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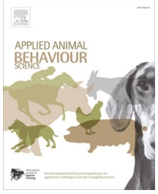

Dark brooder pullets are less active than Non-brooder pullets, but show similar level of synchronisation at the resource areas

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ABSTRACT

Maternal care in commercial chickens can partially be replaced with dark brooders that offer heat and a dark area to rest and possibly avoid negative social interactions. Previous research has established the positive effects of dark brooders on reducing disturbance during resting in young pullets and injurious pecking in pullets and laying hens, which could reduce overall activity levels. The objective of this study was to employ precision livestock farming techniques to compare the overall activity levels and behavioural synchrony at resources in pullets reared with dark brooders until 41 days of age and those reared with whole house heating. Four brooder treatments, varying in size (Small/Large) and deployment method (Raised/Fixed), which could have implications for commercial use, were employed. Activity of the whole pen floor area and behavioural synchrony in drinker and feeder areas were automatically assessed over a 12-hour photoperiod at 10 and 60 days of age. Pixel change detection gauged overall activity across the pen, while an object detection model evaluated pullet behavioural synchrony. The analysis revealed increased activity levels in non-Brooder pullets compared to those in most Brooder treatments at both 10 and 60 days of age. However, no significant differences in behavioural synchrony were observed between Brooder and non-Brooder pullets. The underlying mechanism of dark brooder's effects on the activity levels and behaviour synchrony remains unclear, but the observed reduction in activity levels in brooded pullets likely corresponds to increased resting behaviour and reduced injurious pecking. Furthermore, it is noteworthy that only a small percentage of pullets (up to 57.14%) were observed to use the resources simultaneously. This underscores the importance of conducting additional research to delve into the effects of resource allocation on both behavioural synchrony and activity levels in pullets. We observed minimal differences in the behaviour of pullets raised under different brooder types, suggesting that the simplest (Small-Fixed) brooders may be suitable for commercial use.

1. Introduction

Commercially kept chickens for either meat (i.e., broilers) or egg production (i.e., pullets) are reared without maternal care. Maternal care can partially be replaced with dark brooders, i.e., horizontal heating elements equipped with curtains, which offer heat and darkness. Rearing pullets with dark brooders have multiple short- and long-term welfare benefits as reviewed by Sirovnik and Riber (2022). The main welfare benefits of rearing with dark brooders are a reduction of fear, injurious pecking, and mortality due to cannibalism (Riber et al., 2007; Riber and Guzman, 2017) and improved quality of resting (Forslind et al., 2022).

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Rearing with dark brooders influences the expression of various behaviours in chickens. For example, resting brooder broilers experience fewer disturbances than non-brooder broilers by other individuals, causing them to change position or become active, not only when brooders are present but also for two weeks after the brooders are removed (i.e., until slaughter; Forslind et al., 2022). Similar to broilers, brooder pullets may also be less disturbed while resting, as they show longer active and inactive phases during the first 2 weeks of life compared to the non-brooder pullets, as well as being more synchronised in activity and rest (Riber et al., 2007). Furthermore, during scan sampling a higher proportion of pullets reared with brooders rest and perform fewer active behaviours, including less feeding and drinking in the first four days of life (Riber and Guzman, 2016). Afterwards, brooder pullets perform less of some types of active behaviour (e.g., locomotion and injurious pecking) as compared to non-brooder pullets, whereas other types of behaviour (e.g., drinking and feeding) are unaffected when using a direct observation method. It is difficult to assess the effect of dark brooders on overall activity levels in large groups of chickens when using behaviour scoring by humans. Image analysis utilising automatic detection of change of pixel grey scale value may offer a good proxy for overall activity levels as it captures movement by detecting any change in the body shape. It has been used previously for activity assessment (Fraess et al., 2016; Kristensen and Cornou, 2011; van Leuffen et al., 2023) and was validated for chickens (Rault et al., 2017).

Behavioural synchrony can be defined as performance of a specific behaviour at the same time by multiple individuals in close proximity to each other (Webster, 2002). It has likely evolved as a trade-off response to intra-specific competition and predator avoidance. By synchronising their behaviour, chickens and other animals may reduce individual risk of being targeted by a predator, but at the same time the competition for resources such as feed and water may increase (Clayton, 1978). Although domestic chickens kept indoors on farms or in research facilities are rarely exposed to predation, they have retained the typical characteristics of prey animals, including high levels of alertness towards the environment and human caretakers (Richards et al., 2012). This may be the reason why behavioural synchrony is commonly observed in domestic chickens (Alvino et al., 2009; Eklund and Jensen, 2011; Riber et al., 2007; Savoury, 1980). The presence of a human observer during the observation periods is therefore likely to influence chicken behaviour, including activity levels and access to the feed and water, an effect that is minimised by using video recordings to score the behaviour (Lolli et al., 2013). Additionally, as poultry shows clear ultradian rhythms in activity and feeding behaviour (Nielsen et al., 2008; Formanek et al., 2009), relying on short observation windows may not provide a comprehensive understanding of behavioural synchrony throughout the entire day. The potential long-term impact of dark brooders on behavioural synchrony at resources such as after the removal of brooders has not previously been investigated.

