



Peer Community In Animal Science

The hen, the egg and the chick in conventional and on-farm hatching systems

Florence Gondret based on peer reviews by **Nicolas Bedere**  and **Anna Olsson** 

L. A. Guilloteau, A. Bertin, S. Crochet, C. Bagnard, A. Hondelatte, L. Ravon, C. Schouler, K. Germain, A. Collin (2024) On-farm hatching and contact with adult hen post hatch induce sex-dependent effects on performance, health and robustness in broiler chickens. bioRxiv, ver. 3, peer-reviewed and recommended by Peer Community in Animal Science.

<https://doi.org/10.1101/2023.05.17.541117>

Submitted: 05 June 2023, Recommended: 15 February 2024

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To limit the use of antibiotics in the few days after hatching, it is necessary to improve the robustness of chicks during the early post-hatch period. This can be achieved by ensuring immediate access to feeds, optimizing the implantation and maturation of the microbiota and immune system of each chick, and minimizing exposure of stressors such as transportation. The study conducted by Guilloteau and colleagues (2024) compared the performance and health of chicks raised in conventional hatching systems with those raised in on-farm hatching systems. The authors showed that both systems yielded similar hatching percentage of eggs. Chicks from on-farm hatching systems exhibited higher body weights during the post-hatch period compared to those from conventional hatching, whereas health parameters were not affected by the system. An originality of the study was the examination of the benefits of the presence of an adult hen in hatching systems. The effects on chick traits were interpreted in relation to the hen behavior at hatching and a classification according to maternal or agonistic activities towards the chicks. However, the experimental design did not allow to make statistical correlations between hen behavior pattern and chick traits. Importantly, the presence of a hen decreased the hatching percentage, and this was likely associated with hen aggressiveness in the pen. The presence of the hen deteriorated the quality scores of the chicks in the on-farm hatching system, and increased mortality of chicks at hatching, negatively impacting chick weight gain and feed efficiency during the few days after hatching in both conventional and on-farm hatching systems. Thereafter, the effect of the presence of a hen on chick body weight was different according to the sex of the chicks and the type of hatching system.

The presence of a hen did not reduce the parasitic load of the chicks nor improved clinical signs. No specific characterization of the fecal microbiota of the chicks was conducted, preventing the testing whether or not the presence of the hen affected the early implantation and maturation of the chick microbiome. Altogether, the data indicate that on-farm hatching systems are at least equivalent (in terms of health traits, feed efficiency) or even favorable (for faster growth in the early period after hatching) for chicks. Training the hens (considered as foster adults) to the presence of eggs and chicks or selecting hens according to specific activity behavioral patterns could be ways to establish better interactions between hens and chicks. Although the number and type of environmental stressors tested in the experiment differ from those in commercial farms, the article opens new perspectives for alternative hatching and farming practices.

References:

Guilloteau LA, Bertin A, Crochet S, Bagnard C, Hondelatte A, Ravon L, Schouler C, Germain K, Collin A (2024) On-farm hatching and contact with adult hen post hatch induce sex-dependent effects on performance, health and robustness in broiler chickens. bioRxiv, 2023.05.17.541117. ver. 3 peer-reviewed and recommended by Peer Community in Animal Science.
<https://doi.org/10.1101/2023.05.17.541117>.

Reviews

Evaluation round #2

DOI or URL of the preprint: <https://doi.org/10.1101/2023.05.17.541117>

Version of the preprint: 2

Authors' reply, 12 February 2024

Dear Dr Gondret,

We are grateful for all the reviewing work you have done with the referees. You will find our answers in italics for each point.

Dear authors,

We thank you for corrections and additions already made to the text in response to the first round of revision. The manuscript has been considerably improved and the comments of the referees in round 1 of the revision process have been extensively addressed. The revised manuscript has been sent to the same reviewers that handle the original file in the first round. You will read that they still have some comments to clarify specific points.

