

Reply to recommender of manuscript <https://doi.org/10.1101/2019.12.20.884056>

Dear reviewers and recommender:

Thank you very much for your reviews. We have answered to all questions raised and we think that the manuscript has much improved after addressing the reviews concerns and making appropriate changes in the manuscript.

Please, find below our responses.

Looking forward to hearing from you,

Ignacio Fernandez-Figares

Responses to reviewer #1

The current manuscript shows an interesting metabolic comparison between lean and fatty pigs focus on insulin sensitivity and glucose metabolism. These results increase available data of Iberian breed to improve its characterization. This knowledge could be useful both for Iberian pig production and biomedical studies using the Iberian breed as a biomedical model. In general, the manuscript is well written and has a good explanation of insulin index calculations. These indexes are not common in animal production and with their explanation are easy to understand. However, some main points need to be reviewed to consider the quality of this manuscript.

First, the **number of pigs** of both breeds is low, and metabolite measures usually have high variability. This could be one of reason to draw reliable conclusions because CIs have to be wide. It is only commented in a sentence (L236), and I think this situation could be more discussed at the beginning of Discussion or further studies proposed later to reinforce these results.

We have explained the low number of pigs in the statistical part of this article (L163-170), and we have also modified our conclusion (L394-395).

Second, I missed finding **main data about pigs**. As their age, measures about fat tissues and weight averages separated by breed found in other studies cited below in the Discussion. They are breeds with different growth speeds, so it is difficult to think that they had similar age and weight. These data could be useful to compare their results to other studies. On the other hand, although these breeds are classified as lean and fatty, some variable about their condition, such as subcutaneous backfat, abdominal circumference or BMI (body mass index) could be useful in this metabolism study to improve its interpretation.

Thanks for the comment. We agree with you but, unfortunately, data from their body condition/composition was not taken during this experiment. The comparison between animal breeds with different growth potential is not a simple issue. Because of the substantial changes in e.g. protein turnover occurring during development (Lobley, 1993), it is important that animals are comparable for age or physiological state. The developmental age of the animals may differ, and a decision must be made whether to use pigs of the same BW or age. The study was made on pigs of approximately the same BW because age difference at this early state was considered to be small: (L96-97 and L256; 18 and 14 weeks of age for Iberian compared with Landrace pigs respectively). We have compared in previous occasions Iberian and Landrace pigs of similar weight taking into account these considerations:

Fernández-Fígares, I., Lachica, M., Nieto, R., Rivera-Ferre, M.G., Aguilera, J.F. 2007. Serum profile of metabolites and hormones in obese (Iberian) and lean (Landrace) growing gilts fed balanced or lysine deficient diets. *Livestock Science* 110:73-81.

González-Valero L, Rodríguez-López JM, Lachica M and Fernandez-Fígares I. 2012. Differences in portal appearance of lysine and methionine in Iberian and Landrace pigs. *Journal of Animal Science* 90:110-112.

González-Valero L, Rodríguez-López JM, Lachica M, Fernández-Fígares I. 2016. Contribution of portal-drained viscera to heat production in Iberian gilts fed a low protein diet: comparison to Landrace. *Journal of the Science of Food and Agriculture* 96:1202-1208.

Rodríguez-López, J.M., Lachica, M., González-Valero, L. and Fernández-Fígares, I. 2010. Energy expenditure of splanchnic tissues in Iberian and Landrace growing gilts. *Livestock Science* 133:61-63.

Rodríguez-López JM., Lachica M, González-Valero L, Fernández-Fígares I. 2013. Approaches for quantifying gastrointestinal nutrient absorption and metabolism in a native and a modern pig breed. *Journal of Agricultural Science* 151:434-443.

Lobley GE, Species comparisons of tissue protein metabolism: Effects of age and hormonal action. 1993. *J Nutr* 123: 337-343.