When reared by the mother hen, chickens typically actively leave the space beneath her to engage in active behaviours such as foraging. However, approximately 20% of active bouts are initiated by the mother hen, with a greater impact on chickens in colder ambient temperatures (Sherry, 1981). Additionally, brooded chickens exhibit more feeding and foraging activities compared to non-brooded chickens (Shimmura et al., 2010), likely influenced by the mother hen's encouragement through gestural and vocal food calling elements in her pecking behaviour (Sherry, 1977; Wauters et al., 2002). Commercial chickens lack the guidance provided by a mother hen, potentially impacting their behaviour, such as activity levels and synchrony at feed and water resources. The aim of this study was to estimate activity levels and behavioural synchrony at the resources In pullets reared either with or without dark brooders (Brooder treatment and Control treatment, respectively) using precision livestock farming (PLF) techniques. Studying pixel change and using automatic object detection techniques in the video recordings, we examined activity and behavioural synchrony at two ages: 10 days of age (brooders present) and 60 days of age (brooders removed). We hypothesised that Brooder pullets would be less active and express higher levels of behavioural synchrony at the resources compared to Control pullets. Considering that larger brooders incur higher costs producers may be interested in determining whether there is a size effect of brooders on chicken behaviour. Thus, we investigated whether there was an effect of brooder size or keeping brooders in a fixed position *versus* raising them at regular intervals throughout the first days of life on the activity level and behavioural synchrony.

2. Material and methods

2.1. Animal and housing

Newly hatched non-beak-trimmed female Isa Warren pullets (N = 2254) obtained from a commercial hatchery (TopÆg Aps, Viborg, Denmark) were housed in the experimental poultry facility at AU Foulum (Tjele, Denmark). The pullets were haphazardly assigned to one of 22 pens located in two adjacent experimental rooms, Room A and B. Sixteen pens were located in Room A and six pens in Room B. With the exception of the heating sources explained in section Treatments, the conditions in the rooms were the same.

Each pen (2 m × 4 m, Fig. 1) housed a group of 102–103 pullets at day 1 of age, and due to mortality 88–101 pullets at 10 days of age and 83–101 pullets at 60 days of age. Hence, the stocking density at the commencement of the study was approximately 13 birds/m^2 of usable area, representing approximately two-thirds of that recommended by industrial standards in Denmark for brown pullets destined for barn egg production (Danske Æg, 2020). Visual contact between pullet-groups was prevented using 70 cm high white sheets of hard plastic. During the first 18 days of age, the available floor area was halved using wooden partitions.

Pens were equipped with seven automatic water nipples positioned in a row along a wall and two round feeders (251 cm feeder space, in total). The pullets had unrestricted access to these resources over the course of the study. In addition, pullets were fed on paper during the first 4 days. Pullets were fed a commercial diet (DLG Group, Denmark) according to their rearing stage. The feed was provided *ad libitum*. Bedding was provided in the form of an approximately 5 cm deep layer of wood shavings. On day 18, each pen was provided with two perches (each 2 m long, at 1 m height). Light and ventilation, which followed commercial standard practices, were controlled with a computerised system. The light programme consisted of 24 h and 22 h of light during day 1 and days 2–5, respectively, after which the light duration was reduced to 18 hours and 12 hours during days 6–9 and 10–60 of age, respectively. During the first seven days of age, the light intensity was 12 lux and afterwards reduced to 10 lux.

2.2. Treatments

Pullets were provided either with dark brooders (Brooder treatment) for the first 41 days of life or had whole house heating and no dark brooders (Control treatment). Due to different heating sources, treatments were divided into two rooms: Brooder treatment was used in Room A and Control treatment in Room B. This design enabled us to replicate commercially relevant rearing conditions, as dark brooders allow for lower environmental temperatures compared to whole-house heating systems (Gilani et al., 2012). In Room A, dark brooders (one per pen) were used as the primary heat source and the pullets were placed under the brooders on day 1 of life. The ambient temperature in Room A was kept at 24°C on days 1 and 2, 22°C on days 3 and 4, and then kept constant at 20°C. The temperature under the brooders was adapted to the age of the pullets. The temperature under the brooders was 34°C on days 1–3 and then lowered half a degree each day until 20°C were reached on day 28. On day 1, the brooders were positioned 16 cm above the ground and the height of the brooders was adjusted to 25 cm above the ground at 29 days of age to compensate for the growth

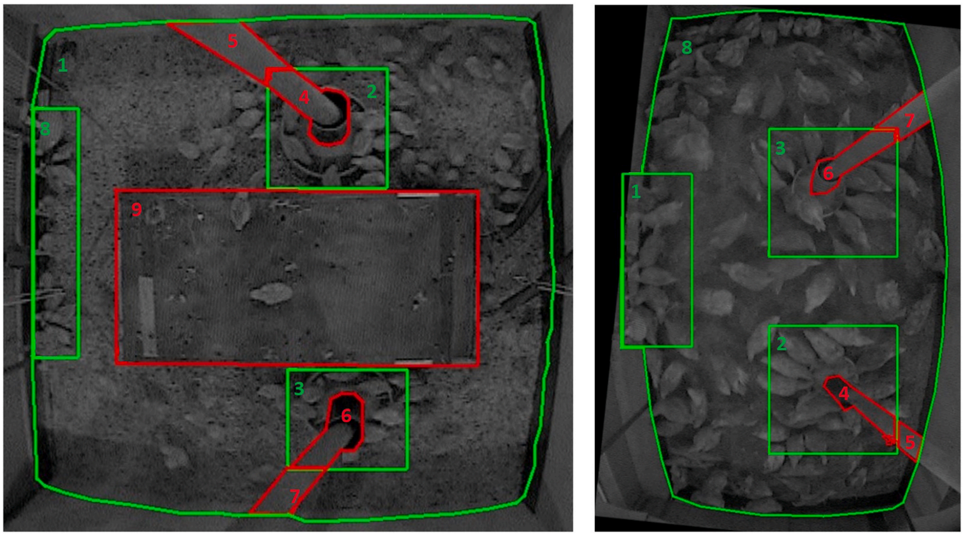

Fig. 1. Pen setup with the areas included in the analysis marked with green colour. Pullets were (a) 10 and (b) 60 days of age. The number of pullets at the resource areas for behavioural synchrony was identified in the drinker area (1) and two feeder areas (2 and 3). Pullet activity was measured on the whole floor area marked with 8 (including areas 1,2,3) but without areas 4,5,6,7,9 marked with red colour. Additionally, two elevated perches were provided in each pen at 18 days of age, but these were not visible in the videos. The marked area 9 indicates the dark brooder. In Control pens, a similarly large area as covered by the dark brooder (area 9) was removed from the activity analysis.

of the pullets. All brooders were permanently raised to 200 cm on day 41 and the heating turned off.

