

Revision Validation

Reply to Arjen van Putten

In general this is a well written and clear paper. My respect for the scale at which this research was done. The tables and figures are clear, as well as the nice pictures. I need some clarification on the method, but aside from that it looks fine.

Thank you. We are happy to clarify.

The scale of the used data with 3 hens and 10 hours of tracking does not reflect the variation in hen behaviour which I expect is available from all other hens. A comparison could have been made between the behaviour of these 3 hens and the movement of all others, but I expect this will be mentioned in another paper.

The validation was done before the start of the proper experiment so we did not analyze the tracking data from other hens for this period. However, density of hens and equipment of the pen was the same as in the following studies with one exception: We later added a second row of antennas on the litter because this validation revealed lower reliability in this area compared with the other areas of the aviary. We added this to the revised version of the manuscript in the discussion.

Frequency of recording rfid is not mentioned but could be found later in the analyses section.

Added.

My main concern is that it's not completely clear to me what has been marked on the video. Did you draw a bounding box and calculate when the box was on average within the area marked above an antenna? This is not clear to me from the text. The orientation and distance of the tags relative to the antennas is of importance for registering the presence of a tag. The fish-eye effect of the camera which I clearly observe in figure q requires some form of calibration and warped image before being able to connect the presence of a hen in one area. Also, did you take into account where the tag is placed on the hen? Currently I am unable to repeat your method due to this missing information.

Yes, the camera had a very wide angle but it could be clearly seen when a hen was walking, standing, or sitting on top of an antenna. However, the exact positions of antennae on the litter were less obvious because they were covered by litter. This could have added to the lower sensitivities of detection on these antennas. Hens in flight above an antenna were not considered and it is very unlikely that the antenna could register the tag in this instance. The reason for that is that hens rarely fly above the antennae because they prefer ramps when they change tiers and the speed of a flying hen would be too high to be picked up by the antenna. As is explained in the methods, the antennae register tags every 0.1 s and the likelihood to have a bird in flight in reading distance at this moment is slim. The tag mounted on the bird was about 1 cm above the ground and the vertical reading distance is up to 15 cm. The horizontal reading distance is about 0 cm. The only complication that arose was that birds sitting on 2 adjacent antennae were sequentially picked up by both (flickering). This information was added in the methods (Barn-setup and RFID system).

Are all antennas tuned exactly the same in the aviary? For instance, what is the minimum distance for a tag to be picked up?

Added.

3a and b could be combined in 1 table, but leaving it like this is fine.

We decided to keep the 2 parts of the table separate for a simpler layout.

Synchronization errors are mentioned between the camera and rfid system. Please mention the video quality and frequency as well as that of the RFID system. If video is collected at a regular 20 fps and the RFID records at 10 Hz, it should be possible when all systems are connected to the same network. Of course having a stable network on farm is impossible and I understand the difference between them.

The frame rate was 30 fps but the problem was that both systems were not connected to the same network. We will try to do that for the future. We added this information in the methods and discussion sections.

Reply to Mona Giersberg

In the present study, the authors validate an RFID system for location tracking of laying hens housed in a semi-commercial environment by means of video analysis. In general, this manuscript is a concise report which highlights the importance of thorough validation when using new technologies in animal behaviour research. As we frequently see a lack of validation of technologies applied both in research and on-farm, this subject cannot be emphasized enough.

Thank you.

... I would suggest to use the terms "semi-commercial (aviary) system/semi-commercial conditions" or "quasi-commercial (aviary) system" when referring to the present set-up.

We agree and changed the title to "Validation of a Radio frequency identification system for tracking location of laying hens in a quasi-commercial aviary system".

L. 53: Not clear what is meant by "invisible". Are the hens occluded/covered/hidden by conspecifics and/or equipment of the aviary?

Yes, there are positions in the middle of the aviary or underneath the aviary where birds cannot be seen on video or by eye. We added this.

L. 58: For which aims or in which situations is it important to track all individuals of a group? For commercial applications I would suppose that knowing whether "the flock" uses a certain functional area would be sufficient. What would be the consequences in such a setting if single individuals would or would not use that area? Tracking of all individual of a group may be of particular interest in research contexts.

Animal welfare is a concept for the individual. We know that individual hens transition through the aviary in an individual manner (Rufener et al., 2018). When some individuals do not use the litter

area this is welfare relevant because access to litter is a basic need for chickens. The sample size depends on the study purpose. For genetic studies, sample sizes should be > 1000 individuals. To clarify, we now added that this is important for genetic studies.

LI. 58-59: How is it possible to validate automated tracking devices with video observations if tracking hens visually is limited (as stated in l. 53)? It is accounted for this limitation in the discussion section. However, at this point it reads as a contradiction.