Regarding **statistical analysis and result presentation**, I am not sure about some points. About the fixed effect of time sampling, time is directly considered in analyses with repeated measures. Then, I think it is not necessary to include it as a fixed factor in their explanation because this might be misleading. It seems that authors did more analysis not well explained using time with categories. I would also like to know why to use pooled SEM for both groups and not separated by groups. I prefer the second option (more informative), so review it in F1-3, I do not know whether SEMs are the same in both data or different. This should also be considered in F4-6, there is no variability measure.

We appreciate your comments. In our opinion, the consideration of time as a fixed effect in mixed model with repeated measures is appropriate (Wang and Goonewardene, 2004) and is often found in the literature: (Agyekum et al., 2016), (Montoya et al., 2019), (Theil et al., 2016), (Regmi et al., 2011).

Regarding the use of a single SEM when Iberian and Landrace had different n, we agree with you that each breed must have its own SEM. We have modified the figures 1-6 accordingly.

On the other hand, in F1-3, their captions say that ‘Comparisons versus basal or control treatment’ were made (it is not described in M&M). I suppose that is a contrast of each time point vs basal point (first time) by breed because it is a default analysis that SAS includes when repeated measures are used. These contrasts are made default vs. the last point. I suppose that authors changed it, but then, why the basal point is compared with itself? And how a comparison with the same values is significantly different? Maybe I misunderstood it, but if this is the situation, they should improve the statistical explanation. Alternatively, these p-values could be the result of the comparison between breeds in each point. I do not know, they must clarify it.

We have changed the title of the figures and we have eliminated “comparisons versus basal or control treatment”, which was misleading. As you point out, figures meant to illustrate differences between breeds in each sampling point. Clarifications have been added in the figures and also in the statistical analysis section. Plasma concentration differences between breeds at each sampling time were analysed by the pdiff (piecewise differentiable) option.

I also think there is a mistake in HOMAB results, please review it.

We have revised the results for HOMA-B and cannot find the mistake. What sort of mistake have you found?

Last questions, why is there a common basal mean of all metabolites if some of them are different by breed? Or is there a reason to give that data in common?

You are right. We have given a common value because for most metabolites, no differences between breeds existed. In any case we have included the basal values for each metabolite in the results sections for further information of readers (L190-195).

After these questions, I would like to know which basal levels were used to correct AUC, joint or separated? There are more requests about this section below as minor reviews.

Basal levels per breed (at time -10 min) were used to calculate the corresponding AUC per metabolite. This information now appears in the results section (L190-195).

L127-128 “Basal levels per breed (at time -10 min) were used to calculate the corresponding AUC per metabolite.”

Related to some doubts described above, I think that some points could be improved in Discussion. The last part of the Discussion is well conducted and is easy to understand and follow. However, I do not have the same feeling reading other parts. I recommend a general review to focus on the main idea, but I propose some specific points. At the beginning (L214-227), some sentences mislead to interpret results and are not clear (such as lower insulin concentrations that are not a result here). I think that the age data would be essential to interpret information from cited papers.

Thank you for this comment. We have made an effort to improve the first part of the discussion following your suggestions and included the weight or age of pigs for comparative purposes.

Regarding lactate, I think that high basal lactate levels might indicate a higher risk of insulin resistant, but it is not considered a fact. Indeed, there is evidence of greater changes in lactate release in control individuals than in obese individual after insulin doses. So, the role of this metabolite is not clear, although it is important to consider it.

Thanks for your comment. I would like to have the reference that you mention regarding the decreased lactate levels in obese individuals after an insulin challenge, to include it in the discussion.

At last, the **conclusion** of this study seems to be that these growing Iberian pigs showed differences that may indicate an early stage of insulin resistance (abstract). However, this idea was not possible to find at the end of the Discussion because there was no conclusion or

summary paragraph. Thus, the end looks like unfinished. On the other hand, the Implications section states the insulin resistance without doubts. I think it is important to unify the study conclusion.

