirmed after FDR correction (Table 4). The model for mortality in the 10th week explained 8% of the variations and 16.9% were explained by the model for mortality in the 16th week. The reactivity score in week 6 was predictive for mortality both in the 10th and 16th week (Table 4), while associations of mortality in the 16th week to bodyweight and presence of down feathers in the 10th week were not confirmed after FDR correction (Table 4).

4. Discussion

By applying our monitoring system in the framework of routine veterinary visits throughout rearing, large variability between flocks in the animal-based indicators was detected and flocks with welfare problems could be identified, thus allowing for counteractions and early intervention. The system proved to be feasible and helpful. The reactivity score in the 6th week was related to later mortality, making it promising for inclusion in daily control by farmers to support earliest detection of welfare problems. This is further supported by its association to bloody lesions as presented in the previous paper (Mels et al., 2022), where also the presence of down feathers on the floor in the 10th week was identified as valuable indicator related to bloody lesions. We also could identify factors influencing uniformity, weight and mortality of the flocks, both flock-specific and farm-specific.

All three indicators as the focus of this paper (uniformity, weight and mortality), showed high variability and problem flocks could be identified: several farms fell below the desired and critical thresholds suggested by Knierim et al. (2020) indicating clear welfare problems and need for intervention. For example, of all flocks, 22.5% in the 6th week and 13.3% in the 10th week had a higher mortality than the critical threshold (6th week: $\leq 1.5\%$, 10th week: $\leq 2.5\%$, Knierim et al., 2020). In the 16th week of age 7.5% of the organic flocks and 6.7% of the conventional had a higher mortality than the critical threshold ($\leq 4\%$ for conventional and $\leq 6.08\%$ for organic flocks when mortality caused by predators is included). 35.7% of all flocks had a uniformity lower than the critical threshold of 80%. The monitoring system also included relevant environmental factors helping to take suitable action.

Regarding risk factor analysis, organic flocks had a higher uniformity and tended to have higher body weight than conventional flocks. These results are in line with (Küçükyılmaz et al., 2012) with respect to higher body weight of organic reared flocks. Regarding uniformity our model results suggest that this may at least partly be caused by breed: all LS flocks of our study were reared in organic farms, and all but one LB flock were reared on conventional farms, and breed got highly significant when deleting farming type from the model. However, genetics likely does not explain completely the farming type effect because other variables confounded with farming type still remained as predictors. In addition to genetic effects, a higher uniformity on organic farms may reflect a more uniform level of welfare within organic flocks due to a lower stocking density that may help minimise effects of social competition and hierarchy (Costa, 1981; Carey, 1987; for review see Meseret, 2016). But other environmental conditions were also confounded with farming type, although to a lesser extent than breed, and may have contributed to these differences.

Generally, uniformity and weight tended to be higher with brighter light intensities. This confirms our hypothesis and is in line with another study suggesting that higher light intensities resulted in higher flock uniformity and better body weight development (Hartini et al., 2002). Low light levels are known to impair a bird's ability to identify environmental cues (Kjaer and Vestergaard, 1999). Brighter light conditions could stimulate feeding behaviour and therefore result in a better body weight development and higher uniformity. However, in a more recent study light intensity (10 vs. 50 lux) did not affect body weight at the end of rearing (Chew et al., 2021). Uniformity was higher with shorter light periods in the model for all farms. This result is in line with a study performed in broiler breeders, however very short light periods (4 vs 8 h) were compared in this study (Griffin et al., 2005). There are only a few studies on lighting regimen impact on body weight in layer pullets and, to our knowledge, none for uniformity. A very short light period (6 vs. 14 h) resulted in smaller pullets (Lillie and Denton, 1965), while differences up to 4 h in week 12-18 (comparing either 8, 10 or 12 h light) did not affect body weight of Pengxian yellow pullets (Han et al., 2017), being in line with our results on body weight. Light hours had no effect on uniformity anymore when breed was included in the model suggesting a low influence if any. Nevertheless, body weight and uniformity should deviate from management recommendations only to a certain extent to avoid negative effects on sexual maturation and laying performance, e.g. reflected in the onset of lay and peak performance (Hudson et al., 2001; Lacin et al., 2008; Abbas et al., 2010). Including parameters such as body weight, uniformity and light intensity into a monitoring system would allow early measures to stimulate weight gain if needed by increasing light intensities.

