Background

- *Mycoplasma hyopneumoniae* is a primary pathogenic bacterium that significantly affects worldwide swine production through enzootic pneumonia and its contribution to porcine respiratory disease complex.

- *M. hyopneumoniae* does not induce a strong and readily detectable systemic immune response, which limits its early detection and prevention. Thus, serologic tests are not sensitive enough to detect antibodies in the initial phase of infection.

- Detection of *M. hyopneumoniae* genetic material early after infection is challenging due to the slow bacterial growth rate and colonization in the lower respiratory tract.

- There is a growing need for surveillance tools that complement diagnostics to demonstrate that populations are *M. hyopneumoniae* negative or to detect early infection in swine populations.

Objective

- The objective of this study was to employ metabolomics analysis as a tool to identify the biomarkers associated with pathological events occurring in early *M. hyopneumoniae* infections in pigs.

Materials and methods

- A subset of serum samples from a previous study (Pieters et al., 2017) were analyzed in this investigation:
  - Serum samples collected from non-infected control pigs.
  - Sera obtained from experimentally infected pigs on 0, 2, 5, 9, 14, 21, and 28 days post-infection (dpi). Pigs were originally experimentally inoculated with 10mL of 1x10^5 CCU/mL of *M. hyopneumoniae* strain 232.
- The metabolites in serum samples were profiled by high-resolution liquid chromatography-mass spectrometry (LC-MS) analysis.
- The metabolite markers were identified by the multivariate statistical analysis of LC-MS data and structural analysis.
- A Random Forest analysis was used to identify metabolites associated with *M. hyopneumoniae* infection.
**Results**

- Principal components analysis (PCA-X) and partial least squares-discriminant analysis (PLS-DA) indicated that experimentally infected and un-infected pigs were in different metabolic status at 14 and 21 dpi.
- A significant increase in the levels of a non-proteinogenic alpha-keto amino acid, alpha-aminobutyric acid (AABA), was identified at 14 and 21 days post infection (p<0.05; Figure 1). This increase in serum AABA was observed in infected pigs, mostly after detected positive for *M. hyopneumoniae* in both LS or TBLF (p<0.05, paired t-test).
- Quantitative analysis of serum free fatty acids (FFA) showed that *M. hyopneumoniae* infection resulted in a greater concentration of total serum FFA (p = 0.058), which was mainly due to the increase of long chain fatty acids in serum. The concentration of FFA such as palmitic, stearic, oleic and linoleic acids in serum samples were significantly elevated at 14 and 21 dpi in infected pigs (p<0.05; Figure 2).
- Stearic and palmitic acids were the most important metabolites for *M. hyopneumoniae* detection and the results were in line with the increased abundance of the long chain fatty acids in infected pigs.

![Figure 1](image1.png)

*Figure 1.* The relative abundance of alpha-amino butyric acid was significantly higher in acutely infected pig serum samples (blue) compared to the un-infected controls (red) at 14 and 21 dpi (p < 0.05).

![Figure 2](image2.png)

*Figure 2.* The abundance of palmitic, stearic, oleic and linoleic acid were significantly higher in acutely infected pig serum samples (blue) compared to un-infected control pigs (red) at 14 and 21 dpi (p < 0.05).

**Conclusions and implications**

- Significant changes in the host amino acid and fatty acid profiles of *M. hyopneumoniae* infected pigs were identified.
- An increased level of fatty acids in the serum was observed to be the predictive variable for detecting *M. hyopneumoniae* at early stages of infection. Nevertheless, the power of the predictive analysis was low with an estimated error rate of 25% due to the limited number of samples.
- Changes observed in the experimental infection need to be validated in the natural course of infection.
- Data from this investigation sheds light on potential pathophysiological mechanisms and host responses to *M. hyopneumoniae* infection.