Agreed, we added that the validation was done for the events when birds were simultaneously tracked and visible on video.

More detail is needed on the RFID system (e.g. were the antennae coated (plastic box) or used as bare wire? With which kind of device were the antennae connected?). Alternatively, a reference to a previous (open access) paper in which the same system is described in more detail could be provided. With the current information given, it is not possible to replicate the study. The same is true for the description of the aviary system. It is stated that 26 antennae (l. 69) were placed in the aviary. In Fig. 1, 12 of these antennae are visible (2 are not visible because they are near the pop holes). If the other side of the aviary would look the same, there should be 28 antennae, or is the veranda only accessible from one side of the barn? Adding a schematic figure of one whole pen of the system may be useful.

We added the requested information and also included both sides of the aviary in Fig 1.

LI. 73: Not clear why birds in 5 pens were fitted with RFID tags. In this study, only two connected pens are used. It should be stated if this validation study was part of a larger experiment (preferably with a reference to a pre-print or paper, if available).

This was a test trial for future, larger experiments. Therefore, we used 5 pens and outfitted all 1125 birds with RFID tags. At the moment, we do not have manuscripts for the follow-up experiments, yet. We added this information.

L. 78: Could the hens move freely between the two connected pens?

Yes, we added this information.

L. 78: Where those back-packs custom made or commercially available? This information would be very helpful for other researchers, as it is often difficult to design suitable backpacks. There is a photo of the leg bands; it would be useful to include one of the back-packs as well.

The backpacks were custom made and we added a picture of it as Fig. 3.

L. 82: How were the times of the video recording system and the RFID system synchronized? Did they use the same time source (e.g. radio clock, web clock)? It is accounted in the discussion section for the limitation of exact synchronization. However, it would be useful to describe the method of initial time synchronization between the two systems.

Unfortunately, the video recording system and the RFID system were not connected to the same network. The systems were manually synchronized at one time but diverged with time. In a future effort, this will be corrected. This information has been added.

L. 104: Is it correct that for initial data processing, R was used and for running the statistical tests/models SAS was used? Why was it chosen to use both programmes?

Yes, initial data processing was done in R and SAS was used for a summary of the frequencies. The reason was that different people worked on the project with different preferences for software.

L. 107: Table header: sentence structure does not seem correct.

Thank you, yes, the word 'from' was added.

L. 110: Table 2: I wonder whether “test” is the right word here. Which calculations were made to create the variables?

We modified the heading of Table 2.

Table 2: “Side correction needed”: How was this done precisely? A second person checked all data from the video observations for plausibility?

Yes, we added this to the text.

Results

L. 117: ...was detected within 1 min. By the RFID system?

Yes, added.

L. 130: “...including the litter...”

Added.

L. 134: And other occasions: please be consistent using either “1 min” or “one min”.

Corrected.

Table 3 a: It is interesting that the sensitivity of the observer is quite low. Would you expect this to be the case with other observers as well (e.g. because of occlusions, low video-quality)?

This really depends on the circumstances and cannot be generalized, I think. I do not think that the quality of the videos was the problem because only instances with clear sightings were considered. It is important to note that observers make mistakes. This fact can be generalized but the extent is influenced by many factors. We added a reference for this topic.

Table 3 b: last row seems to be incorrect (same results as in a, but n = only 158).

You are right, we corrected it.

Discussion

L. 150: “Non-RFID systems” is quite broad. At least one example should be given, e.g. what was the sensitivity when Montalcini et al. 2022 validated their low-frequency tracking system with active tags in the same barn?

Added.

L. 156: Full stop missing after reference.

Corrected.

L. 158-159: Very useful practical recommendation.

Thank you.

L. 164: In which contexts/cases is this level of accuracy necessary?

This would very much depend on the research question. E.g. it might be interesting if a (sick) hen moves at all or if always the same nest location is preferred. When constructing social networks this might also be of interest. We added that the research question determines the required level of accuracy.

L. 176: Not clear what is meant by "...the system's physical and spatial configuration."

We modified the sentence.

L. 180: Are there any results available on this improved design or is the research still ongoing?

We are planning a new validation with this improved design in a couple of weeks.

L.193: Is the cause only not obvious or can it also not be explained?

We replaced not obvious by unknown.

L. 196: Is this due to the level of experience etc. of the observer or is it due to the set up (e.g. low video quality, frequent occlusions)?

We included more information and gave a reference.

L. 199: Not clear where the 96% come from. The sentence is also a bit misleading as it suggests that tracking in the litter had a sensitivity of 96%. The concluding statement of the abstract is preferable.

We modified the sentence and dropped the number of 96%.