In Room B, no brooders were used, and the ambient temperature in the room was kept at the same temperature schedule as used for the temperature under the brooders positioned in Room A. The heat sources used for keeping the schedules for the ambient temperature in Rooms A and B were water heated vertical radiators mounted on the outer walls, which is the traditional way to heat poultry houses in Denmark. Air humidity was not manipulated.

2.3. *The four brooder treatments*

Pens in Room A were randomly assigned to one of four treatments that differed in the size of the brooder placed in each pen (Large or Small) and in the management of the height of the brooders (Raised or Fixed). Hence four size and management combinations were tested: Large-Raised, Large-Fixed, Small-Raised and Small-Fixed. The Small brooders measured 60 cm × 90 cm and corresponded to 54 cm^2 available area per pullet; the size was based on a recommendation of a company producing brooders. The Large brooders measured 60 cm × 120 cm corresponding to 72 cm^2 per pullet available area under the brooder, which is a 33% increase compared to the Small brooder. We primarily compared brooders of different sizes for practical reasons, as larger brooders are linked with higher costs, while still ensuring that all pullets could utilise the area beneath them simultaneously. The Raised brooders were automatically raised for 10 min every 4 hours during the photophase on Days 1–4 to simulate the behaviour of the mother hen and to compare the effect of stimulating activity by raising the brooder on the feed and water utilisation in the first days of life, as described in Riber and Guzmán (2016, 2017). Fixed brooders maintained at the same height during brooding.

2.4. *Pullet-group relocation*

All pullet-groups were relocated among the 22 available pens of the rooms on Day 44 (3 days after the brooders had been permanently raised). This was done to minimise potential undesired differences between rooms/pens influencing the behaviour. Relocation was performed in a balanced way so that one pullet-group from each of the four Brooder treatments and two pullet-groups from the Control treatment were housed in Room B and the remaining pullet-groups in Room A. The allocation of pullet-groups in pens within each room was semi-haphazard with the only limitation that a pullet-group could not be allocated to its original pen.

2.5. *Ethical note*

The study was conducted according to the guidelines of the Danish Animal Experiments Inspectorate with respect to animal experimentation and care of animals under study. This study was part of a larger project that also evaluated the effects of dark brooders on time budget and fearfulness of layers (Riber and Guzman, 2016), injurious pecking and productivity (Riber and Guzman, 2017) and on the brain and HPA-axis (Nordquist et al., 2020). On the days when video recordings for this study were taken, the animals received only daily care and were not subjected to any other manipulations.

2.6. *Data collection*

Each pen was continuously video recorded on days 10 and 60 of age from 06:00 in the morning to 18:00 in the late evening (i.e., during the photo phase). The field of view of cameras used to record video in the experiments covered the whole floor area in each pen (marked with 8 (green) in Fig. 1) and excluded the space that contained feed delivery tubes as well as area covered by dark brooders and was consistent across pens (red marked areas in Fig. 1: 4,5,6,7, and 9). Video recordings were made with a Monacor camera positioned in the middle of the pen, approximately 2.30 m above the ground so that the whole pen floor was visible with the exception of the area under the brooder, feeders and drinker (Fig. 1). Perches, which were raised above the ground, were also not visible. Video footage was recorded continuously for all pens using MSH Video Server software (S-VIDIA™, https://svidia.com/) via 2 computers (one computer per room) at a mean frame rate of 2.6 (SD = 0.8) fps in 768–576 pixels resolution. In total, 528 hours of videos were recorded and used for subsequent analysis.

2.7. *Data analysis*

2.7.1. *Activity levels*

Custom software was developed - “Pullet Monitor” (version

20220106, Leading Edge Research, Moggill Qld Australia), to analyse activity levels. Activity was measured as a pixel change between consecutive frames, which has been used elsewhere to assess pullet activity level (Fraess et al., 2016; Kristensen and Cornou, 2011; Rault et al., 2017) and has previously been validated through concurrent manual observations by an experienced observer during the photophase of the day (Rault et al., 2017). Pixel change between frames depends on the degree of data aggregation and is related to the size proportion of the area covered by the animal body/ies vs other structures, which amongst other factors depends on the stocking density. The studies differ in the degree of data aggregation and stocking density; thus we are unable to compare the activity levels in our pullets with those in other studies.

The activity level of the pullets was estimated per frame of video over the whole 12-hour video recording period at both ages and assessed with an activity index previously described by Rault et al. (2017), Bloemen et al. (1997), and Oczak et al. (2014). In the first step, grey values of pixels in recorded images, which were stored in an 8-bit format (0−255), were subtracted from grey values of pixels in the previous image of the video. If the difference of the pixel's grey value between consecutive images was higher than a threshold, then it was classified as a pixel with moving pullets. The threshold was empirically determined to be 35 by random sampling with human verification. This threshold provided the highest signal to noise ratio between actual movement and error sources such as lighting changes or image defects. In the second step, the activity index was calculated using the following formula (1):

$$Activity\ index = \frac{number\ of\ pixels\ with\ moving\ animals}{number\ of\ pixels\ of\ pixels\ in\ the\ floor\ zon\ of\ the\ pen} \times \frac{100}{duration\ between\ frames}[\%] \tag{1}$$

The output was quantified as the number of pixels that indicated animal movement in each frame of the recorded videos in relation to the total number of pixels in the image.