You can read that the two reviewers and I (see below) also questioned the statistical models. I think however that this is not mandatory for the revision, since we can accept the type of statistics you have selected for participar purposes.

Besides reviewers'commmts, you will find below my additional requests (especially format) at some points:
Abstract (L22): removed "certified JA757". This is not necessary at this place of the text.

OK

Abstract (L36): add "(CH + AB)" after the words "treatment of CH chicks with antibiotics"

OK

Abstract (L48-49): the conclusion sounds incorrect considering the fact that health status of the hens and their behavior have not been specifically related to health and welfare of chicks. Behaviors of hens have been

scanned, but the different categories (agonistic/maternal) were not statistically related to performance of chicks. One of the reviewers also has dedicated comments about the recorded health status.

We agree and removed the last sentence.

Introduction (L62): what is "overall" health ?

"overall" has been removed.

M&M (L274): remove "certified JA 757". This information about the genotype has been already given L153. Add a reference to Fig. 1.

OK

M&M (L290): remove "without inducing pathology or mortality". You cannot ensure that these stressful conditions were not associated with any causes of mortality. I think that the sentence quoted in reference could be rewritten as "these conditions are stress factors that chickens may encounter on farms"

OK

L566: there is a mismatch in the groups considered for evaluating chick quality. CH + H was not included.

Yes indeed.

L569: The BW during the rearing period was not independent of the animal (= repeated measures for a given animal). A MIXED model is likely better suited for this type of data.

OK

L601: "5.6% +/- 5.9%" ? I think there is a misspelling figure ?

The number of died chicks was very variable according to the pen (and the hen present), (from 0 to 16.7%).

Which figure?

L617: "the deterioration of chick quality with hens was due to the hen aggressiveness". I think that this sentence should be moved in the discussion. See also similar comment in one of the reviewer's evaluation.

The sentence has been removed and interpretation was already present in the discussion.

L650-680: this paragraph should be moved to the beginning of the result section. Indeed, the hatchability was related to the aggressivity of the hens, and this classification of hens between aggressive/tolerant/maternal was shown in Table2.

That is true, but the hens' behaviour was defined during the first 9 days of interaction with the chicks. It doesn't seem logical to us to present these results before the hatching and chick quality results.

According to the comment of one of the referee, we did not find any information regarding how the budget time (Table 1) may influence or not hatchability and chick performance. If you did not try to relate both traits, then Table 1 can likely be removed or provide as an additional file.

We were unable to make any significant link between hen behavior and chick hatchability and quality, but it does seem interesting to present the hens' time budget which was used to categorize hens.. We propose to put Table 1 in a supplementary table as suggested.

Figure 5: I question the opportunity to show the weight of CH+AB chicks at day 0, considering that the antibiotics treatment was applied from D2 onwards. Considering that there was no difference between BW of chicks between CH and CH+AB groups at Day 19, I think that it is relevant to consider the differences of BW at D34 between the 5 groups.

We don't fully understand the comment. There is no difference between the weight of CH and CH + AB at D0 (there was no antibiotic treatment at that time), but there is a difference at D19. Nevertheless, we have kept the 5 groups for each analysis time.

Figure 7 is very hard to understand

We moved this figure as Figure S1 in an additional file.

Discussion L944 "there was possibly communication between hens and embryonated eggs before hatching". Please add a literature reference for this assumption.

We added the reference to the review of Edgar et al, 2016 which reports that the communication between the mother and chicks begin as early as the day before hatching.

Discussion L955-956: " it does not reflect the rearing environment". Please rewrite. Do you mean that environmental and vaccine challenges applied in the experiment are less complex than the environmental pressure combining multiple sources of stress ?

Yes, we have completed the sentence to clarify the message.

L958-960: these sentences can be deleted to avoid unnecessary speculation

Yes and no, it is important to remember that experimental sanitary conditions are not the same as those on farms.