Body weight was higher when the pre-rearing period was shorter (including the flocks that were not pre-reared at all i.e. with a duration of 0). This result is in line with our result on plumage damage reported earlier (Mels et al., 2022): plumage damage was lower with shorter pre-rearing as well. Animals were confined in the aviary for some time after being transported to the actual raring farm; stocking density in kg per m² was the higher the older animals were and thus longer pre-rearing can result in higher competition and stress during this aviary-confined period which may result in lower weight gain (Carey, 1987; for review see Meseret, 2016). In addition, transportation can cause stress and result in weight loss (Lalonde et al., 2021); older animals may react stronger to transport and the novel environment due to age dependent increase in fearfulness (Ghareeb et al., 2008). Finally, all specialised pre-rearing farms raised their pullets on the floor with access to litter, while pullets that were raised on the actual rearing farm from day one (no pre-rearing on another farm) were confined in the aviary for the first few weeks before being released from the aviary (except on one farm which practiced floor-rearing in the first weeks). Confinement in the aviary at an early age could ease access to feed since all aviaries were equipped with feeding chains. In addition, movement in the aviary is also restricted compared to floor rearing. Both aspects might have contributed to a better weight development in the first few weeks of life that might have had lasting effects and might have contributed to our results of higher weight with shorter (including no) pre-rearing. For example feed conversion rate (FCR) was better with lower space allowance in pullets (Ahammed et al., 2014) and in conventional cages compared to aviaries in laying hens (Aerni et al., 2005). Nevertheless, pullets benefit from having more opportunities for locomotor behaviour in aviaries compared to conventional cages with regard to better muscle growth and bone strength (Casey-Trott et al., 2017) and benefit from access to litter from day 1 on during floor-rearing (Huber-Eicher and Sebö, 2001; Mels et al., 2022). Regarding the provision of environmental enrichment during rearing, Liebers et al. (2019) did not find an effect of whether litter was provided or not on body weight. In our study, litter quantity was also not associated with either weight or uniformity.

Mortality was higher when pullets were diagnosed with some kind of disease. Fossum et al. (2009) reported that diseases (e.g. colibacillosis, red mites, erysipelas) and cannibalism were the most frequent cause for higher mortality in layers. However, piling, while infrequent, can cause high mortality as well, if occurring (Sparks et al., 2008). We excluded two flocks with very high mortality of about 15%, which was caused by piling events, because we could not fit a valid model otherwise. Causes for piling (e.g. panic or heat stress; Gray et al., 2020) are different from causes for disease. All flocks that were diagnosed with a disease were, depending on the diagnose and severity, treated with antibiotics, vitamins and/or probiotics for disease control. Mortality tended to be higher

when the cleaning and disinfection score was higher. Cleaning and disinfection are fundamental steps for biosecurity programs to reduce infection pressure from pathogen destruction as well as transmission from one flock to the next (Meroz and Samberg, 1995; Maertens et al., 2018). Farmers' that already experienced problems with occurring diseases may have taken increased measures in cleaning and disinfection in an attempt to control outbreaks of diseases. Thus the cleaning/disinfection effort may have been rather a result of former disease outbreaks than an influencing factor. In organic flocks mortality was higher if pullets did not have access to a covered veranda. This could be due to the resulting overall higher stocking density if pullets have to stay inside the barn. Higher stocking density was associated with higher mortality in pullets (Carey, 1987) and in broilers (for review see Meseret, 2016). Organic pullets should have had access to a covered veranda from the 8th week of age (Bio Austria 2019). Compliance with the guidelines (e.g. providing access to a covered veranda and to free range) should be monitored regularly and might be beneficial for animal health and welfare.

