

Decision for round #1 : Revision needed

Revision Request

Dear Dr. Nicolas Bédère,

Bonjour,

Thank you for submitting a manuscript to PCI Animal Science.

Manuscript entitled “Genetic background of body reserves in laying hens through backfat thickness phenotyping” which you submitted to PCI Animal Science has been reviewed.

Sorry for the late reply. I would like inform you several reviewers were assigned to your manuscript and the reviewing process was a little long. But, we received comments of two reviewers, they acknowledged that your manuscript had valuable results. The reviewers highlighted a number of issues with the manuscript that need to be addressed before the paper can be considered for publication.

I look forward to receiving your revision.

Cordialement,

S A Rafat

Authors:

Dear recommender,

Thank you for handling our submission. We have addressed all the comments from the reviewers. In this document, you will find our answers to their comments. Our preprint has been updated, changes are highlighted in the text to make tracking easy (yellow for reviewer1, green for reviewer2).

Best regards,

Nicolas Bédère, on behalf of all authors

Review by anonymous reviewer 1, 25 Sep 2023 21:45

This study aimed at validating backfat thickness as an indicator of fatness in laying hens, estimated its genetic parameters, and examine its relationships with other traits. Overall, this study provided useful information that is of potential interest to egg layer breeders and researchers. My main concern is that an indirect measurement of fatness (CT scan) rather than a more direct measurement (such as abdominal and mesenteric fat weight or chemical analysis) was used to validate the backfat thickness method . So, I would recommend being less assertive with the conclusion, as suggested below (L325)

Authors:

Dear reviewer,

We are grateful for your time and advice to improve our paper.

We agree with you: additional indicators for body fatness such as chemical analyses of the shredded body or the abdominal fat pad weight would have further supported our findings. We have followed your suggestion and edited the conclusion.

Best regards,
Nicolas Bédère, on behalf of all authors

Comments/suggestions/questions:

L35: “extend the career of laying hens” should be reworded, as “career” is typically used in reference to people, and its use for layer hens is uncommon.

Authors: we have changed “career” to “egg production period” (L35).

L41: Usage of “...” is uncommon in the scientific literature. Please consider removing these throughout the text. For example, you could use “such as x, y and z” to indicate that there are more items than x, y and z.

Authors: OK, changed (L41).

L110: change “soon after death” to “immediately after euthanasia”

Authors: OK, changed (L117).

L115: “Fatty components were located between -130 and -90 HU.” Was this established based on prior studies? If so, please provide citations. If the answer is no, please provide explanation as to how this has been established. Some readers like myself may not be familiar with this technique.

Authors: We have provided details about the determination of the thresholds (L120-128), and added two references in the bibliography (L 477 and 523)

L134-135: “A first blood sample was collected from the wing vein at 17 weeks of age and a second blood sample was collected during the neck bleeding at the slaughter process, at 53 weeks of age” since blood from neck bleeding contains a mix of arterial and venous blood, as oppose to blood samples collected from the wing vein, do the authors expect no impact of blood source on the levels of compounds measured in this study?

Authors: In another experiment, Joelle Dupont (one of the authors), did not see any difference in adipokines concentrations between neck bleeding samples and venous blood. Unfortunately, these observations are not published. We have added a sentence to mention this limit (L145).

L187-190: this sentence is difficult to understand; it could be revised and split to shorter sentences

Authors: OK, changed (L196-200).

L185: “Descriptive statistics of the data suggested a bimodal distribution of backfat.” Was this observed within both R- and R+ lines, or just within one of these lines?

Authors: In both lines, especially at 40 and 53 weeks (Figure 3). We edited the manuscript (L203).

L186: change “Reported heritabilities” to “Reported heritability estimates”

Authors: OK, changed (L194).

L237: change “consistent whith the findings previously” to “consistent with the findings previously”

Authors: OK, changed (L246).

L268: change “latter alining with” to ““latter aligning with””

Authors: OK, changed (L280).

L283: change “incluce in our setup” to “influence in our setup”

Authors: OK, changed (L295).

L315: change “few studies mentionning” to “few studies mentioning”

Authors: OK, changed (L341).

L318: change “a targetted neither” to “a targeted neither”

Authors: OK, changed (L344).

L325: change “backfat thickness is an accurate indicator of the overall fatness of laying hens” to “backfat thickness is a potentially accurate indicator of the overall fatness of laying hens”

Authors: OK, changed (L355).

Review by anonymous reviewer 2, 28 Sep 2023 20:05

Review on: Genetic background of body reserves in laying hens through backfat thickness phenotyping

General comments:

The overall purpose of the manuscript is to investigate backfat thickness phenotyping and investigating its heritability and relation to phenotypes in laying hens. This is a well-prepared manuscript and carefully prepared and carried out in opinion of the reviewer. Overall, the authors highlight the potential to utilize backfat thickness for additional genetic information, but given that the association was “moderate and negative”, the authors could potentially shift the discussion to show how backfat thickness could serve as a “more\less/easier/harder” efficient technique to drive selection? The materials and methods section can be improved for repeatability of the research work being presented. Overall a well written and thoroughly cared for manuscript.