L979-980: "these practices offer possible evolutions ... to continue to decrease the use of antibiotics". This sentence should be removed. There was no OFH+AB group in this study, so it is difficult to conclude.

This sentence has been removed.

I hope that all these comments combined with the detailed evaluations of the two reviewers would be helpful to prepare a revised version of the manuscript.

We thank you again for choosing PCI Animal science community and open science.

Sincerely yours

Florence Gondret

Dear Dr Olsson,

You will find the answers in italics for each point.

Review PCI #199

Thank you for providing a revised version of your manuscript. The experimental design is now clear, and so are other methodological details that were missing in the previous version. I have a few remaining concerns.

1) The behavioural characterization of the hens. Did you try to use this measure to understand the relation between hen behaviour and chick performance? I can't find this information in the text. If you did indeed perform any analysis of how hen behaviour affected chicks, this needs to be reported.

Alternatively, if analysis was limited to the effect of hen presence independently of how she behaved, you need to reconsider how to refer to that analysis. I find it a little confusing and potentially misleading that you refer to the behavioural characterization when you describe the effect of hen presence if this measure was not used. I would remove "categorised according to their behaviour" from lines 32-33 and line 55, or alternatively, add detail on how hen behaviour affected the chicks. The statement on lines 327-328 that "The deterioration of chick quality with hens was due to the hen aggressiveness" is also problematic if there was no analysis of hen behaviour effect. You could reword it to "The deterioration of chick quality with hens was probably related to hen aggressiveness"

Our study focused on the effect of the hens' presence with the chicks. Characterizing the hens' behaviour is a complementary indicator, but the number of different profiles (agonistic, tolerant, maternal) is too limited and variable to study their effects on chicken performance. Nevertheless, we mention the information available to help discussion of the results.

We removed "categorised according to their behaviour" from lines 32-33 and line 55

The sentence "The deterioration ... hen aggressiveness" has been removed. This comment is already present in the discussion.

2) How did you correct for multiple comparisons in the statistical analysis? You have five treatment groups and the analysis is repeated for multiple time points so this correction is definitely needed. Unlike some post-hoc tests, Fisher's LSD doesn't automatically correct for multiple comparisons, as far as I understand.

Fisher's LSD post-hoc test used in this study included correction for multiple comparisons (type FWER).

Minor issues:

Line 54 The Ecoantibio 2017 plan needs a reference or further explanation

A reference has been added.

Line 339 At which timepoint were these hens removed?

It was during the following day after the overnight physical contact between hens with chicks, when they had access to the whole pen even if the chicks had access to their own space. This has been specified in the text.

Lines 452-454 This sentence is ambiguous and it's difficult to understand which treatment is referred to with "reported higher for the hatchability, lower for the quality score of chicks and lower for the mortality".

This has been reworded for greater clarity.

Line 463 change "their rather tolerant behaviour, their brooding behaviour could be optimised" to something "their rather tolerant behaviour, it may be possible to optimise their brooding behaviour »

The sentence has been modified.

Dear Dr Bédère,

You will find the answers in italics for each point.

Dear Dr Gondret,

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

Sincerely,

Nicolas Bédère

Review:

Important comment:

As I already mentioned in the previous round, I strongly recommend to remove speculative statements from the manuscript. I considered these speculative because they are not tested in the study, these are conclusion drawn by the authors based on their expertise, but they do not come from results reported. These are:

- L48 & throughout (including conclusion): About the effect of the health status of the hens on health and welfare status of the chicks: there is no difference in health status of the hens recorded or reported in your paper, it is even a criterion for enrollment in the experiment. To conclude about the effect of health status of the hens on the chicks I think you need variation in the health status of the hens. Since they're all in good health, there is no variation in health of the hens, so variation in the health and welfare of the chicks can not be related to that of the hens.

This sentence has been removed.