There might be a higher risk for health problems in organic layer production leading to a higher mortality compared to conventional production (Fossum et al., 2009; Stokholm et al., 2010; Bestman and Wagenaar, 2014). However our finding does not support this in pullets: we did not find an association between farming type and mortality rates. However, the abovementioned studies refer to adult laying hens and losses due to predators (which can be higher in free range systems) and infectious diseases that, in our study, did not occur during rearing (e.g. histomoniasis or brachyspira). Our results therefore point to the importance of an adapted management to control disease and losses, independent from farming type.

Stockpeople have a considerable effect on animal productivity (including weight gain in growing animals), health and welfare both via their management decisions and via their behaviour and handling practices and subsequent animals' level of fear of humans (Hemsworth and Coleman, 2011; Waiblinger, 2019). Positive handling resulted in lower or no fear of humans and higher weight gain in several species: in calves (Lürzel et al., 2015), in pigs (Hemsworth et al., 1981) as well as in poultry (Barnett et al., 1992). Early gentle handling of chickens did not only reduce their fear of humans, but also improved disease resistance, weight gain and feed conversion efficiency (Gross and Siegel, 1979, 1980, 1982), although these effects are not always seen (e.g. Collins and Siegel, 1987). In our study, weight was higher with only one stockperson taking care of the pullets, when flocks were visited more often per day and when avoidance distance was lower, indicating a better human-animal relationship (Waiblinger et al., 2006). Further, flocks that were calmer in the 6th week had a lower mortality in the 10th and 16th week of age. Our results support the above mentioned studies: regular (positive or neutral) contact with the same stockperson(s) decreases fear of humans as reflected in lower avoidance distance (Mels et al., 2022) and might enhance growth performance and lower mortality, likely due to reduced fear and stress and consequently improved immune responses and reduced piling events due to panicking. This line of reasoning also accounts for the reactivity score (reflecting the level of fear of humans but also being affected by general fearfulness; Waiblinger et al., 2006) which was associated with later mortality. Fear responses such as flight reactions, escape or panic can cause injury or even death of animals (for review see Jones, 1996). Generally, higher levels of stress are known to increase susceptibility for diseases (for review see Rosales, 1994; Umar et al., 2016), and thus may lead to increased mortality. The assessment of flocks' reactivity and avoidance distance (as indicators for the human-animal relationship) can be performed easily and quickly and provide valuable information; thus they are worth monitoring regularly.

The confounding of farming type and breed or other factors is a limitation of our study regarding the overall models. However, it displays the situation in practice where some conditions are inherent to farming type. Further, we dealt with this confounding during model calculation as described in the methods and, finally, for the models with the subset of only organic flocks this problem is inexistent. In general, the breeds, housing systems and management are comparable to other European countries, especially for the organic farms, supporting a relatively high external validity of our results, which is supported also by results being in line with earlier studies.

Implementing the monitoring system on a regular basis and monitoring the animal-based indicators and potential influencing factors such as management conditions (e.g. light intensity, access to covered veranda) and human-animal relationship can contribute to early detection of problems and their causes and enable early counteractions, to safeguard the welfare status of the flock at a later age. The effectiveness of the monitoring system and associated early intervention was shown with respect to bloody lesions where the number of affected flocks largely decreased from the 10th to the 16th week (Mels et al., 2022). Examples of possible interventions in case of noticeable problems (e.g. high reactivity score, no down feathers present) would be, for example, administration of minerals and/or vitamins, control and adaptation of feed or encouraging farmers to increase contact to their animals; depending on the identified problem and the current situation on the farm. In the meantime, the monitoring system is used as described in this paper and in Mels et al. (2022) in the veterinary practice, in the integration company and used on farm by the stockpersons. All data are collected in an electronic data base with access for involved persons (farmer, veterinarian and integration staff). The first author noticed that communication became more intense and efficient which contributed, in her and her veterinary company's opinion, to a faster adaption in management and treatment, if problems (e.g. weight loss, increasing mortality rates) occurred. The monitoring system can thus be recommended to be used for surveillance of pullet flocks by veterinarians in cooperation with integration staff and/or farmers; if necessary it would allow easy adaptation to specific local factors not yet included.