Thanks for your input. Conclusion has been modified according to the abstract. Implications have been rewritten from a different perspective.

Moreover, some details should also be considered to improve the quality of the manuscript or its understanding.

First, I recommend changing the two last **keywords**. Maybe using fatty and lean swine breeds could be easier to find.

Ok; we have changed the last two words: fatty pigs, lean pigs.

Second, I suggest reviewing possible **misspelling** and some expression in English. I suppose there is one in some p-values (ex. $0.01 < P < 0.001$; L25-26, 28, 30, 167-69,). I think '> P <' or ' $0.001 < P < 0.01$ ' are correct.

You are right, thank you. We have corrected the misspellings.

There are also some sentences in parentheses without closing.

Ok; done.

Regarding English, I propose to change 'associated to' to 'associated with', 'animals cared' to 'animals were cared' and 'over a 1 min' to 'over one min' and do a general revision. Another point is the use of Iberian alone (without pigs, breed...) because sometimes makes readability difficult.

Ok; thank you.

Regarding **Material and Methods**, I would like to know the intra- and inter-assay CVs rest of molecules, even if they are common (manuscript or annexe). I also think units of fasting insulin and glucose should be indicated the first time they are named, in HOMA index calculations or previously.

Thanks for your comment. For RIA and ELISA, it is customary to report CV intra and inter-assay when you validate the technique according to the matrix you are using. It is not the case for routine analysis, so your request is surprising for us. All the biochemical parameters were analysed in a single assay, so only CV% intra-assay are following: Glucose 4.9%, albumin 3.4%, cholesterol 4.5%, creatinine 5.4%, lactate 3.8% and urea 8.0%.

Also the units for fasting plasma glucose (mM) and fasting plasma insulin ($\mu\text{U}/\text{mL}$) have been indicated at HOMA index calculations. L147-148.

At last, I would like to know if the standard diet and the energy level of maintenance after surgery were the same for both breeds or if different diets and calculation were used for the Iberian breed.

Yes, both breeds were fed exactly the same diet. It was a standard barley-soybean meal diet formulated to cover nutritional requirements of Iberian pigs (145 g crude protein / Kg; 17.8 MJ / Kg). Pigs were fed close to *ad libitum* intake.

Regarding **Results**, I think that the interaction of time and breed is the most interesting difference and would be indicated after fasting values and not to give all the priority to breed differences.

Thanks for your comment. The interaction Breed x Time was significant only for insulin. We agree that this interaction is very interesting and deserve a detailed discussion. However, I cannot see the convenience of including the insulin Breed x Time interaction after fasting values without showing the breed and time effect.

I would also like to know whether there are differences by breed in the fasting values of the rest of metabolites. They gave some common basal levels that have different between both breeds. At last, naming in figure captions the variability measure used in figures would improve their understanding.

Thanks for your comment. We have included the fasting values of all metabolites in the results section.

Finally, there are some points in the **Discussion**. In L239, a common pattern in many models of obesity is commented, please one citation at least.

Thanks for the comment. We have included a reference for this statement. L285.

No significant interaction of breed and time in lactate concentrations were found, so its levels were always higher, please rewrite the sentence (L263-6).

We cannot understand the connexion between the statement in L263-266 and the non significant interaction for lactate between Breed and time. ("Compared with Landrace, the increased lactate AUC in Iberian pigs after the IAGTT could therefore be a consequence of a larger adipose tissue (Nieto et al., 2002) instead of greater insulin sensitivity.")

Cholesterol concentrations in dyslipidemia may be increased. This was not discussed only its HDL and LDL fractions, although their values are not available.

Thanks for the comment. Although cholesterol concentration differences occurred between breeds after the glucose challenge, the levels found were low for both breeds and it would not make sense to state that Landrace pigs were hypercholesterolemic. We have written to clarify:

"In any case the cholesterolemia for both breeds in the present experiment was in the lower range of published values (Fernández-Fígares et al., 2007) and it cannot be considered that Landrace pigs were hypercholesterolemic." L329-332.

