1 Phenolic acid-derived metabolite profile in blood (S1)

2 Statistical analyses

3 The data was filtered prior to analysis and only metabolites measured in at least two samples

- 4 within each diet group and for at least 24 samples (including start, adaption period and end)
- 5 were included in further analysis. Of the original 15 metabolites, only 6 passed this filter. The
 6 phenolic acid-derived metabolites passing the filter were: caffeic acid (CA), ferulic acid (FA),
- 7 4-hydroxyphenylacetic acid (PHPA), dihydrocaffeic acid (HCA), benzoic acid (BA),
- 8 phenylpropionic acid (PPA) as well as the internal standard o-coumaric acid (o-CA)¹. The
- 9 data was normalized with respect to the standard (o-CA), and fourth root transformed due to
- 10 heteroscedastic noise. The fourth root was selected instead of for instance the log-transform as
- 11 it can deal with zeroes.

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13 Results and Discussion

14 Phenolic acid-derived metabolites in plasma

Effects of grain, treatment and their interaction on phenolic acids and their metabolites was 15 tested after one week (W1), after three weeks (W3), for difference to start (W3-W0) and for 16 difference between end and adaption (W3-W1). After three weeks there were no significant 17 differences between the diets. After one week FA (Ferulic acid) was significantly higher in 18 oat than barley (p=0.01), whereas PPA (3-phenylpropionic acid) (p<0.01) was higher in 19 barley than oat. This was also observed for PHPA (2-(4-hydroxyphenyl) acetic acid) (p=0.06). 20 For the difference between week3 and week 1, HCA (Dihydrocaffeic acid) was significantly 21 higher in untreated than extruded grain (p=0.01), whereas BA (Benzoic acid) (p=0.03) was 22 significantly higher in oat than barley, the interaction term was nearly significant (p=0.09), 23 indicating an effect of extrusions within barley (BU>BE). Even though significant effects of 24 grain and treatments on phenolic derived plasma metabolites was observed, these differences 25 in plasma metabolites could not be correlated to OTUs. 26

No correlation was found between the data for phenolic acids-derived metabolites in blood
and the microbiota at the end of the experiment (week 3). This could be explained by large
individual differences in the phenolic acids-derived metabolites in blood and that the observed

- 30 effect described by Hole et al. ¹ was due to a shift in phenolic acids-derived metabolites in
- 31 blood in the pigs fed extruded grain from start to end.

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34 **References**

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