5. Conclusion

The developed monitoring system is a promising tool for surveillance of pullet flocks allowing for early interventions if needed. The system can easily be implemented in regular veterinary visits but also used by integration staff and the farmers themselves. Several easy to record animal-based indicators of animal welfare were identified; they could be analysed more frequently by the farmers' which would increase early detection of problems. Implementation of such a routine-based monitoring system with easy-to-assess parameters can contribute to better animal health and animal welfare in pullets.

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2023.105929.

References

- Abbas, S.A., Gasm Elsei, A.A., Ahmed, M.K.A., 2010. Effect of body weight uniformity on the productivity of broiler breeder hens. Int. J. Poult. Sci. 9 (3), 225–230. https:// doi.org/10.3923/ijps.2010.225.230.
- Ahammed, M., Chae, B.J., Lohakare, J., Keohavong, B., Lee, M.H., Lee, S.J., 2014. Comparison of aviary, barn and conventional cage raising of chickens on laying performance and egg quality. Asian-Australas. J. Anim. Sci. 27 (8), 1196–1203. https://doi.org/10.5713/ajas.2013.13394.
- Alonso, M.E., González-Montaña, J.R., Lomillos, J.M., 2020. Consumers' concerns and perceptions of farm animal welfare. Animals 10 (3), 385. https://doi.org/10.3390/ ani10030385.

Barnett, J.L., Hemsworth, P.H., Newman, E.A., 1992. Fear of humans and its relationships with productivity in laying hens at commercial farms. Br. Poult. Sci. 33 (4), 699–710. https://doi.org/10.1080/00071669208417510.

Bestman, M., Wagenaar, J.P., 2014. Health and welfare in dutch organic laying hens. Animals 4 (2), 374–390. https://doi.org/10.3390/ani4020374.

