Responses to reviewers:

We thank the editor and both reviewers for their useful suggestions, which will certainly improve the manuscript. Our responses to the reviewers' comments are below. The line numbers in this document refer to the line numbers in the PDF with track changes, not the version on bioRxiv.

Mathieu Monziols

General comments:

This paper presents the potential of Dual X-ray absorptiometry to predict chemical composition of living pigs. Even if there are already papers on the subject showing that DXA is a suitable methods to predict chemical composition, this paper is clearly original because it shows the absolute need of DXA calibration in order to obtain accurate prediction and also shows that carcass analysis can be used for empty body chemical composition (with an increase in the prediction error). Furthermore, another originality is the comparison with already published prediction models and the differences observed. That is suggesting the possibility to use a single model for closed DXA systems which is quite interesting.

The paper is well written, clear and very understandable. The figures are also clear and support the text.
The paper can be published with few minor revisions

Thank you very much for your positive remark.

Form modifications :
- Line 55 : It is the first line where the different chemical measurements are presented. It would have been kind for a non chemical composition measurements initiate reader to have the full name of the different terms as for example : Bone minerals (ash), total Calcium (Ca), total Phosphorus (P), Total Crude proteins (CP), total nitrogen (N) and total lipids (lipid)
- Line 56 : same remark for body weight (BW)
- Line 56 : same remark for Empty body (EB)

We agree that the use of abbreviations might make it more difficult for readers not so familiar with these to understand the implications statement. We changed the section accordingly.

- Table 1 : the EBW-3 /carcass weight variable can be understood as a ratio which is not, maybe it can be changed by total mass (EBW-3 or carcass)

This is a good point. We replaced the slash by "or" as suggested

- Table 3 : RMSE is expressed in the same unit as the predicted variable, I suggest to add the units of the variable for the RMSE

We added the unit of measurement in Table 3.

Minor remark for paper improvement : As stated in the paper, the bw range used for a predictive regression model calculation is extremely important. But there is no mention in the paper of specific regression models for each BW groups used in the global trial. It would have been interesting to have a quick remark on the different models (intercepts and slopes) obtained at within different target body weight groups (20, 60 and 100) to ensure the relationships between chemical measurements and DXA are closed to the global one (and that the global one is indeed linear). Even if the 20 kg and 60 kg groups are composed of 6 and 18 individuals respectively, it would be interesting to have a word in the paper about such intra bw groups models and if their prediction results would differ from the global model ones.

We thank Reviewer 1 for this suggestion. We have conducted the proposed analyses and present them in the supplementary material (section 5). In the methods section, we added a brief description of how the regional regressions (that is the regressions on subsets of the data for 20, 60 and 100 kg slaughter weight) in lines 292-302. We discuss the similarity or difference of the regional and global regressions in the results and discussion section directly after the presentations of the several groups of variables (lines 378-381, 402-404 and 414-419).

Since the original data (DXA value on the x-axis, chemical value on the y-axis) is now presented in Figures S3 and S4, together with regional and global regressions, we decided to change Figures 2 and 3. The latter now show the measured variable, i.e. the chemical value, on the x-axis, and the value we predicted using the calibration equations we built, on the y-axis.

Reviewer 2:

The work by Kasper et al is an attempt to predict body composition in growing pigs using dual-energy X-ray absorptiometry.

Major comments

- *It is difficult to extract the original information from the manuscript since the subject is not new and very little advanced data is supplied by this work.*

We believe that this manuscript offers new insights in the potential use of DXA for animal science. First, we show that not only body composition can be estimated with high accuracy and precision, but also the content of single nutrients can be assessed. Second, we show that empty body chemical composition can be estimated from carcass scans. Third, we compare the fit of published regressions on our data, which has, to the best of our knowledge, not been done before. This is a critical first step of assessing the validity of DXA calibrations across laboratories. Taken together, we are convinced that this study has merit for an animal science readership.

- *Furthermore, recognised pitfalls associated with DEXA measurements, while extensively published, are not take into account.*

We are not sure which pitfalls Reviewer 2 meant exactly, since this comment is rather unspecific. In this study, we tried to prevent common pitfalls, such as the influence of

body position or time since last meal, by standardizing the procedure as much as possible.