2.8. *Behavioural synchrony*

The software program Pullet Monitor was used to designate resource zones in each pen, i.e., drinker area, feeder area 1 and feeder area 2. The size of each resource area was decided based on the size of the pullets included in the experiments: a whole pullet length perpendicular to the drinker (row of nipples) and diagonally from the feeder had to fit in the drinker and feeder area, respectively. The sizes of the drinker area were 45 × 250 pixels and 100 × 250 pixels and of each feeder area were 120 × 120 pixels and 185 × 185 pixels at 10 and 60 days of age, respectively. To assess the synchrony of pullets in a resource area, pullets were detected with an object detection model You Only Look Once X (YOLOX) (Ge et al., 2021) and their number in each resource area was summarised for the three previously defined resource areas for each frame of the recorded videos. To train the YOLOX-extra large model for bird detection it was necessary to select images from the recorded video that could be later labelled by a human labeller. The term extra large indicates the size of the used model, which has 99.1 million parameters and is the largest of the YOLOX models. The k-means algorithm was used to select 481 images with the least correlation out of the total number of 4942,080 images recorded in the experiment as described in Pereira et al. (2018) and Oczak et al. (2023). These images were then randomly split into training and validation sets with 337 (70%) and 144 (30%) images respectively.

YOLOX-extra large was trained with parameters set in MMDetection (version 2.25.2), i.e., optimiser stochastic gradient descent (SGD), with learning rate 0.01 and momentum 0.9 (Chen et al., 2019). SGD is an iterative method for optimising an objective function with suitable smoothness properties. Especially in high-dimensional optimisation problems SDG reduces the very high computational burden, achieving faster iterations in exchange for a lower convergence rate. Learning rate is a tuning parameter that determines the step size at each iteration while moving toward a minimum of a loss function. Since it influences to what extent newly acquired information overrides old information, it metaphorically represents the speed at which a machine learning model "learns". Momentum is a method of smoothing out model parameter updates and allowing the optimiser to continue advancing in the same direction as previously, minimising oscillations and increasing convergence speed.

Images were augmented with standard augmentation techniques implemented in MMDetection i.e., mosaic, random affine, mixup, random horizontal flip, and colour jitter. Image augmentation is the process of generating new transformed versions of images from the given image dataset to increase its diversity. No changes were made to the architecture of YOLOX methods, optimizer, or augmentations provided in MMDetection. Python version 3.8 was used together with MMDetection for training, validation, and visualisation of results of application of the YOLOX object detection model.Fig. 2

YOLOX-extra large was trained for 1000 epochs and reached the best performance on the validation set at 515 epochs with 52.2 AP (Fig. 3a). Number of epochs represented the number of times the algorithm passed through the whole dataset in the training process, while AP stands for mean Average Precision, which is a metric of performance of object detection models. The best performing model was used to detect birds in resource zones on recorded videos at 60 days of age. Subsequently birds were counted automatically as inside a resource zone if 2/3 of the rectangle indicating the bird's body was inside a resource zone (Fig. 3b).

To validate the model for the purpose of counting birds in the resource areas, we compared the number of automatically detected pullets in each resource area to the results of counting by a human. For the validation purpose, we randomly selected 100 images from videos of 60 days old pullets and 100 images from videos of 10 days old pullets. The images were evenly distributed across the time of the day and type of treatments. Pullets were counted on 200 images in total in all resource areas. A pullet was counted as inside a resource area if 2/3 of the pullet's body was inside a resource area. From 100 images in each age group, 70 were randomly selected to optimise the confidence threshold of the trained YOLOX-extra large model in each resource area. This was done as object detection model returned various confidence thresholds for each detected pullet, i.e., a score from 0 to 1, where 1 indicated the absolute certainty of the model that the detected object is a pullet. We optimised the confidence threshold and counted only those objects for which the algorithm achieved confidence above the threshold level. The reaming 30 images out of 100 were used to test the model performance in counting pullets in one final test. The Mean Absolute Percentage Error (MAPE) between counts of the human and model's detection on 30 test images for the drinker area were 10.5%, 16.7% for feeder area 1 and 20.5% for feeder area 2 in 60 days age group and 12.8% for the drinker area, 17.2% for the feeder area 1 and 12.8% for feeder area 2 in the 10 days age group. Finally, we calculated the MAPE between the counts of the human and the automated method on 30 test images for all 3 resource areas grouped together. MAPE for 60 days age group was 11.1% and for the 10 days age group it was 10.3%.

2.9. *Statistical analysis*

Frames where no pixel changes were observed (compared to the respective previous frames) and the single frames right before and right after these frames were removed. Frames with 0 frame rate (compared to the respective previous frames), the single frames right before and right after these frames and frames with a frame rate larger than 1 second were also removed. In total, 404 frames were removed. 5126,106 frames remained for the statistical analysis of the activity levels and behavioural synchrony. All analyses were performed in R (version 4.1.2) (R Core Team., 2021) on data that were aggregated at 5-minute intervals per pen and age, using the mean for the pixel fraction, frame rate and the

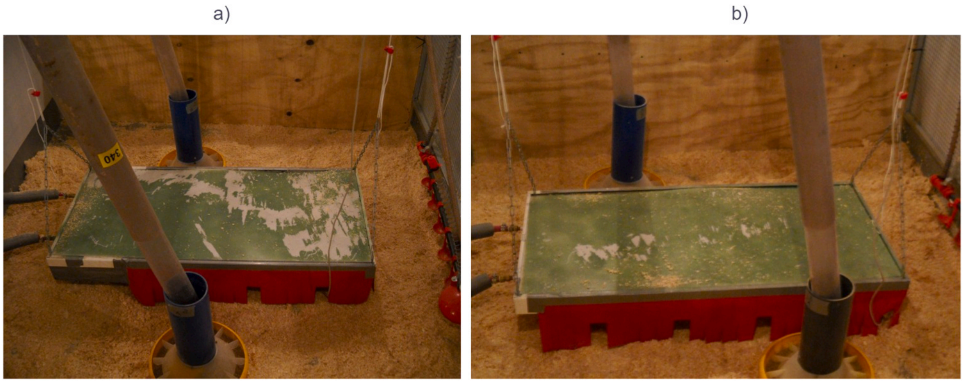

Fig. 2. Large (a) and Small (b) dark brooders in the down position. In half of the pens, the brooder (Small or Large) stayed down (Fixed brooder) and in the remaining pens the brooder was raised above ground every four hours in the first four days of life (Raised brooder). Large (a) and Small (b) brooders required the same floor area in the pen, but part of the Small brooder was made inaccessible to the chicks by a coated polystyrene block (leftmost side on the brooder in Fig. 2b).