- Blokhuis, H.J., van Niekerk, T., Bessei, W., Elson, A., Guémené, D., Kjaer, J.B., 2007. The Laywel project: welfare implications of changes in production systems for laying hens. World'S. Poult. Sci. J. 63 (1), 101–114. https://doi.org/10.1017/ S0043933907001328.
- Butterworth, A., Arnould, C., van Niekerk, T. (Eds.), 2009. Assessment protocol for poultry. Welfare Quality® Consortium, Lelystadt, Netherlands.
- Carey, J.B., 1987. Effects of pullet-stocking density on performance of laying hens. Poult. Sci. 66 (8), 1283–1287. https://doi.org/10.3382/ps.0661283.
- Casey-Trott, T.M., Korver, D.R., Guerin, M.T., Sandilands, V., Torrey, S., Widowski, T.M., 2017. Opportunities for exercise during pullet rearing, Part I: Effect on the musculoskeletal characteristics of pullets. Poult. Sci. 96 (8), 2509–2517. https://doi. org/10.3382/ps/pex059.
- Chew, J., Widowski, T., Herwig, E., Shynkaruk, T., Schwean-Lardner, K., 2021. The effect of light intensity on the body weight, keel bone quality, tibia bone strength, and mortality of brown and white feathered egg-strain pullets reared in perchery systems. Poult. Sci. 100, 101464 https://doi.org/10.1016/j.psj.2021.101464.
- Collins, J.W., Siegel, P.B., 1987. Human handling, flocksize and responses to an E. coli challenge in young chickens. Appl. Anim. Behav. Sci. 19 (1–2), 183–188. https://doi. org/10.1016/0168-1591(87)90215-2.
- Costa, M.S., 1981. Fundamental principles of broiler breeders nutrition and the design of feeding programmes. World'S. Poult. Sci. J. 37 (3), 177–192. https://doi.org/ 10.1079/WPS19810013.
- De Haas, E.N., Kemp, B., Bolhuis, J.E., Groothuis, T., Rodenburg, T.B., 2013. Fear, stress, and feather pecking in commercial white and brown laying hen parent-stock flocks and their relationships with production parameters. Poult. Sci. 92 (9), 2259–2269. https://doi.org/10.3382/ps.2012-02996.
- Decina, C., Berke, O., van Staaveren, N., Baes, C.F., Harlander-Matauscheck, A., 2019. Development of a scoring system to assess feather damage in canadian laying hen flocks. Animals 9 (7). https://doi.org/10.3390/ani9070436.
- Eder, V., Hofer, D., Gala, M., Gansinger D., Kober, W., Niebuhr, K., Edlinger S., 2016. Leitfaden Tierwohl Geflügel. BIO AUSTRIA, Linz, Austria. https://www.bio-austria. at/app/uploads/2017/04/Leitfaden-Tierwohl-Geflu%CC%88gel-201609.pdf (accessed 07 February 2023).
- Ensminger, M.E., 1980. Poultry science, second ed. Danville, IL.
- European Commission, 2016. Attitudes of Europeans towards animal welfare: report. Directorate-General for Health and Food Safety. https://data.europa.eu/doi/ 10.2875/884639.
- Fossum, O., Jansson, D.S., Etterlin, P.E., Vågsholm, I., 2009. Causes of mortality in laying hens in different housing systems in 2001 to 2004. Acta Vet. Scand. 51 (1), 3. https://doi.org/10.1186/1751-0147-51-3.
- Fraser, D., 2008. Understanding animal welfare. Acta Vet. Scand. 50 (1), 1–12. https:// doi.org/10.1186/1751-0147-50-S1-S1.
- Ghareeb, K., Awad, W.A., Nitsch, S., Abdel-Rahe, S., Bohm, J., 2008. Effects of transportation on stress and fear responses of growing broilers supplemented with prebiotic or probiotic. Int. J. Poult. Sci. 7 (7), 678–685. https://doi.org/10.3923/ ijps.2008.678.685.
- Graml, C., Niebuhr, K., Waiblinger, S., 2008. Reaction of laying hens to humans in the home or a novel environment. Appl. Anim. Behav. Sci. 113 (1–3), 98–109. https:// doi.org/10.1016/j.applanim.2007.10.004.
- Gray, H., Davies, R., Bright, A., Rayner, A., Asher, L., 2020. Why do hens pile? Hypothesizing the causes and consequences. Front. Vet. Sci. 7, 616836 https://doi. org/10.3389/fvets.2020.616836.
- Griffin, A.M., Renema, R.A., Robinson, F.E., Zuidhof, M.J., 2005. The influence of rearing light period and the use of broiler or broiler breeder diets on forty-two-day body weight, fleshing, and flock uniformity in broiler stocks. J. Appl. Poult. Res. 14 (2), 204–216. https://doi.org/10.1093/japr/14.2.204.
- Gross, W.B., Siegel, P.B., 1979. Adaption of chickens to their handlers and experimental results. Avian Dis. 23, 708–714. https://doi.org/10.2307/1589747.
- Gross, W.B., Siegel, P.B., 1980. Effects of early environmental stresses in chicken body weight, antibody response to RBC antigens, feed efficiency and response to fasting. Avian Dis. 24, 549–579.
- Gross, W.B., Siegel, P.B., 1982. Socialization as a factor in resistance to infection, feed efficiency, and response to antigen in chickens. Am. J. Vet. Res. 43 (11), 2010–2012.
- Han, S., Wang, Y., Liu, L., Li, D., Liu, Z., Shen, X., 2017. Influence of three lighting regimes during ten weeks growth phase on laying performance, plasma levels- and tissue specific gene expression- of reproductive hormones in Pengxian yellow pullets. PloS One 12 (5), e0177358. https://doi.org/10.1371/journal.pone.0177358.
- Hartini, S., Choct, M., Hinch, G., Kocher, A., Nolan, J.V., 2002. Effects of light intensity during rearing and beak trimming and dietary fiber sources on mortality, egg production, and performance of ISA brown laying hens. J. Appl. Poult. Res. 11 (1), 104–110. https://doi.org/10.1093/japr/11.1.104.
- Hemsworth, P.H., Coleman, G.J., 2011. Human-livestock interactions. The stockperson and the productivity and welfare of intensively farmed animals, second ed. CABI, Wallingford.
- Hemsworth, P.H., Barnett, J.L., Hansen, C., 1981. The influence of handling by humans on the behavior, growth, and corticosteroids in the juvenile female pig. Horm. Behav. 15 (4), 396–403. https://doi.org/10.1016/0018-506X(81)90004-0.
- Huber-Eicher, B., Sebö, F., 2001. The prevalence of feather pecking and development in commercial flocks of laying hens. Appl. Anim. Behav. Sci. 74 (3), 223–231. https:// doi.org/10.1016/S0168-1591(01)00173-3.

