

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.
- 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?).
- 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.

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.

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

INTRODUCTION

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

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

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

M&M

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

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

L167: I am simply wondering: 16h of light is the lightning 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?
- + could you comment on this type of strategies that can be used to decrease aggressive behavior in your discussion?

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.

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.

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.

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

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?

DISCUSSION

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

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.

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

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

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?

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

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?

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