Need better table descriptions so they are standalone items for text

Authors:

Dear reviewer,

We are grateful for your time and advice to improve our paper.

We have edited the M&M section according to both reviewers' suggestions and we hope it is clearer now. We have also improved the table descriptions as suggested.

In this study, the moderate and negative genetic correlation between backfat and total egg number suggests a trade-off between body reserves and egg production. There appears to be an even greater trade-off between body weight and egg production. Body weight is partly made of fat, as indicated by the moderate and positive genetic correlation between backfat and body weight. This correlation means that other components of body weight are also parts of the trade-off, such as minerals (bone vs eggshell) and protein (muscle vs egg white). Body weight is a trait that breeders aim to stabilize, maintaining consistent and uniform body size among the birds within a flock. When discussing with layers breeders,

fatness would be the same: an optimal target, aiming at neither fat nor lean hens. In fact, aiming at an optimal body fatness allows to face perturbations (cold wave, food supply shortage...), breeders report that too lean hens would stop producing or die from their condition. In the other hand, obese condition is associated with reduced reproductive performance (egg number, egg quality, sperm concentration and sperm quality, fertility and hatchability; Zhang et al., 2018), higher incidence of metabolic disorders (Bain et al., 2016), and reduced feed efficiency, meat quality, and carcass yield in chickens (Baéza and Le Bihan-Duval, 2013). We have added these motivations (opportunity to preserve health and welfare) in the perspectives (L333-340, 347-352).

Since the genetic correlation between backfat and total egg number is moderate and negative in our study, selecting the R+/R- on egg number would result in an indirect selection against backfat.

Commercial lines are intensively selected for egg number, if a similar genetic correlation between egg number and backfat is observed in these populations, it would be important to include backfat in the selection index to maintain sufficient fatness. We are currently studying a population from a breeding company to confirm these findings from the R+/R- lines, which are unusual for the egg industry.

Best regards,

Nicolas Bédère, on behalf of all authors

Baéza, E., Le Bihan-Duval, E., 2013. Chicken lines divergent for low or high abdominal fat deposition: a relevant model to study the regulation of energy metabolism. *Animal* 7, 965–973.

<https://doi.org/10.1017/S1751731113000153>

Bain, M.M., Nys, Y., Dunn, I.C., 2016. Increasing persistency in lay and stabilising egg quality in longer laying cycles. What are the challenges? *British Poultry Science* 57, 330–338.

<https://doi.org/10.1080/00071668.2016.1161727>

Zhang, X.Y., Wu, M.Q., Wang, S.Z., Zhang, H., Du, Z.Q., Li, Y.M., Cao, Z.P., Luan, P., Leng, L., Li, H., 2018. Genetic selection on abdominal fat content alters the reproductive performance of broilers. *Animal* 12, 1232–1241. <https://doi.org/10.1017/S1751731117002658>

Specific comments:

Lines 46-52: what are the nutritional effects/confounding effects and how could it be adjusted to deal with these issues, is this a management issue or a reality across all production systems?

Authors: According to Bain et al. (2016) it seems to be a global issue, encompassing genetics, physiology, nutrition, management... We edited the manuscript to make this clear (L49-50)

Lines 60-62: Aren't these techniques developed through time-point slaughter through different slaughter points in different physiological stages? Perhaps making the argument differently could avoid this confusion, but allometric sequential slaughter studies are a good way to determine dynamics of body reserves.

Authors: OK, that is what we meant with the statement "except as collateral information". We stated it differently (L62-63).

Lines 79-81: citing of ideal physiological body conditions from literature

Authors: Unfortunately, to our knowledge, this can not be found in the literature and has yet to be defined by the egg industry. Indeed, breeders do not evaluate body reserves yet, therefore they do not have a targeted value for fatness. What they mostly do is to monitor and manage body weight, the

targeted mean weight can fluctuate according to the different commercial lines (e.g. a White Leghorn line would be close to 1.6 kg while a Rhode Island line would be close to 1.9 kg around 1 year of age).

Lines 101-103: more details on facilities, diet, operations would allow for reproducibility

Authors: We added information about the temperature of the building and the time of diet distribution and the protein and energy content of the diet (L106-107). Further information about the diet can be found in Appendix 1.

Lines 101-105: Would suggest creating a table with the diets for the animals, also need more details on housing of the animals and feeding management for reproducibility

Authors: The diet composition can be found in Appendix 1. Laying hens are fed this diet ad-libitum as indicated (L106-107)

Line 113: recommend "hens were placed dorsally on the x-ray"

Authors: OK, changed (L119).