- Hudson, B.P., Lien, R.J., Hess, J.B., 2001. Effects of body weight uniformity and pre-peak feeding programs on broiler breeder hen performance. J. Appl. Poult. Res. 10 (1), 24–32. https://doi.org/10.1093/japr/10.1.24.
- Janczak, A.M., Riber, A.B., 2015. Review of rearing-related factors affecting the welfare of laying hens. Poult. Sci. 94 (7), 1454–1469. https://doi.org/10.3382/ps/pev123.
- Jones, B.R., 1996. Fear and adaptability in poultry: insights, implications and imperatives. World's Poult. Sci. J. 52 (2), 131–174. https://doi.org/10.1079/ WPS19960013.
- Kjaer, J.B., Vestergaard, K.S., 1999. Development of feather pecking in relation to light intensity. Appl. Anim. Behav. Sci. 62 (2–3), 243–254. https://doi.org/10.1016/ S0168-1591(98)00217-2.
- Knierim, U., Andersson, R., Keppler, C., Petermann, S., Rauch, E., Spindler, B., Zapf, R., 2016. Tierschutzindikatoren: Leitfaden für die Praxis – Geflügel. Vorschläge für die Produktionsrichtungen. KTBL-Sonderveröffentlichung 12618 or P_12618, Darmstadt. KTBL.
- Knierim, U., Gieseke, D., Michaelis, S., Keppler, C., Spindler, B., Rauch, E., 2020. Tierschutzindikatoren: Leitfaden für die Praxis – Geflügel. Vorschläge für die Produktionsrichtungen Jung- und Legehenne, Masthuhn, Mastpute, second ed. Darmstadt: KTBL.
- Küçükyılmaz, K., Bozkurt, M., Herken, E.N., Cinar, M., Catlı, A.U., Bintaş, E., Cöven, F., 2012. Effects of rearing systems on performance, egg characteristics and immune response in two layer hen genotype. Asian-Australas. J. Anim. Sci. 25 (4), 559–568. https://doi.org/10.5713/ajas.2011.11382.
- Lacin, E., Yildiz, A., Esenbuga, N., Macit, M., 2008. Effects of differences in the initial body weight of groups on laying performance and egg quality parameters of Lohmann laying hens. Czech J. Anim. Sci. 53 (11), 466–471. https://doi.org/ 10.17221/341-CJAS.
- Lalonde, S., Beaulac, K., Crowe, T.G., Schwean-Lardner, K., 2021. The effects of simulated transport conditions on the muscle tissue characteristics of white-strain layer pullets. Poult. Sci. 100 (1), 103–109. https://doi.org/10.1016/j. psi.2020.09.064.
- Liebers, C.J., Schwarzer, A., Erhard, M., Schmidt, P., Louton, H., 2019. The influence of environmental enrichment and stocking density on the plumage and health conditions of laying hen pullets. Poult. Sci. 98 (6), 2474–2488. https://doi.org/ 10.3382/ps/pez024.
- Lillie, R.J., Denton, C.A., 1965. Effect of lighting systems in the grower and adult periods upon the over-all performance of White Leghorns. Poult. Sci. 44, 809–816. https:// doi.org/10.3382/ps.0440809.
- Lürzel, S., Münsch, C., Windschnurer, I., Futschik, A., Palme, R., Waiblinger, S., 2015. The influence of gentle interactions on avoidance distance towards humans, weight gain and physiological parameters in group-housed dairy calves. Appl. Anim. Behav. Sci. 172, 9–16. https://doi.org/10.1016/j.applanim.2015.09.004.
- Maertens, H., Reu, K., van Weyenberg, S., van Coillie, E., Meyer, E., van Meirhaeghe, H., 2018. Evaluation of the hygienogram scores and related data obtained after cleaning and disinfection of poultry houses in Flanders during the period 2007 to 2014. Poult. Sci. 97 (2), 620–627. https://doi.org/10.3382/ps/pex327.
- Main, D.C.J., Mullan, S., Atkinson, C., Bond, A., Cooper, M., Fraser, A., Browne, W.J., 2012. Welfare outcomes assessment in laying hen farm assurance schemes. Anim. Welf. 21 (3), 389–396. https://doi.org/10.7120/09627286.21.3.389.
- Marchewka, J., Watanabe, T.T.N., Ferrante, V., Estevez, I., 2013. Welfare assessment in broiler farms: transect walks versus individual scoring. Poult. Sci. 92 (10), 2588–2599. https://doi.org/10.3382/ps.2013-03229.
- Marchewka, J., Estevez, I., Vezzoli, G., Ferrante, V., Makagon, M.M., 2015. The transect method: a novel approach to on-farm welfare assessment of commercial turkeys. Poult. Sci. 94 (1), 7–16. https://doi.org/10.3382/ps/peu026.
- McAdie, T.M., Keeling, L.J., 2000. Effect of manipulating feathers of laying hens on the incidence of feather pecking and cannibalism. Appl. Anim. Behav. Sci. 68 (3), 215–229. https://doi.org/10.1016/S0168-1591(00)00107-6.
- Mels, C., Niebuhr, K., Futschik, A., Rault, J.L., Waiblinger, S., 2022. Predictors for plumage damage and bloody lesions indicative of feather pecking in pullets reared in aviaries. Appl. Anim. Behav. Sci. 250, 105607 https://doi.org/10.1016/j. applanim.2022.105607.
- Meroz, M., Samberg, Y., 1995. Disinfecting poultry production premises. Rev. Sci. Et. Tech. 14 (2), 273–291. https://doi.org/10.20506/rst.14.2.839.
- Meseret, S., 2016. A review of poultry welfare in conventional production system. Livestock Research Rural Development 28(12), 234 ref.37. http://www.lrrd.org/ lrrd28/12/mese28234.html (accessed 07 February 2023).
- Mullan, S., Szmaragd, C., Cooper, M.D., Wrathall, J.H.M., Jamieson, J., Bond, A., 2016. Animal welfare initiatives improve feather cover of cage-free laying hens in the UK. Anim. Welf. 25 (2), 243–253. https://doi.org/10.7120/09627286.25.2.243.
- Nicol, C.J., 2019. Feather pecking in laying hens: why they do it, and welfare implications. In: Olukosi, O.A., Olori, V.E., Helmbrecht, A., Lambton, S., French, N.S. (Eds.), Poultry Feathers and Skin: The Poultry Integument in Health and Welfare. CABI, Wallingford, pp. 31–46.
- Niebuhr, K., Waiblinger, S., Raubek, J., Zaludik, K., Gruber, B., 2007. Farmers' attitudes, daily working and handling practices on grower and laying hen farms and their relationship with hen welfare. Rep. EEC-Proj. Welf. Quality 22.
- Olsen, R.H., Frantzen, C., Christensen, H., Bisgaard, M., 2012. An investigation on firstweek mortality in layers. Avian Dis. 56 (1), 51–57. https://doi.org/10.1637/9777-051011-Reg.1.
- Petitte, J.N., Hawes, R.O., Gerry, R.W., 1982. The influence of flock uniformity on the reproductive performance of broiler breeder hens housed in cages and floor pens. Poult. Sci. 61 (11), 2166–2171. https://doi.org/10.3382/ps.0612166.
- Robinson, F.E., Robinson, N.A., 1991. Reproductive performance, growth rate and body composition of broiler breeder hens differing in body weight at 21 weeks of age. Can. J. Anim. Sci. 71 (4), 1223–1231. https://doi.org/10.4141/cjas91-145.