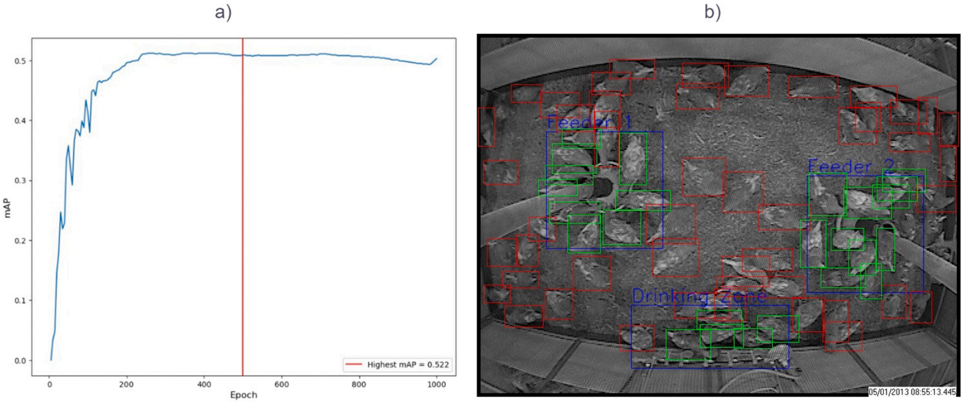

Fig. 3. Results of validation of YOLOX-extra large model a) AP on 1000 epochs. Highest AP was 52.2, AP_{50} was 93.3 and AP_{75} was 52.4. b) Detection of birds within resource zones i.e., green bounding boxes indicate that birds were counted as within a zone and red that the birds were counted as outside of a resource zone.

time of the day, and the sum for the number of pullets around resources and the total number of pullets. The average time of the day per time interval, measured in seconds, was transformed into radians (2):

$$radians = \frac{2 \times \pi \times average\ time\ of\ the\ day\ per\ time\ interval\ in\ seconds}{86400(seconds\ inaday)} \tag{2}$$

The sine and cosine of the time radians were fitted as predictors in the models below to evaluate periodic effects of the time of the day. Finally, the last 30 minutes of the 12-hour (photophase) window showed a biased behaviour of the pullets and were excluded from further analysis.

2.10. Activity levels

Effects on pullet movement were evaluated via a beta regression mixed model with the logit link function [package *glmmTMB*, version 1.1.7, function *glmmTMB*, options 'family = beta_family(link = "logit"), control = glmmTMBControl(optimizer = optim, optArgs = list(method = "BFGS"))'] (Brooks et al., 2017). The average pixel fraction per time interval was fitted as the response. No transformation was performed on the response as there were no 0% or 100% measurements. The average frame rate per time interval, the interaction between treatment (4 Brooder treatments and Control treatment) and age (10 and 60 days of age) and the interactions between age and each one of the sine and cosine of the time radians were fitted as fixed effects. The room ID was not used as a predictor due to multicollinearity with the treatment variable. The combination between pen ID and age, and the pullet-group ID were fitted as random intercepts to account for the covariance structure in the data. The frame rate and the sine and cosine of the time radians were fitted as uncorrelated random slopes within both random intercepts. A dummy coded (0/1) and centred variable for the age was fitted as an uncorrelated random slope within the group ID.

Assumptions about the residuals were evaluated via a simulation-based approach (package *DHARMa*, version 0.4.6, function simulateResiduals, option 'n = 1000') (Hartig, 2022). Multicollinearity was evaluated via variance-inflation factors (package *car*, version 3.1–1, function *vif*) (Fox and Weisberg, 2018). Using custom scripts provided by Dr. Roger Mundry, Leibniz ScienceCampus, Primate Cognition, we evaluated overdispersion and model stability (visual evaluation for the latter via a leave-one-out strategy for the levels of the random intercepts). No obvious violations were observed.

2.11. Behavioural synchrony

The data set was treated as compositional data by assigning the total number of pullets from each pen, age and time interval to three activities: "drinking", "feeding" (both feeder areas combined) or "other". To evaluate effects on the behaviours of interest (drinking and feeding), the frequencies were transformed using an additive log-ratio transformation (package *compositions*, version 2.0–6, function *alr*) (van den Boogaart et al., 2023) to deal with the constrained sample space (the simplex) and project the data into a multivariate real space (Aitchison, 1986). The 'other' group was used as the base for the additive log-ratio transformation.

Effects on the additive log-ratio transformed counts for the feeding and drinking behaviours were evaluated via linear mixed models [package *lme4*, version 1.1–32, function *lmer*, options 'REML = false, control = lmerControl(optimizer = "bobyqa", optCtrl = list(maxfun = 10000))'] (Bates et al., 2015). The aforementioned variables were treated as the response in each model. The models' structure (fixed/random effects/slopes) is similar to the model described in Section 3.7.1 (Activity levels) – the frame rate was not used here as a fixed effect. Assumptions about the residuals, random intercept and random slopes were visually evaluated. Multicollinearity and model stability were evaluated as described above. No obvious violations were observed.