I hope this manuscript can be improved. Reading this paper was a pleasant experience allowing me to learn more about insulin sensitivity and other physiological processes in a fatty pig breed, so I encourage authors to do it.

References

Agyekum, A. K., E. Kiarie, M. C. Walsh, and C. M. Nyachoti. 2016. Postprandial portal fluxes of essential amino acids, volatile fatty acids, and urea-nitrogen in growing pigs fed a high-fiber diet supplemented with a multi-enzyme cocktail. *Journal of Animal Science* 94: 3771-3785.

Montoya, C. A., S. J. Henare, P. Zhu, S. M. Rutherford, and P. J. Moughan. 2019. Adaptation of intestinal fermentation over time in the growing pig is influenced by the amount of kiwi fruit consumed. *British Journal of Nutrition* 121: 601-614.

Regmi, P. R., B. U. Metzler-Zebeli, M. G. Gaenzle, T. A. T. G. van Kempen, and R. T. Zijlstra. 2011. Starch with High Amylose Content and Low In Vitro Digestibility Increases Intestinal Nutrient Flow and Microbial Fermentation and Selectively Promotes Bifidobacteria in Pigs. *Journal of Nutrition* 141: 1273-1280.

Theil, P. K., U. Krogh, V. Bjerre-Harpoth, and A. C. Storm. 2016. Hepatic metabolism of anaesthetized growing pigs during acute portal infusion of volatile fatty acids and hydroxy-methyl butyrate. *Journal of Animal Science* 94: 324-327.

Wang, Z., and L. A. Goonewardene. 2004. The use of MIXED models in the analysis of animal experiments with repeated measures data. *Canadian Journal of Animal Science* 84: 1-11.

Responses to reviewer #2

Dear authors,

Thanks a lot for submitting your text to PCI Animal Science.

Please find below my comments about your text.

General comments:

This text present the results on the difference in insulin sensitivity of two breeds of pigs, the Iberian pig (native breed of the Mediterranean basin) and the Landrace pig (conventional breed). The low number of pigs in this study aiming to study the breeding effect is a question. Please justify - and assume - the low (and non-balanced) number of pigs, and the potential consequences on the statistical analyses and results (statistical power of the analysis for example). Then, it seems there is small incoherence between the text and the figures. Please check carefully the graphs, and correct if necessary. If there is no mistake on the graphs, please consider the comments and explain the inconsistency.

[Thank you. We have taken in consideration all your comments. You will find more details below](#)

Details:

Abstract L.19 : Please use the same definition for IAGTT between the abstract and the text. You use intra-arterial glucose challenge in the abstract and intra-arterial glucose tolerance test in the text. Prefer “tolerance test” which is more precise. In order to increase understanding, please use the same words all over the text.

[You are right. We have used exclusively “glucose tolerance test” in the abstract and also in the text.](#)

L. 31 : Please define QUICKI and HOMA-%B. In general, do not use abbreviation at the beginning of a sentence.

[We have changed this sentence. You can now read “Indices for estimating insulin sensitivity in fasting conditions indicated improved \$\beta\$ -cell function in Iberian compared with Landrace pigs,”L33-35.](#)

Implications

L. 51 : Could you please precise exactly what are the implications for the breeds (and the breeders).

[We have changed the perspective of the implications. L48-54.](#)

Introduction

L. 66 : could you please precise the two levels of CP.

Done. We have indicated the levels of CP in the text: 12% and 16% CP. L71.

Materials and methods

L. 80 : I guess it was originally planned to use the same number of animals of each breed ? If yes, could you please indicate it and precise that results were available for only 9 animals. If not, could you discuss the unbalanced design?

Yes, the original idea was to use 5 pigs per breed. We have explained it in the text.