- L48 & throughout: a similar comment, yet less strong, for brooding behavior of the hens: the hens were not offered the possibility to express brooding behavior towards eggs (this may be worth mentioning in the discussion). Have you checked the literature: is there a relationship between brooding the eggs and the chicks? Are they both links of the same behavioral chain? Concerning brooding behavior towards the chicks, as far as I understood the paper brooding behavior was included in maternal behavior, and there was not significant relationship between maternal behavior and health and welfare of the chicks (except if you oppose maternal and aggressive behaviors?).

An egg was present in the nest to encourage brooding behaviour, this information has been added in M&M. This

sentence has been removed.

- L870: you deleted the indirect response on maternal behavior, to selection on egg production, yet this is still suggested by this small sentence and it remains entirely speculative. Remove the sentence or report evidences (meaning genetic correlation, differences between lines...) with citations.

This sentence has been removed.

Suggestions for details improvement:

ABSTRACT

L38: I would avoid the use of "best" (or "worst" if any) for comparison and prefer "highest" to report neutral comparison.

OK

L47: I would remove "The effects of" and start the sentence with "The presence of the hen" to make this sentence easier to read

OK

INTRODUCTION

L70: "at between" is wordy, I would remove "at"

OK

L105: I miss a link between the ideas: can you shortly explain how antibiotics can play the role of growth promoters?

We added the following sentence and another reference in addition to Broom, 2018.

"Growth promotion induced by antibiotics is associated with effects on the caecal microbiome at taxonomic, metagenomic, and metabolomic levels, which might be targeted via its contribution to host-microbiota crosstalk, particularly by acting on the gut barrier function (Broom, 2018; Plata et al, 2022)."

L116: Is the fact that they are their mothers that is important or any adults would also fulfill this role?

We have not found any comparative data to answer this question

M&M

L150: Please improve the legends... Tables and figures are supposed to stand alone, separately from the manuscript.

Explanation of hatching conditions has been added

L167: can you recall here the citation of the protocol that promotes maternal behavior?

OK

L167: I am simply wondering: 16h of light is the lighting program usually found on farms for laying hens.

Light color and intensity are also known to influence social interaction between hens, and tuning both the color and the intensity is a management strategy used to decrease aggressive behavior such as pecking. I was wondering:

+ are both the color and intensity used in your experiment appropriated?

The light intensity was 16h for the hens before the arrival of the chicks. In order to conciliate the light requirement for the chicks, we increased the duration to 20h at the time of hatching/placement of the chicks, then gradually decreased the duration to return to 16h of light. Light color was that used on the farms.

+ could you comment on this type of strategies that can be used to decrease aggressive behavior in your discussion?

The following sentence has been added " Light color and intensity are also known to influence social interaction between hens, and tuning both the color and the intensity could be a management strategy to decrease aggressive behaviour such as pecking but whose effects vary according to age, genetics and activities (Du et al, 2022)"

L563: I disagree with the authors' opinion about GLM based on my comment during the first round. GLM are a generalization of linear model meant for other distribution than the Normal distribution, including either the Poisson or Categorical distribution, that can be approximated by using a log or logit link functions respectively (https://en.wikipedia.org/wiki/Generalized_linear_model). Then several factors can be accounted for in the analyses jointly, decreasing potential biased by accounting for interactions or confounding effects. Nevertheless, regarding the power of the experimental design, it is possible that similar conclusions will be drawn anyway between a GLM and the nonparametric tests. I do not recommend to perform the analysis again.

OK

RESULTS

L618: I would be interesting to relate the quality scores of the chicks with the behavioral data to further support this result with an actual test. Otherwise, I recommend to tune down the sentence "The deterioration of chick quality with hens was due to the hen aggressiveness", using conditional mode, and moving it to the discussion section.

We performed a correlation test between the chicks' subtotal score for appearance and the hens' agonistic or maternal behaviours for the OFH + H group, but there is too little data to conclude. We have therefore removed the sentence, this comment is already present in the discussion.