2.12. *Final statistical inference*

For the three models described above, multiple pairwise comparisons between treatments at each age (package *emmeans*, version 1.8.5, function *emmeans*, options 'pairwise ~ treatment|age, adjust = "none", type = "response"') (Lenth et al., 2023) were collectively accounted for via the false discovery rate (FDR) method (function *p.adjust)* (Benjamini and Hochberg, 1995). The same approach was used for the comparison between the two ages for each treatment. The tests for sine and cosine effects of the time radians at each age (package *emmeans*, functions *emtrends* and *test*) were also collectively accounted for via the FDR method. Significance was declared at a 5% FDR cut-off.

To evaluate differences between the two rooms, we re-ran the models and analysis pipeline described above using the interaction between room (instead of treatment) and age as fixed effect. No significant effect of room was identified at either age.

2.13. *Correlation tests*

Correlation tests between the response variables (log10-transformed pixel change and additive log-ratio transformed counts for the feeding and drinking behaviours) within combinations of pen ID and age were evaluated via repeated measures correlation (package *rmcorr*, version 0.6.0, function *rmcorr*) (Bakdash and Marusich, 2023) and accounted for via the FDR method.

3. Results

3.1. *Activity levels*

Pullets in the Control treatment exhibited higher activity than pullets from most of the Brooder treatments at both ages ($FDR < 0.01$). The exceptions were the Large-Fixed Brooder treatment at 10 days of age and the Small-Raised Brooder treatment at 60 days of age (Fig. 4, Table S1). Our analysis also showed that the pullets were more active in the morning (starting from 06:00) and evening (up to 18:00) than at midday at 60 days of age (cosine effect of time radians = 0.47, $FDR < 0.0001$, minor sine effect at both ages, Table S2, Figure S1). Furthermore, older pullets were more active than younger pullets in all treatments ($FDR < 0.02$, Table S3, Figure S2).

3.2. *Behavioural synchrony*

Behavioural synchrony at the resources for each pen, age and time interval was low as no more than 57.14% of pullets were simultaneously present in the drinker (μ ± SD: 4.92% ± 2.75%, max: 25.00%) and feeder areas (both feeder areas combined: μ ± SD: 10.39% ± 3.54%, max: 44.90%) at 10 days and 42.27% of pullets were simultaneously present in the drinker (μ ± SD: 5.70 ± 2.01%, max: 18.56%) and feeder areas (μ ± SD: 13.67% ± 3.72%, max: 35.23%) at 60 days of age. No treatment effect was detected ($FDR > 0.4$, Table S4 and S5). A greater number of pullets was observed in the resource areas in the morning (starting from 06:00) and evening (up to 18:00) than at midday at 60 days of age (cosine effect of time radians = 0.62 and 0.37, FDR = 9.85e-16 and 4.03e-15 for drinker and feeder areas, respectively, Tables S6 and S7, Figures S3 and S4). Furthermore, pullets in the Control treatment were using the resources more at age 60 than at age 10 days (contrast = − 0.35 and − 0.54, FDR = 0.033 and <0.0001 for drinker and feeder areas, respectively). A similar age difference was also observed for the feeder areas in the Small Brooder treatment (contrast = − 0.28 and − 0.34, FDR = 0.036 and 0.013, for Small-Raised and Small-Fixed, respectively, Tables S8 and S9, Figures S5 and S6).

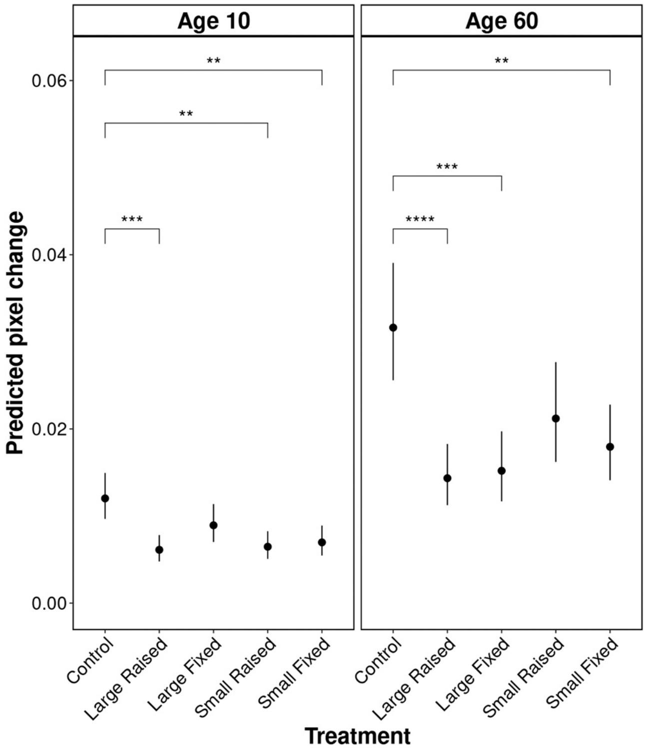

Fig. 4. Treatment differences in regard to activity levels at 10 and 60 days of age. Activity levels were evaluated via the detection of pixel changes between consecutive frames in 12-hour videos, aggregated at 5-minute intervals. Estimated marginal means and their 95% confidence intervals, inferred by the beta regression mixed model, were back-transformed from the logit scale and are presented as predicted pixel changes (response). Pairwise comparisons between ages for each treatment were accounted for via the FDR method (collectively with other similar tests – see section Final statistical inference). Only significant results at an FDR cut off of 5% are presented (**: $FDR \leq 0.01$, ***: $FDR \leq 0.001$, ****: $FDR \leq 0.0001$).

3.3. *Correlations*

We observed weak correlations between pullet activity level and number of pullets in the drinker (r = 0.28, FDR <0.0001) and feeder areas (r = 0.28 FDR <0.0001). The number of pullets in the drinker area was correlated moderately with the number of pullets in the feeder area (r = 0.39, FDR <0.0001).