- Rosales, A.G., 1994. Managing Stress In Broiler Breeders: A Review. J. Appl. Poult. Res. 3 (2), 199–207. https://doi.org/10.1093/japr/3.2.199.
- Savory, C.J., 1995. Feather pecking and cannibalism. World'S. Poult. Sci. J. 51 (2), 215–219. https://doi.org/10.1079/WPS19950016.
- Sparks, N.H.C., Conroy, M.A., Sandilands, V., 2008. Socio-economic drivers for UK organic pullet rearers and the implications for poultry health. Br. Poult. Sci. 49 (5), 525–532. https://doi.org/10.1080/00071660802290416.
- Stokholm, N.M., Permin, A., Bisgaard, M., Christensen, J.P., 2010. Causes of mortality in commercial organic layers in Denmark. Avian Dis. 54 (4), 1241–1250. https://doi. org/10.1637/9375-041910-Reg.1.
- Tierhaltungsverordnung (1.) Österreich 2022. 1. Tierhaltungsverordnung in der Fassung der Bekanntmachung vom 27. Januar 2006 (BGBI. II Nr. 25/2006), das zuletzt durch am 27. Juli 2022 (BGBI. II Nr. 296/2022) geändert worden ist. https://www.ris.bka. gv.at/GeltendeFassung.wze?Abfrage=Bundesnormen&Gesetzesnummer=20003820 (accessed 02 March 2023).
- Tierschutzgesetz Deutschland 2022. Tierschutzgesetz in der Fassung der
- Bekanntmachung vom 18. Mai 2006 (BGBI. I S. 1206, 1313), das zuletzt durch Artikel 2 Absatz 20 des Gesetzes vom 20. Dezember 2022 (BGBI. I S. 2752) geändert

