#### Supplementary material S1

Relation between feeding behaviour and energy metabolism in pigs fed diets enriched in dietary fibre and wheat aleurone.

K. Quemeneur, L. Montagne, M. Le Gall, Y. Lechevestrier, E. Labussiere

*animal* journal

Blood collection

On day 7, blood was sampled to analyse plasmatic concentrations of metabolites (glucose, lactate, triglycerides, free fatty acids, α-amino nitrogen and urea) and insulin. The catheter inserted in the external jugular vein of the pig was externalized outside of the respiration chamber to collect blood samples without disturbing the animal. The first voluntary meal of day 7 that occurred between 0900 h and 1430 h was considered as a test meal. Blood (10 mL) was sampled at 0830 h and 0845 h (referred as pre-prandial), at the beginning of the meal and 15, 30, 45, 60 minutes after the meal. Blood samples were stored on ice before centrifugation during 10 min at 3000 g (4°C). Plasma was then harvested and stored at -20°C until metabolites and hormone concentration analyses. For each blood sampling, the haematocrit (H) was measured in order to adjust plasma concentrations of metabolites and hormones. For each pig, a basal haematocrit (BH) was calculated as the mean of pre-prandial haematocrits. Plasma metabolite and insulin concentrations were adjusted as follows:

Data analyses

Data were further analysed by multiple factorial analysis and hierarchical classification on principal components to discriminate clusters of pigs using the R statistical package “FactoMineR” (Lê et al., 2008). As blood metabolites and insulin concentrations are affected by feeding behaviour (Reynolds et al., 2010, Le Naou et al., 2014), size of the test meal and pre-prandial metabolites and insulin concentrations, pigs were discriminated on these different factors of variation to analyse their effects on plasma metabolites and insulin kinetics. Three classes of variables were included in the clustering analysis: pre-meal plasma concentrations of metabolites (glucose, lactate, free fatty acids, α-amino nitrogen, urea and triglycerides) and insulin, variables characterizing feeding behaviour over the previous days (number of meals, average meal size (g/kg BW0.60), average duration of meal (minutes), total duration of feed intake per day (minutes), and average daily feed intake (g/kg BW0.60)) and the size of the test meal (g/kg BW0.60). After cluster identification, data were further tested by ANOVA with MIXED procedure of SAS for a cluster effect on feeding behaviour, blood kinetics of metabolites and hormones and energy and nitrogen balances.

Missing data

For pigs without metabolic data (n=10), due to catheter malfunctionning, pre-prandial concentrations of the first meal of the 7th day of measurements were predicted with a PLS procedure. In the respiration chamber, physical activity and gas exchanges (O2, CO2) were recorded continuously. On the basis of gas exchanges, respiratory quotient was calculated as the ratio of volume of CO2 to volume of O2 for 30 minutes before the first meal of the 7th day of measurement. The PLS procedure used feeding behaviour on the week of measurements, physical activity since the last meal, respiratory quotient and gas exchanges (O2, CO2) during 30 minutes before the meal as predictive variables. When predicted pre-prandial concentrations were obtained, pigs were attributed a cluster considering the lowest distance of the pig to the barycentre of each cluster.

References

Le Naou T, Le Floc’h N, Louveau I, Van Milgen J and Gondret F 2014. Meal frequency changes the basal and time-course profiles of plasma nutrient concentrations and affects feed efficiency in young growing pigs. Journal of Animal Science 92, 2008-2016.

Lê S, Josse J and Husson F 2008. FactoMineR: An R Package for Multivariate Analysis. Journal of statistical software 25, 1-18.

Reynolds CB, Elias AN and Whisnant CS 2010. Effects of feeding pattern on ghrelin and insulin secretion in pigs. Domestic Animal Endocrinology 39, 90-96.