4. Discussion

This study used automatic behaviour detection to analyse activity levels and behavioural synchrony at resources in layer pullets reared either with dark brooders or without (Control) at 10 (brooders present) and 60 days of age (brooders removed). Previous studies (Forslind et al., 2022; Riber et al., 2007), including the study from which we obtained the video material for our analysis (Riber and Guzman, 2016), identified short-term (i.e., until the brooders were permanently raised) effects of rearing with brooders on the activity and behavioural synchrony using the direct behaviour observation method within short observation periods. The current study used automatic detection of activity and pullet location to remove the influence of the human observer and to obtain a comprehensive understanding of the pullet behaviour throughout the day. In line with our hypothesis, we observed greater activity levels in Control pullets compared to pullets in the three Brooder treatments, with the exception of Large-Fixed Brooder pullets at 10 days and Small-Raised Brooder pullets at 60 days of age, which did not differ from Control pullets. Contrary to our hypothesis, we did not find a treatment effect on behavioural synchrony identified with the number of pullets present in the drinker and feeder areas at either age.

4.1. Activity level

The treatment-related activity level variations were likely not due to feeder and drinker use, as indicated by the lack of a treatment effect on behavioural synchronisation at the resources and a weak correlation between activity level and pullet numbers at the resources. Our study lacks specification regarding the type of behaviour exhibited by pullets or its impact on pixel change, as observed in other species (Hesse et al., 2010). To address this gap, we propose future research that explores pixel change in relation to different behaviours in chickens. This is particularly relevant, considering that behaviour type correlates with the floor area covered by chicken bodies (Bokkers et al., 2011), with a potential increase in pixel change corresponding to a greater area covered by the body during specific behaviours.

Prior investigations into this cohort of animals in adulthood, highlighted a tendency towards elevated mean counts of arginine vasotocin-positive neurons in the hypothalamus when reared with brooders (Nordquist et al., 2020). Increased levels of arginine vasotocin result in a diminished locomotor activity in birds (Nephew et al., 2005; Oliveira, 2022). Thus, behaviour differences between Brooder and Control pullets may at least partly originate from brain changes. Further research investigating brain development in chickens reared with brooders is needed to reveal the mechanisms responsible for behavioural differences in Brooder and Control pullets.

The reduced activity level in the Brooder pullets is potentially attributed to enhanced resting behaviour. Notably, broiler chickens reared with brooders, even when these were permanently removed at a later age, experienced less disturbance during rest (Forslind et al., 2022). Prior behavioural analysis of the pullets used in our study revealed a higher proportion of resting behaviour in Brooder pullets compared to Control pullets, though an age and brooder type interaction on resting behaviour was observed (Riber and Guzman, 2016). These findings suggest that brooders may reduce overall activity by improving rest in pullets and may explain the reduced activity levels observed when using the automatic method for assessment of pullets raised with Small brooders at 10 days of age. Yet, pullets in the Large-Raised Brooder treatment also exhibited lower activity than non-Brooder pullets when estimated by the PLF techniques, whereas the proportion of resting pullets in this treatment was only non-significantly higher at 9 days of age when using the direct observations (Riber and Guzman, 2016). Due to the lack of behavioural scoring, it is unclear whether resting behaviour accounted for the treatment difference in activity levels at 60 days of age.

Apart from the brooders, all pens were managed similarly regarding light conditions, resource allocation, and space. However, space utilisation likely varied between brooder and non-brooder treatments, as the area under the brooder is dark and typically utilised for resting (Forslind et al., 2022). Nevertheless, the brooders covered less than 50% of the floor area, allowing the pullets sufficient space to engage in natural behaviours. Additionally, to facilitate comparison, we excluded an area of the same size and location from the activity analysis of Control pullets. It is worth noting a difference in environmental temperature and relative humidity levels between Brooder and non-Brooder pullets, which may have influenced the observed variations in activity levels, particularly considering young chickens' reliance on external temperature sources for homoeostasis. While investigating these underlying mechanisms of behavioural change is intriguing, our aim was to simulate rearing conditions feasible for commercial farm use. (e.g., Gilani et al. (2012)). Furthermore, at 10 days of age, treatment and room were confounded, despite efforts to minimise differences between the rooms (e.g., using the same lighting source, pen size, equipment, insulation, etc.). However, we did not observe a systematic difference between non-Brooder pullets and the pullets in all Brooder treatments, suggesting that the room likely had minimal impact on pullet behaviour. In addition, we statistically evaluated differences between the two rooms and did not identify a significant effect of the room at either age.

There was no difference in the body weight of the pullets across various treatments at 7 days of age as described in Riber and Guzmán (2017). Thus, it is unlikely that dark brooders influenced the physical fitness of the pullets in a manner that could have impacted their activity levels at a young age. Information concerning the body weight of the pullets at 60 days of age is lacking. Pullets were more active at 60 days of age compared to 10 days. Given the absence of activity analysis under the brooders and on the perches, an underestimation of pullet activity at both ages may potentially exist, when brooders and perches were deployed, respectively. Previous research indicates that chickens predominantly utilise brooders (Forslind et al., 2022) and perches (Skånberg et al., 2021) for resting and engage in active behaviours on the ground outside the brooders. Consequently, the impact of this study's limitation on estimating activity levels is anticipated to be marginal.

Feed and water resource use is an unlikely explanation for the age difference in the activity levels as only Control treatment led to an increase in the number of pullets at the resources as the pullets aged. Moreover, 60-day-old pullets spent less time feeding compared to 10-day-old individuals and drinking was not age related, further supporting our suspicion that increased activity in older pullets was likely a consequence of behaviours other than feeding and drinking, such as litter directed behaviour and preening, which increase with age (Savory, 1980). Another explanation is the increasing size of the pullet bodies as they age, as the larger bodies of pullets at 60 days of age covered a greater floor area than at 10 days of age. It can be assumed that the proportion of floor area covered with an animal body influences the amount of pixel change resulting from the movement.

