

Dear Dr Gondret,

Please, find below the review I made of the preprint entitled "On-farm hatching and contact with adult hen post hatch induce sex-dependent effects on performance and welfare in broiler chickens" (<https://doi.org/10.1101/2023.05.17.541117>).

Sincerely,
Nicolas Bédère

Review:

My understanding of the article:

With the development of antibiotics resistance in some bacteria populations, it has been discouraged, if not forbidden, to use antibiotics as growth enhancers in livestock. The chicken sector is looking for preventive actions to promote robustness and performance of the birds without antibiotics supply. In addition, chicks are usually hatched in hatcheries and moved to farms thereafter. This has been documented to be stressful for them and to induce long-lasting metabolic change, affecting their performance. There is a lack of knowledge about alternative hatchery systems, that could also promote gut health and thus animals' performance and robustness.

L. A. Guilloteau and her collaborators have investigated the effect of alternative farming practices, consisting in on-farm hatching and contact with an adult bird, on the performance of the chicks.

To do so, they conducted an experiment involving 700 fertilized eggs or day-old chicks distributed among 5 conditions : hatchery hatching, hatchery hatching with antibiotics, hatchery hatching with an adult hen, on-farm hatching, and on-farm hatching with an adult hen. There were about 18 individuals per treatment, each condition was repeated 8 times summing up to about 700 individuals. On day 27 (approximately half-way in the rearing period) the birds were challenged with a stress: they were transported in a box to a new room, with a lower temperature, and experience a 4h food deprivation. When they came back to their original pen, there were vaccinated against the Gumboro disease and the available space was twice as small as it was originally.

Body Weight was recorded for each chick at day 1, 19, 34, and 55. About 25 chicks for 3 out of the 5 experimental conditions were scored for quality. Dead birds were examined to identify the cause of death. Feed intake and gut parasite infestation were recorded for each pen. Parasite load and behavior (qualifying the global activity as well as interaction with the chicks) was recorded for each hen.

Diverse statistical analyses were performed according to the dependent variable.

The authors reported that despite a faster growth for the on-farm chicks compare to the hatchery ones at the beginning of the experiment, the body weight was similar in the second half of the experiment. Both the presence of a hen and antibiotics treatment impaired growth. Feed intake was a little lower in the presence of an adult hen and no difference was found in terms of parasite load. The distribution of behaviors of the hens were similar with chicks from both hatching conditions, as well as the proximity between the chicks and the hen.

The authors conclude that on-farm hatching is no different (if not better) than conventional hatching in hatchery.

Merits of the paper:

I think the research is interesting, investigating disruptive farming practices such as on-farm hatching, and the presence of foster adults with the chicks. The experimental set up is complex (I will come back to this later) but appropriate to address the research questions. I think most of the information to repeat the study is described in sufficient

details. I think the results reported in the paper are a valuable contribution to a knowledge gap.

flaws of the paper:

- The experimental set up is complex. I ended up drawing a diagram and it helped me a lot to understand the paper. In the details below, I suggest removing some figures, this will give room to add a diagram explaining the experimental set up.
- The introduction is explaining why there was an antibiotic challenge quite late, I would refer earlier to antibiotic resistance and the urge to change some farming practices from the beginning.
- Concerning antibiotics, can you explain why the experimental set up did not include an on-farm hatching + antibiotics treatment?
- The data made available for behaviour is already processed, I don't think it is very useful "dataset" as such.
- I don't understand the rationale behind the challenge, please explain it in the paper. Why did you do such a challenge, why don't you report a comparison of the performance before and after the challenge to relate to robustness? I know growth is a dynamic process, but there are way to tackle this difficulty and make the best use out of the experimental design.

Concerns:

I don't have major concerns about the research, I think that important improvements in writing or presenting it are needed.

Suggestions for general improvement:

- It is not always clear to me when you compare all treatments with each-other and when you take some of them only (e.g. L224, or figure 2, 3 and 4). Particularly, I have the feeling that CH refer to different data subsets throughout the paper.
- I was confused about the ordering of the result section: the first results reported are the behaviour of the hens, which seems (to me) to be an additional question in the study that focuses on chick performance. Therefore, I recommend some structural changes: starting with the hatchability and chick quality (L307...), secondly with the growth(L344...), thirdly with the health (L402...), forth and lastly with the behaviour (L272...).

Suggestions for details improvement:

TITLE

L2: I don't think the paper is about welfare, please remove it from the title. The paper, however, is about antibiotics, and this is not mentioned. I think this point is deeper than just the title, I would tune down interpretations about welfare and tell more about the challenge and the antibiotics.

ABSTRACT

L25-28: why did you do such a challenge? What are you aiming for in terms of animal response? Why don't you report it?

L28: you could change "performance" to "growth" to be more specific

L28: you could change "robustness" to "survival" to be more specific

L34: I did not understand this sentence, which other groups than OFH are you referring to, CH ? So there is one other group only ?

L38 an 40: I would avoid the use of “eventually” to give a clear message.

L42-43: what do you mean with this sentence “In conclusion, the OFH system was a hatching system at least equivalent to the CH system, if not better in this study.” ? Be specific please.

L45-46: how did you conclude that “The health status and brooding behaviour of the hens are essential to ensure the health and welfare of the chicks” based on you results ? I did not see any variation in the health status of the hens reported in the paper, so how can you estimate its effect and conclude about it ? I have the same question about brooding, the eggs were not in a nest, and the hen did not have any access to the eggs nor to the resting place of the chicks (except for the night with the adoption protocol) so can you explain how they had the opportunity to show brooding behaviour?

