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Reply

Response to the letter to the editor from Dr. Markus Lacorn

Dear Editors,

We appreciate the interest of Dr. Lacorn in our study "Effects of raw potato starch and live weight on fat and plasma skatole, indole and androstenone levels measured by different methods in entire male pigs" (Food Chemistry, 101, 439–448, 2007). However, most of Dr. Lacorn's comments are in line with our discussion and could not be considered as a criticism.

Dr. Lacorn stated that "growth is linked to maturity and therefore steroid concentrations" There is no disagreement about that in our study. Moreover, we have made a similar comment in the Discussion section (see Section 4.3) of our paper. We have mentioned that the faster growing pigs slaughtered at 90 kg might had reached sexual maturity, thus having high androstenone concentrations in plasma.

Dr. Lacorn stated that the addition of 0.6 kg of starch in the diet without compensation in the control group is questionable. We would like to clarify again that resistant starch is "the sum of starch and products of starch digestion that are not absorbed in the small bowel" (Cummings & Stephen, 2007). It is difficult to know how much starch is resistant in raw potato starch and, therefore, it cannot be completely compensated for. We could assume that an enlarged part of resistant starch in the diet could reduce energy and protein intake, thus negatively affecting growth performance. It is, however, unlikely that those effects would be significant since the pigs were fed resistant starch only two weeks prior to slaughter.

Dr. Lacorn acknowledged that "a real cross-validation needs different assays with different detection principles from different laboratories". Again, there is no disagreement about this in our paper. We compared some available methods to measure skatole and androstenone, but never called this comparison "cross-validation between laboratories". Comparison of the results obtained by the HPLC and GC–MS methods, which was actually performed in different laboratories, was used as a part of method validation after adopting the HPLC method of Hansen-Møller, 1994 in our laboratory.

As described in the Section 2.2.3. *Analysis of androstenone by ELISA*, the calibration standards were spiked with a blank fat and subjected to the same extraction procedure as samples. This means that eventual losses during sample preparation were taken into account when androstenone concentrations were measured. We are surprised that Dr. Lacorn stated that the ELISA method is not repeatable. Intra- and inter-assay variations did not exceed 10% (see Section 2.2.3), thus showing good repeatability and reproducibility of the method. The definitions of repeatability and reproducibility that we used are provided by Causon (1997). The reproducibility in our study was expressed as variability among the measurements of the same sample on different days (inter-assay variations). However, the variability among the measurements of the same sample by different equipment in different laboratories by different operators (also referred to reproducibility) is missing and needs to be performed, as we stated in the Introduction section.

As Dr. Lacorn correctly supposed and as we stated in the Section 2.2.1. *Chemicals*, the antibodies used in our study were the same as those used by Squires and Lundström, 1997. As discussed in our study, the cross-reactivity of the antibodies against 3β- and 3α-androstenols was not measured and this is a potential limitation of the method (see Section 4.1. *Comparison of different methods*). The cross-reactivity of the antibodies against androstenone sulphate has not been determined because (i) androstenone sulphate is not commercially available and (ii) the existence of androstenone sulphate is not completely established, as also stated by Dr. Lacorn.

Dr. Lacorn doubted that some figures and tables were interpreted correctly. According to the equation on Fig. 2, the colorimetric method underestimates skatole values compared to HPLC. Obviously, the opposite would be expected because other indolic compounds also contribute to values obtained by the colorimetric method. We discussed the comparison of those two methods in the Section 4.1. *Comparison of different methods*. Dr. Lacorn stated that "colorimetric determinations ... should not be used in relevant analytical problems". We have made exactly the same point in the discussion section of our paper, although we do not call it an "old-fashion method" since it is still used in Denmark as an on-line method in slaughter houses to sort out tainted carcasses.

We are grateful to Dr. Markus Lacorn for detecting an error in the equation on Fig. 3. The correct equation is as follows: $y = 1.3605x + 0.4366$ (x: HPLC; y: ELISA) and $r = 0.94$. Fortunately, our conclusion on the correlation between those two methods is still valid; the ELISA results overestimated fat androstenone levels compared to the HPLC results.

It is well known that the relationship of skatole to androstenone differs between different studies. The conflicting results from different studies may be due to several reasons: (i) differences in environmental factors which would affect skatole production but would not affect androstenone; (ii) the relationship between skatole and androstenone is age-related and possibly due to the effect of androstenone on skatole hepatic metabolism. Thus, a positive correlation between skatole and androstenone cannot be observed constantly.

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Dr. Lacorn pointed out that our suggestion about a possibility of androstenone sulphation in the intestine is not verified by additional chemical analysis and therefore unnecessary. We would like to emphasise, that the Discussion section in scientific publications should include thoughts and speculations in order to interpret the results and open up new avenues of research. Accordingly, we suggested several alternative explanations based on already published results. To test whether those explanations are correct or not, more research is needed, as stated in the Discussion.

Dr. Lacorn has also made assertions that, based on our paper, incorrect political decisions can be made. The main conclusion of our paper is that "dietary composition and genetic background are important determinants of skatole levels. Future research should address the possibility of reducing other boar taint compounds, especially androstenone". We honestly do not see how this conclusion can be interpreted in other ways than stated. It was previously shown that reduction of live weight (LW) of pigs used in Swedish industry from 115 kg to 90 kg would not eliminate boar taint due to androstenone (Zamaratskaia, Babol, Andersson, Andersson, & Lundström, 2005). The present study demonstrated that even skatole levels can be high in pigs at 90 kg LW, at least in fast-growing pigs.

We believe that our study provides useful results on the factors involved in the regulation of the boar taint compounds, skatole, indole and androstenone. Additionally, we have demonstrated that results on boar taint compounds might differ, depending on the analytical method used for the measurements.

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