4.2. Behavioural synchrony

The lack of treatment effect on the number of pullets simultaneously in the drinker and feeder areas may have been linked to the number of drinking nipples and feeder space allocated (Sirovnik et al., 2018, 2021). The maximum proportion of pullets simultaneously in the feeder area was smaller than in previous studies, where up to 80% of adult laying hens were simultaneously in the feeder area (Eklund and Jensen, 2011; Sirovnik et al., 2021). A similarly low proportion of laying hens simultaneously in the feeder area was only seen when the feeder space was scarce in the study by Sirovnik et al. (2021). It is thus possible that in our study the resource allocation has limited the number of pullets having access to the resources simultaneously and so adversely affected the study outcome, although the allocation of feeder space was larger than what is recommended in most management guides and standards (2.5 cm vs. 2.0 cm feeder space per pullet, e.g., Lohmann 2023 and RSPCA 2018). The drinking nipple allocation was lower than in most management guides and standards (14.3 pullets vs. 12.5 pullets per drinking nipple (e.g., Lohmann, 2023; RSPCA, 2018)), but to our knowledge, no previous study measured behavioural synchrony at the drinker making any comparisons impossible.

Chickens are known for a well-established drinking-to-eating ratio (Williams, 1996), thus a correlation between the number of hens in the feeding and drinker areas was expected. The moderate correlation indicates that pullets were likely not only performing feeding and drinking behaviour in the resource areas, but also other behaviours. The observed weak correlation between pixel change (activity levels) in the whole pen floor area and the number of pullets in the resource areas indicates that the behaviours performed might have differed between the resource areas and other parts of the pen so that the performance of behaviour resulting in greater pixel change could differ between areas in the pen. The pens in our study were relatively barren and provided pullets only with feeders, drinking nipples and two perches. However, pullets prefer a structured housing environment including visual barriers (Ross et al., 2020). While drinkers did not offer any visual barrier, feeders may have acted as visual barriers to avoid potential injurious pecking. If this was the case, a greater number of the Control pullets was expected in the feeder area compared to the Brooder pullets due to the problem with injurious pecking occurring in the Control pullets, as previously described in Riber and Guzman (2017). As this was not the case, we can assume that the feeder areas were not used for avoidance of pecking, but likely for other behaviours such as exploration, foraging or dust bathing.

The Control treatment led to an increase in the number of pullets at the resources as the pullets aged, but behavioural synchrony was not affected by age in any of the Brooder treatments. Our results for the Control pullets are in line with those of Kozak et al. (2016), who showed that activity, including feeding and drinking, increased with age in pullets reared without brooders. However, there are inconsistencies in the effect of age on feeding and drinking behaviour as one study did not find an age effect on behavioural synchrony in these behaviours (Keeling et al., 2017). The differences between studies of age effects on behavioural synchrony are possibly also attributable to the different behaviours investigated. Keeling et al. (2017) studied specific behaviours (i.e., feeding and drinking behaviour), Kozak et al., (2016) grouped various behaviours for the analysis, and in our study, we only have knowledge of the occupancy in the resource areas and no information on the types of behaviour performed.

In line with previous research, we found that pullets at 60 days of age are more active and show a greater behavioural synchrony in the resource areas in the morning and evening than at midday (Eklund and Jensen, 2011; Riber et al., 2007; Savory, 1980). However, feeding activity and resource use in chickens depend on the light regime (Weaver and Siegel, 1968) and light intensity (Alvino et al., 2009) and can follow other diurnal patterns than the one observed in our study (Alvino et al., 2009; Savory, 1980). Furthermore, to our knowledge, the ontogeny of the diurnal behaviour pattern in chickens is not yet known, impeding the interpretation of the age effect on diurnal patterns in behavioural synchrony.

5. Conclusion

We confirmed our hypothesis that pullets subjected to Brooder treatment exhibit decreased activity levels compared to their Control treatment counterparts both before and after the permanent removal of dark brooders. The observed reduction in activity levels likely corresponds to increased resting behaviour, although the underlying mechanism remains unstudied. The variation in the effectiveness of different Brooder treatments in lowering pullet activity levels lacks a clear explanation. Contrary to our expectations, we found no treatment effect on behavioural synchrony in drinker and feeder areas. However, the low proportion of pullets simultaneously present in resource areas raises questions about resource accessibility. Given the unidentified nature of behaviours in these areas, it is plausible that pullets may engage in activities beyond their intended purpose. We highlight the necessity of exploring pixel change in relation to various behaviour types to enhance the interpretation of activity based on this metric. Furthermore, we underscore the importance of continued development of automated methods for chicken behaviour detection.

CRediT authorship contribution statement

Albert Martin-Cirera: Writing – review & editing, Visualization, Data curation. **Manolis Lyrakis:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Anja B. Riber:** Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Maciej Oczak:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Mathias Gosch:** Writing – review & editing, Validation, Formal analysis. **Mark Dunn:** Writing – review & editing, Methodology, Formal analysis. **Janja Sirovnik:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Conceptualization.

Author contribution statement

Albert Martin-Cirera: data preparation, manuscript revision, figure preparation. Manolis Lyrakis: statistical analysis, manuscript preparation and revision. Anja B. Riber: study design, data collection, manuscript preparation and revision. Maciej Oczak: study design, algorithm development, manuscript preparation and revision. Mathias Gosch: algorithm development, manuscript revision. Mark Dunn: algorithm development, manuscript revision. Janja Sirovnik: study design, manuscript preparation and revision

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.

Data Availability

None of the data was deposited in an official repository. Information can be made available from the authors upon request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2024.106283.

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