Line 137-140: Why were blood samples collected from different locations, would adipokine concentration be distributed in similar concentrations in both tissues?

Authors: Reviewer 1 also has commented on this, please find our answer below:

In another experiment, Joelle Dupont (one of the authors), did not see any difference in adipokines concentrations between neck bleeding samples and venous blood. Unfortunately, these observations are not published. We have added a sentence to mention this limit (L146).

Table 1. Standardize decimal places for all values reported

Authors: OK, we changed a few numbers per column, especially in the "estimated effect" row. We did not standardize the decimal across columns because each variable has its scale of values.

Line 212: Fort "For" ?

Authors: OK, changed (L221).

Line 214: "performed on one batch only", also can this be fixed with additional sample analyses?

Authors: OK, changed (L223).

Yes, we think this is a clear example of a lack of power in the statistical analysis, additional observations are required. Unfortunately, this is not possible on the studied lines.

Line 221: "leaner"

Authors: OK, changed (L230).

Line 235-237: need to cite the papers you are referring to

Authors: OK, changed (L245).

Line 238: The reviewer would suggest the need to establish and justify highly, and moderately, different fields will have different thresholds on what is a good/high correlation value, list results and p values.

Authors: OK, we defined it in the M&M (L 195)

What we usually find in the literature regarding genetic parameters in livestock is the following interpretation of heritability estimates: 0.00-0.25 is low, 0.25-0.50 is moderate and > 0.50 is high.

Lines 281-283: would ghrelin and adipokine levels shift at different physiological stages? Is the association linear or are there allometric coefficients attributable to different life stages? Diet/management effect on these markers is overlooked if not discussed further, would you believe this could influence the results you observed?

Authors:

It has been observed in turkeys that plasma concentrations of adiponectin, chemerin, and visfatin are lower at the end of the laying period than at the beginning (Diot et al., 2015). We could not find any information in the literature about the trajectory of changes in plasma concentrations over time (e.g., linear or allometric). In the present study, time was added as a fixed regression coefficient as indicated in L198. These results were not presented in the first version of the manuscript and have been added (L295-302):

We observed a significant increase in blood levels of adiponectin ($P < 0.001$) and visfatin ($P = 0.007$), a significant decrease in chemerin ($P < 0.001$), and no significant change in ghrelin ($P = 0.14$) between 17 and 53 weeks of age.

In broilers, feed restriction is known to decrease plasma levels of adiponectin, fish oil supplementation is known to decrease plasma levels of chemerin (Mellouk et al., 2018), and grape seed extract is known to decrease plasma levels of chemerin and increase those of adiponectin and visfatin (Barbe et al., 2020). However, our experiment is designed for genetic studies, so environmental factors such as dietary or management effects are not tested and are even controlled to induce as little variability as possible. This is the reason why we did not discuss these sources of variation extensively in the paper and emphasized the fact that our results were observed in our setup, where adult hens are housed in individual cages and fed *ad-libitum*. We have added a sentence to mention these elements (L295-302).

The additional references were added (L402 and 440).

Barbe, A., Mellouk, N., Ramé, C., Grandhay, J., Staub, C., Venturi, E., Cirot, M., Petit, A., Anger, K., Chahnamian, M., Ganier, P., Callut, O., Cailleau-Audouin, E., Metayer-Coustard, S., Riva, A., Froment, P., Dupont, J., 2020. A grape seed extract maternal dietary supplementation in reproductive hens reduces oxidative stress associated to modulation of plasma and tissue adipokines expression and improves viability of offsprings. PLOS ONE 15, e0231131. <https://doi.org/10.1371/journal.pone.0231131>

Diot, M., Reverchon, M., Rame, C., Froment, P., Brillard, J.-P., Brière, S., Levêque, G., Guillaume, D., Dupont, J., 2015. Expression of adiponectin, chemerin and visfatin in plasma and different tissues during a laying season in turkeys. Reproductive Biology and Endocrinology 13, 81. <https://doi.org/10.1186/s12958-015-0081-5>

Mellouk, N., Ramé, C., Marchand, M., Staub, C., Touzé, J.-L., Venturi, É., Mercierand, F., Travel, A., Chartrin, P., Lecompte, F., Ma, L., Froment, P., Dupont, J., 2018. Effect of different levels of feed restriction and fish oil fatty acid supplementation on fat deposition by using different techniques, plasma levels and mRNA expression of several adipokines in broiler breeder hens. PLoS ONE 13, e0191121. <https://doi.org/10.1371/journal.pone.0191121>

Line 308 and throughout: the reviewer would suggest not starting sentences with abbreviations.

Authors: OK, changed throughout the manuscript.