L685: I would remove "Independently of the treatment" because it is a confusing statement since this is how an ANOVA with type II or type III sums of squares works (even type I if the effect of treatment is before the effect of sex in the analysis). Default options for softwares is often type III sums of squares so that's OK.

OK

L784 -786 from "the pen with the lightest" to "were observed": is that important? I would remove it.

We feel it is important to show these BW values at the end of the rearing period in relation to the hens' behaviour, even if the differences are not very great. We moved the Figure 7 as Figure S1 in an additional file.

L841: Due to chance, or rather "bad luck", 11 chicks were eliminated in groups with hens. Given the size of the

groups, could this have biased the comparison between conditions with or without hens?

The actual group size is included in the statistical analysis.

DISCUSSION

L864: "However...whereas" is wordy, I would remove "whereas"

OK

L893-894: I would remove "and the presence of hens at hatching harmed chicken BW regardless of the hatching condition and on FCR" because it is repeated and better stated L 901-905.

OK

L909: Was there a dose-response issue with your experiment: was the provided dose similar to the dose usually used as growth promoter?

We used the doses prescribed by the veterinarian.

L929: you could replace "but the fact is that there is" by "in fact there is"

OK

L935: Results conflicting with other studies could be due to limits of your experimental design such as the power (complex design + limited number of animals).

L946: do you have any reference to further support this idea?

We added the reference to the review of Edgar et al, 2016 which reports that the communication between the mother and chicks begin as early as the day before hatching. No other references were found to support our hypotheses.

L947-951: do you have any reference to further support this idea?

No

L981: Retrospectives about the experiment could be valuable: now that you have experience about raising together adults and chicks, what would you do differently to repeat the experiment with the same research questions?

It would be necessary to better prepare the hens to induce a brooding and maternal state, or at least a state of chick tolerance (hens at the end of their laying cycle, season). This has already been mentioned in the discussion. The sex effect in the hen-egg/chick interaction could be better characterized by in ovo sexing.

Perspectives would also be much appreciated: what new research questions popped up?

Further studies should be done to assess the effects of these hatching and chick-starting conditions, in the presence or absence of hens, on the implantation and maturation of the chicks' gut microbiota and mucosal immunity. New devices enabling interactions between hens and chicks should also be tested.

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Decision by [Florence Gondret](#), posted 12 January 2024, validated 12 January 2024

Revisions

Dear authors,

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Sincerely yours

Florence Gondret

Reviewed by Nicolas Bedere , 12 January 2024

[Download the review](#)

Reviewed by Anna Olsson , 15 December 2023

Please see the attached file.

[Download the review](#)

Evaluation round #1

DOI or URL of the preprint: <https://doi.org/10.1101/2023.05.17.541117>

Version of the preprint: 1

Authors' reply, 04 December 2023

Dear Florence Gondret,

I've been waiting for the preprint update to go online. It is now online. We are grateful for the reviewing work that has been done to ensure a better understanding of our work. We have modified the form of the article as much as possible, taking into account the recommendations of the referees. We hope that in this form, the article will be recommended by PCI Anim. Sci.

All the best,

Laurence Guilloteau

[Download author's reply](#)

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Decision by Florence Gondret, posted 09 September 2023, validated 09 September 2023

Revise

Dear Dr Guilloteau,

We are pleased to let you know that your manuscript has now passed through the review stage and is ready for revision. Many manuscripts require a round of revisions, so this is a normal but important stage of the process.

Two reviewers suggested thoroughly correction of your manuscript. They found merit in your study, but suggested several modifications and requested information or structural changes to make much easier the understanding of the experimental design.

If you decide to revise it to continue in the process of recommendation, please address all comments and prepare the manuscript.

Sincerely yours,
Florence Gondret

Reviewed by Nicolas Bedere , 08 September 2023

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Reviewed by Anna Olsson , 04 July 2023

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