INTRODUCTION

L60: You could be more specific than the broad terms “development, performance, and welfare” ?

L64: are there more stressors than the ones already mentioned? If so, cite them please.

L75-81, there is a lot of information, some of it (e.g. fear) is already mentioned L59, and some information does not seem to be crucial (age of the parents’).

L89-93: these few lines are quite wordy. Can you explain facts, what is already known, where is the knowledge gap concerning gut health and microbiota?

M&M

L114-119, please remove this paragraph. It is about ethics (already stated in the ethic section L528), and about giving credit to the experimental unit (already stated in the acknowledgments L548).

L130-132: as already mention, you need to explain why such a challenge was applied: including a rationale in the introduction, explaining how you analyzed it in the methods, and presenting the results of the challenge. I assume every individual is it’s own control because every chicks were challenged? This implies methods about longitudinal data or prediction of an unperturbed performance.

L141, about the comparison between OFH and CH: among the things that differ between the two treatment there is the lighting regime, the temperature (and eventually the humidity). Can you comment on that in the discussion since you give those details in the M&M?

L163: the laying hens are 31 weeks, this should be around their laying peak period. Is there any relationship between laying and brooding like in other birds when one comes after the other? Would that explain the relative aggressiveness of the hens toward the chicks, meaning it could be the wrong time for them to adopt chicks? Can you comment on that?

L191: why were the hens removed?

L222 and 224: it is not always clear to me if CH always refer to the same thing (the CH treatment, which is different from CH+AB and CH+H) or if it sometimes refers to all of CH chicks. Can you please make sure that CH abbreviation stands for one thing only, and that it is clear for the reader?

L226: Do you think the type of funding and name of the project of the chick quality grid is a valuable information ? I would remove "CASDAR QUALICOUV project" and keep only intelligible information, for any reader, in the M&M. If necessary, you can mention this project in the funding or acknowledgment section.

L240-241: Can you cite which disorders and the causes of death please?

L251: I found the statistical analysis section a little blurry, I don't think a reader can repeat the same analysis using the text. It is always difficult for me with a plain text, would you try to write it in formula syntax please?

L253: I think it could be valuable to use a GLM instead of a Kruskal-Wallis test. That would enable to test the effect of the treatment, while taking the experimental design into consideration as fixed effect (e.g. pen). Using a Poisson distribution, you would take into account the fact that the variable is discrete and is a score.

L255: you don't need to mention you checked the distribution of the residuals, checking the initial conditions of statistical analyses should be a common practice. In addition, this comes before the model, thus it is confusing.

L260-261: I think there is a typo with the P-value threshold, it should be $0,05 < P < 0,10$, isn't it?

L263: same remark for behaviour than for chick quality variables: are you sure you can't use parametric models? In addition, the data for behaviour is preprocessed, thus it is hard to have an opinion about alternative statistical approaches.

RESULTS

L287: Is table 1 really needed?

L288-294: I think this paragraph should be in the M&M section, L295 only is a result.

L304: Is Figure 2 really needed?

+ Part of the legend is actually some M&M elements, the legend is not so clear. Why not simply say "Mean number of chicks in the hen's zone according to the hatching condition (CH.... OFH....)?" That comment about legends or title could be applied to other tables and figures.

L313: so there were significantly more eggs in the pen next to the pen with one of the 3 aggressive hens? Is it really significant?

L322: Is figure 3 needed ?

L328-333: please split the ideas in different and short sentences.

L335 & Figure 4: I don't understand this results, I am very puzzled about this. What was compared : all CH against OFH and OFH+H? What about the CH+H then?

L336: so is this due to the hens' aggressiveness or not specifically?

L363-366: please split the ideas in different and short sentences.

Figure 5&6: I think the diagram is not appropriate. If you want to refer to growth, which is a dynamic process, I recommend drawing growth curves instead of these different barplots. This would enable to check the effect of the challenge on growth. This would also enable to understand if CH chicks have a compensatory growth. This phenomenon is documented, yet not discussed in your paper. Could you please add few words about compensatory growth in your discussion?

DISCUSSION

L423 please keep a constant vocabulary, what is a "OFH-certified JA757" chick in your experiment?

L429: is this a reminder of OFH compared with CH, or is this compared with other studies?

L432: can you write differently, in simple syntax "These degraded indicators could be since in our experimental design, very few hens expressed a clear maternal behaviour towards the chicks (n = 2/16), and some even showed agonistic behaviour" please?

L436: Is that so? I think this is a very strong statement... Is really one of the breeding goals of the breeders to reduce brooding? If so how do they record it to select against it in their selection index? If it's not intended, do you have evidence that selection for laying resulted in an indirect genetic selection against brooding (that's related to my comment about L163 in the M&M)? If you do have evidence please cite them and report the genetic correlations. If it is speculative, I would strongly recommend removing this statement because it would be flawed or to tune it down while explaining the rationale that makes you think there is an indirect selection against brooding. This is possible, if the breeders don't check, they don't know.

L483: This is where I would mention that CH chick may have display a compensatory growth response induced by the starvation between the hatchery and the farm.

L497-498: do you have evidence that the fact they are not their mother is the cause? Or is it a question?

L515: indeed you did not set the unchallenged condition: why is that? Please explain your rationale.

L520: I would remove the "if not better"