• *Finally, critical papers e.g. Mitchell J.Anim.Sci, 1998, 76:2392-2398 are not even cited.*

We agree that the article fits well with our study and cite it now in line 110, following the statement that it is necessary to relate chemical values and DXA measurements via prediction equations. We added a discussion of the finding that, while body weights correspond well between DXA and scales, this is not true for body composition in lines 317-320: " As noted previously, the exact correspondence of body weight measured by DXA and weigh scales in no way means that the body composition determined by the two measuring methods is equally accurate (Mitchell et al., 1998)." In lines 388-395 we now discuss the finding that fat content is underestimated in small pigs: "The DXA values for fat were consistently lower than chemically measured lipid values for the pigs in the 20-kg slaughter category, which was more pronounced in the empty body than in the carcass. The same phenomenon was observed in a study comparing DXA and chemical analyses in small pigs (5 to 27 kg live weight; Mitchell et al., 1998). It appears that DXA overestimates the fat content of pigs with a high percentage of body fat (> 20%) and underestimates the fat content in lean pigs (Mitchell et al., 1998). Similar findings have also been reported in humans (Genton et al., 2006)."

• *Data about anesthesia and pre-anesthesia are not given*

We are not sure if we correctly understood the comment. Lines 153-160 in the manuscript describe the anaesthesia procedure. No specific measurement or data sampling was collected prior anaesthesia and no measurement was conducted for anaesthesia monitoring.

• *Data about DEXA calibration and quality control are missing altogether*

We thank reviewer 2 for drawing our attention to this omission and inserted information on how the calibration and quality control using the phantom provided by the manufacturer was carried out in lines 188 to 190.

• *The methods for ROI calculation are missing while they are essential for the accuracy of DEXA (the word ROI is even not present in the manuscript)*

We inserted the abbreviation after "regions of interest" in line 214. We added a description of the placement of ROI in the present study in lines 214 to 217 and we provide a figure illustrating the placements of the reference points in the scans of live animals in the Supplementary Information (Fig. S1).

• *The influence of body position (for longitudinal studies) is not investigated*

The investigation of the influence of body position was not a goal of this study. In our study, we took great care to place the live pigs or the carcass cuts on the DXA table in a standardized manner as documented in Figure 1 throughout the study, which lasted less than seven months. We added "standardized" to the description of the placement to highlight this in lines 200 and 204.

• *The influence of the delay between the meal and the imaging is not investigated while known to be of significant importance in body composition analysis at least in human.*

Our study also did not seek to investigate the effect of the time between the last meal and the scan. In the present study, pigs were fastened for at least 16 h before anaesthesia or slaughter to standardize this aspect. Similar to the body position, this is a factor that can and should be controlled in studies in order to reduce variability between measurements.

Minor comments

• *Scale sensitivity and reproducibility is mandatory (or a supplier and reference is necessary)*

We added the trade mark, model, supplier and sensitivity of the two scales used in the experiment in the text (L152f and 164f).

• *As the name implies, DEXA need at least two energies. Only one is presented in the manuscript (100KV). what about the second one. Some data are not presented adequately e.g DEXA with a narrow-angle fan beam. The word collimator is missing*

We thank Reviewer 2 for drawing our attention to this omission and added both energies (~39 keV and ~71 keV X-ray effective energies) in line 187-188. We added the collimator model used in the GE iDXA in line 186.

• *Scan speed and percentage of overlap for the detector cells are also mandatory metrics.*

We added the scan speed (80mm/sec) for Thick Mode in the manuscript in line 191. The percentage of overlap for the detector cells remains unknown because GE does not provide this information in the technical information sheet. We are not aware of any articles that include this information for the model we used. The higher the overlap, the more information can be acquired behind a bone. The narrow-angle fan beam technique was developed to improve this aspect in comparison to the wide-angle fan beam technique. Lunar i-DXA uses multi-view image reconstruction technique in which narrow fan beam sweeps overlap, and the overlap is taken into account in the software to produce distortion-free images.

For a given DXA model/technology, certain parameters, such as the angle of the fan beam, the size, resolution and the percentage of overlap of the detector array are fixed and cannot be modified by the users. Thus, they matter when comparing between techniques, but not within.