L. 92 : sterile saline : add solution ?

Thank you, you can read now “sterile saline solution”

L. 101 : delete RIA (only use one time)

Ok, done.

L. 112-113 : You used trapezoidal geometry in order to calculate AUC. Could you please give more details concerning this method. How did you process, with R, with Excel ?

Thanks for the comment. Trapezoidal rule is a method for approximating a definite integral: it decomposes the area under the curve into trapezoids (which are decomposed into square, rectangles and triangles) and then you calculate the area of each of them. It can be done manually with Excel but we did it using one of several available computer programs that do it automatically for you (GraphPad Prism, Version 5.02. San Diego, CA). We double checked for some animals determining the AUC using Excel to assure that the calculations were correct.

L. 115 : specify the last AUC. AUC180 ?

Done: “and so on, until the last time AUC0-180”

L. 117 – 118: Please precise IAGTT before «challenge».

Done

Could you explain why you decided to limit the analysis from 0 to 30 min. If we consider the Figure 1, the insulin level is not returned to its basal level at t=30 min, especially for the Landrace breed.

Ideally, we should have analysed the data for 0-45 min for Landrace or 0-30 min for Iberian, so we had to decide which time to use to compare both breeds. We were more interested on the Iberian and so decided to use the 0-30 min time frame. Additionally, for comparative purposes, other authors used 0-30 min interval to calculate β -cell function and insulin sensitivity indexes after an intravenous glucose tolerance test (Christoffersen et al., 2009), (Amoikon et al., 1995).

Amoikon EK, Fernandez JM, Southern LL, Thompson DL, Ward TL and Olcott BM 1995. Effect of chromium tripicolinate on growth, glucose-tolerance, insulin sensitivity, plasma metabolites, and growth-hormone in pigs. *Journal of Animal Science* 73, 1123-1130.

Christoffersen B, Ribel U, Raun K, Golozoubova V and Pacini G 2009. Evaluation of different methods for assessment of insulin sensitivity in Gottingen minipigs: introduction of a new, simpler method. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* 297, R1195-R1201.

L. 116-119 : Could you please cut this sentence in two sentences?

Done

L. 127 : used instead of utilized ?

Yes, thank you

L. 146 : I am bored because there are more sampling by pigs than animals... I suppose you used the autoregressive option in order to consider the pig effect has no interest, and to define a model in repeated measures by assuming that the residuals of the measurements of the same pig are correlated? Could you please precise?

Thanks for the observation. You are right, it is a mistake. When checking the best covariance structure, SIMPLE, CS, AR(1), ANTE(1) and UN covariance structures were assayed. As we are presenting unequally spaced time points in our sampling design, AR(1) is not the appropriate covariance structure. Instead, we have used first-order ante dependence covariance ANTE(1), which allows unequal variances over time and unequal correlations and covariance among different pairs of measurements. In other words, equal spacing between times is not a must. We have clarified it in the text. L175-178.

Moreover, I think it is very ambitious (on a statistical point of view) to test a breeding effect on 4 vs. 5 animals. Could you please justify more the low number of animals used?

We have justified the low number of animals in the statistical section. Five pigs per breed were used in this experiment according to the principle of the three R as requested by the ethical committee. This number has been used in previous works (e.g. Stoll *et al.*, 1999) studying insulin sensitivity in pigs.

However, we agree that the limited number of pigs is a weakness of this work (see conclusion L394-395) and more research is needed to confirm our findings with a larger number of animals.

L. 149 : Did the pig consider as the random effect ? Could you precise.

Yes, the pig was considered a random effect. We have clarified this in the text.

L.152. ad a space between the point and «Homogeneity”, and correct «Homogeneity» in Homogeneity.

Done. Thank you.

L. 156 : Did the pig consider as fixed effect ? Could you explain why you used a GLM model in this case, and a MIXED model in the other case?