worden ist. https://www.gesetze-im-internet.de/tierschg/BJNR012770972.html (accessed 07 February 2023).

- Vasdal, G., Granquist, E.G., Skjerve, E., de Jong, I.C., Berg, C., Michel, V., Moe, R., 2019. Associations between carcass weight uniformity and production measures on farm and at slaughter in commercial broiler flocks. Poult. Sci. 98 (10), 4261–4268. https://doi.org/10.3382/ps/pez252.
- Vasdal, G., Marchewka, J., Newberry, R.C., Estevez, I., Kittelsen, K., 2022. Developing a novel welfare assessment tool for loose-housed laying hens - the Aviary Transect method. Poult. Sci. 101 (1), 101533 https://doi.org/10.1016/j.psj.2021.101533.
- Veissier, I., Butterworth, A., Bock, B., Roe, E., 2008. European approaches to ensure good animal welfare. Appl. Anim. Behav. Sci. 113 (4), 279–297. https://doi.org/10.1016/ j.applanim.2008.01.008.
- Waiblinger, S., 2019. Agricultural animals. In: Hosey, G., Melfi, V. (Eds.), Anthrozoology: human-animal interactions in domesticated and wild animals. Oxford University Press, Oxford, pp. 32–58.
- Waiblinger, S., Boivin, X., Pedersen, Vivi, Tosi, Maria-Vittoria, Janczak, A.M., Visser, E. K., Jones, R.B., 2006. Assessing the human–animal relationship in farmed species: a critical review. Appl. Anim. Behav. Sci. 101 (3–4), 185–242. https://doi.org/ 10.1016/j.applanim.2006.02.001.