Thanks for the comment. For variables without repeated measurements over time (fractional turnover rate, indices of insulin resistance and AUC), both GLM and MIXED are models could be used. However, GLM has traditionally been used although it requires balanced data compared to MIXED. As we have a missing pig (Iberian), we have analysed the parameters with no repeated measures using the MIXED procedure. No differences have been found using any of the approaches. Therefore, we have suppressed in the text the mention to the GLM procedure, which could be misleading to the readers.

Results

L. 163 : use L instead of I for liter abbreviation

Ok, done.

L. 171 : please avoid abbreviation at the beginning of the sentence

Ok, done.

L. 177 : Only tendency for time 90 if we consider Figure 1.

Correct. We have specified that as follow: "such that concentration of insulin was greater in Iberian pigs from -10 to 15 min and from 90-180 min ($P < 0.05$, with $P < 0.1$ at times 0 and 90 min)." L206-207.

L. 178 : Only significant (tendency) at time 25 if we consider Figure 1.

You are right. We have corrected this in the text.

General comments about the insulin results on Figure 1: I wonder about the increase in plasma insulin between -10 and 0 min. Could you please explain what's happen? The increase in plasma insulin is greater than those which occurs after IAGTT. Is there a mistake in the Figure 1? I suppose this is due to the fact that your first sample is not exactly at the same time than the glucose infusion. Can you estimate the exact delay and indicate it in the Figure ?

Good point. Time 0 corresponds to the blood sample taken immediately after the bolus of glucose and subsequent flushing with 5mL of saline solution. In practice, it was very fast and we can estimate 20-30 seconds after the bolus injection.

Details have been given in the text (L102) and also in the Figure: "Concentrations at time 0 correspond to 20-30 s after glucose bolus injection"

L. 187 : Indicate the peak values of glucose for each breed. If we consider a basal glucose level at 4.68 and 5.85 mM, respectively for Iberian and Landrace, and an average increase of 288%, we obtain a glucose level equal to 18.2 and 22.7 mM respectively for the two breeds. It seems inconsistent with the Figure 2, especially for the Iberian pigs which appear greater. Could you check and give the respective increase for both breeds.

Your calculations are corrects. The 288% value is an average between the two breeds. The actual value for Iberian pigs is 19.6 Mm, consistent with Figure 2. However, it is true that this is difficult to appreciate due to the dotted lines and the error bars.

Real values have been indicated in the text L218.

L. 189 : Check it is well 25 min for the Landrace pigs. It is not the case on the Figure 2.

Thank you! You are right. It was a mistake. Now it reads: "Subsequently, glucose concentration gradually decreased to values below fasting levels after 25 min and 30 min, respectively for Iberian and Landrace pigs" L220

L. 190: give the value for each breed and precise the time the nadir occurred for each breed. I think it would be interesting to define nadir.

Ok, done. "The lowest plasma glucose concentrations (glucose nadir) were found at 45 min (2.95 mmol/L and 3.70 mmol/L for Iberian and Landrace pigs, respectively)." L221-223.

General comments about the glucose results on Figure 2: It seems there is an inconsistency between the Figure and the text. In fact, you write glucose peaked immediately after glucose infusion. It is not the case in the figure, which an increase occur from time -10 min to 0. I guess there is the same explanation than for the Figure 1. If there is no problem on the Figure, please explain this increase during fasting, and adjust the text.

Thank you for this interesting comment. We have explained it in the Figures as a footnote: "Concentrations at time 0 correspond to 20-30 s after glucose bolus injection"

L. 195 : not exactly for the Landrace pigs, in which an increase occurred from time -10 (in Figure 3).

Please, see our answer to your previous comment.

L. 201 : and 0-10

Good catch. Thank you!

Discussion

L. 208 : I am surprised by the term "obese". In the introduction, you did not define them as well.

We have included references supporting the use of Iberian pigs as a model for obesity related disorders (Torres-Rovira et al., 2012). The Iberian pig has a greater fat deposition than other pig breeds and is genetically considered an obese breed (Torres-Rovira et al., 2012). As a historical hint, Ossabaw pigs, well known breed in comparative obesity studies, are descendants of the Iberian pig ancestors brought by Spaniards to America in the XVI century. We have included the term "obese" in the introduction in relation to Iberian pigs (L59-60).

Torres-Rovira L, Astiz S, Caro A, Lopez-Bote C, Ovilo C, Pallares P, Perez-Solana ML, Sanchez-Sanchez R and Gonzalez-Bulnes A 2012. Diet-Induced Swine Model with Obesity/Leptin Resistance for the Study of Metabolic Syndrome and Type 2 Diabetes. Scientific World Journal 2012, 510149.

Torres-Rovira L, Pallares P, Vigo E, Gonzalez-Anover P, Sanchez-Sanchez R, Mallo F and Gonzalez-Bulnes A . 2011. Plasma Leptin, Ghrelin and Indexes of Glucose and Lipid Metabolism in Relation to the Appearance of Post-Weaning Oestrus in Mediterranean Obese Sows (Iberian Pig). Reproduction in Domestic Animals 46: 558-560.

L. 221-222: "The limited growth and development of slow growing pigs could result as least partly from lower concentrations of plasma insulin" : This seems not consistent with your results showing greater fasting (and after the IAGTT after 90 min) plasma concentration in Iberian pigs. Please discuss or precise

We have been more precise. You can now read "The limited growth and development of slow growing pigs could result at least partly from disturbances in insulin secretion and/or in insulin binding," L264-266.

L.228 : infusion instead of "ingestion" ?

Yes, thank you!

L. 233 : not 20 min for the Landrace ?

For Landrace plasma glucose concentration was below fasting values within 25 min. This precision is now indicated in the text. L277-278.

L. 235 : replace "challenge" by "tolerance test" or by IAGTT

Ok, done.

L. 239 : please ad a reference

We have included a reference (Kay et al., 2001). L285.

Kay JP, Alemzadeh R, Langley G, D'Angelo L, Smith P and Holshouser S. 2001. Beneficial effects of metformin in normoglycemic morbidly obese adolescents. Metabolism-Clinical and Experimental 50: 1457-1461.

L. 244-245: I don't understand well the interpretation between the greater gastrointestinal tract and the decreased AUC of glucose.

Cells from the intestinal mucosa use a large amount of glucose as energy. So, as Iberian have greater gastrointestinal tract than Landrace pigs, a greater requirement of glucose may be expected and hence lower AUC glucose after IAGTT as shown in Figure 5.

L. 260 : ad “here” or “in the present work” after “we report”.

Ok, done.

L.268 :close the parenthesis.

Ok, done.

L. 285-289 : precise the references

Ok done.

L.307 : indicate the QUICKI index (0.5 vs 0.6) directly after “QUICKI index”

Ok, done. L362.

L. 310-316 : please replace your results in this paragraph

We are not sure of understanding your request. Do you mean to include the results within the paragraphs? We have done so. L368-370.

L. 327-328 : this sentence is not really consistent with the abstract.

Abstract : “may indicate an early stage of insulin resistance”

Discussion : “insulin resistance has not yet been fully established”

Thanks for the observation. We have rewritten the paragraph in discussion and now it reads:

“Although Iberian pigs may be considered an obese breed in terms of body composition (Nieto et al., 2002; Barea et al., 2007), insulin resistance mechanisms have not yet been fully established at the development stage of the pigs in this experiment.” L387-390.

General comments on discussion: it is sometimes difficult to understand if you write about your results or from the bibliography. Could you please precise in each case. If I understand well you make the hypothesis that unexpected results concerning “no insulin resistance evidence in Iberian pigs” is partly due the age of the animals? Could you precise. Could you please take a more assumed position on your results and their discussion.

Thanks for your comment. We have included a reference when citing literature information and stressed when the results are from our experiment.

We have carried out our experiment with young pigs (about 4months for Iberian), but we can guess that as pigs gain weight (and proportionally more fat in the case of the Iberian pigs), insulin sensitivity will decrease.

References :

Check the notation of the pages. There is sometimes space, sometimes no.

We have checked all references and corrected when necessary.

L. 357 : Homogenize the citation of Animal between this citation and this in L. 373 (Gonzales-Valero et al., 2014)

Ok, done.

L. 360 to 365 : references are not in the alphabetical order

You are right. Thank you.

L. 366 to 371 : references are not in the alphabetical order

You are right. Thank you.

L. 377 : Is this a book ? Indicate the chapter, the pages and the editor(s)

Yes, it is. We have completed the reference properly including the chapter consulted.

L. 381 : replace & by and ?

Ok, done

L. 399 : indicate the chapter and pages used

It is chapter 21 Lipid Biosynthesis, subchapter 21.4 Cholesterol, Steroids, and Isoprenoids: Biosynthesis, Regulation, and Transport.

Nelson DL and Cox MM 2017. Cholesterol, Steroids, and Isoprenoids: Biosynthesis, Regulation, and Transport. In Lehninger principles of biochemistry. 7th ed.; W.H. Freeman; New York, NY; pp.816-832.

L. 417 : there is no indication of pages, editors. Is there a book ?

We apologize for the wrong citation. Yes, there is a book but we do not have access to it right now (Covid-19) so we cannot complete the reference (chapter, editor and pages). Instead, we'd rather use the following reference: (Shipley and Clark, 1972; cited by (Gopinath and Etherton, 1989).

Gopinath R and Etherton TD 1989. Effects of porcine growth-hormone on glucose-metabolism of pigs .2. Glucose-tolerance, peripheral tissue insulin sensitivity and glucose kinetics. Journal of Animal Science 67, 689-697.

L. 422 : there is no page

Ok, done

Table 1 :

Please change in accordance with the text “challenge” by “tolerance test”

Ok, done

IAGTT is not necessary because not used after

Ok, done

ns : indicate the p value instead of not significant

Ok, done

Homogenize the notation between mmol/L and mM in accordance with the text

Ok, done

Replace l by L for liter

Ok, done

Table 2 :

Replace «challenge» by «tolerance test»

Ok, done

Indicate the amount of infused glucose

Ok, done.

ns : indicate the p value instead of not significant

Ok, done.

Figure 1 :

Replace «challenge» by «tolerance test»

Ok, done.

Modify the indication : “comparison versus basal or control treatment”. You compared the breeds and not a control to a treatment. And I wonder if it would be better to indicate this in legend instead of in the title.

You are right. We have changed the title of Figure 1 as follows: “Plasma insulin concentrations before and after intra-arterial glucose tolerance test (500 mg/kg BW) in growing Iberian (n = 4) and Landrace (n = 5) pigs”

I don't understand the mean of ***. Is it the p value associated to breed, Time and breed x time ? You could indicate it by a sentence in legend.

The information is not clear. We have deleted the p-values associated to the overall analysis because the reader can find it in table 1.

Homogenize the unit with the text and the Tables.

Ok, done.

ns : indicate the p value instead of not significant

I think you mean for the Figure 2. We have deleted the p-values associated to the overall analysis because the reader can find it in table 1.

Figure 2 :

Consider the same comments than Figure 1

Ok, done.

Figure 3 :

Consider the same comments than Figure 1

Ok, done.

Figure 4 :

Replace « challenge » by « tolerance test »

Ok, done.

Figure 5 :

Replace « challenge » by « tolerance test »

Ok, done.

Replace l by L for liter

Ok, done.

Figure 6 :

Replace « challenge » by « tolerance test »

Ok, done.

Replace l by L for liter

Ok, done.