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Metabolomic changes in lactating multiparous naturally MAP-infected Holstein-Friesian dairy cows suggest changes in mitochondrial energy pathways

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Supplementary Figures and Tables

Supplementary Table 1 Diet and parity for MAP infected and control cattle.

| Cow | MAP Status | Diet | Parity | Early lactation DIM ¹ | Mid lactation DIM ² |
|------|------------|------|--------|----------------------------------|--------------------------------|
| 3547 | Pos | A | 3 | 75 | 145 |
| 2881 | Pos | B | 5 | 82 | 152 |
| 3544 | Pos | C | 3 | 78 | 148 |
| 3269 | Pos | D | 4 | 55 | 125 |
| 3350 | Pos | D | 4 | 79 | 149 |
| 2239 | Neg | A | 7 | 72 | 142 |
| 3190 | Neg | B | 4 | 83 | 153 |
| 3893 | Neg | C | 2 | 87 | 157 |
| 3983 | Neg | D | 2 | 50 | 120 |
| 3014 | Neg | D | 4 | 73 | 143 |

¹ = Days in milk on the early lactation sampling date

² = Days in milk on the mid lactation sampling date

Supplementary Table 2 Composition of the experimental diets used kg/DM/day.

| | A | B | C | D |
|---------------------------------------|--------------|--------------|--------------|--------------|
| Red clover silage | 6.30 | 6.30 | 6.30 | 6.30 |
| Grass Silage | 6.30 | 6.30 | 6.30 | 6.30 |
| Barley ¹ | 3.50 | 3.50 | 3.50 | 6.40 |
| Soyabean meal ¹ * | 0.00 | 1.20 | 0.00 | 0.00 |
| Soypass ¹ | 0.81 | 0.20 | 0.81 | 0.81 |
| Soya hulls ¹ | 3.70 | 3.70 | 3.70 | 2.80 |
| Molassed sugar beet pulp ¹ | 2.05 | 0.95 | 2.05 | 0.00 |
| Rapeseed meal ¹ | 0.00 | 1.00 | 0.00 | 0.00 |
| NovaPro ¹ | 0.89 | 0.40 | 0.89 | 0.89 |
| Metasmart ² | 0.04 | 0.00 | 0.00 | 0.00 |
| Megalac ³ | 0.35 | 0.35 | 0.35 | 0.35 |
| Minerals and Vitamins ⁴ | 0.12 | 0.12 | 0.12 | 0.12 |
| Total | 24.00 | 24.00 | 24.00 | 24.00 |
| Predicted Composition | | | | |
| CP (% DM) | 15.9 | 17.6 | 15.8 | 15.9 |
| MP-N (g/kg DM) ⁵ | 110 | 121 | 110 | 110 |
| MP-E (g/kg DM) ⁶ | 100 | 103 | 100 | 102 |
| MP (limiting)(% requirement) | 97 | 100 | 97 | 99 |
| Methionine (g/100g CP) | 2.3 | 2.0 | 2.0 | 2.0 |

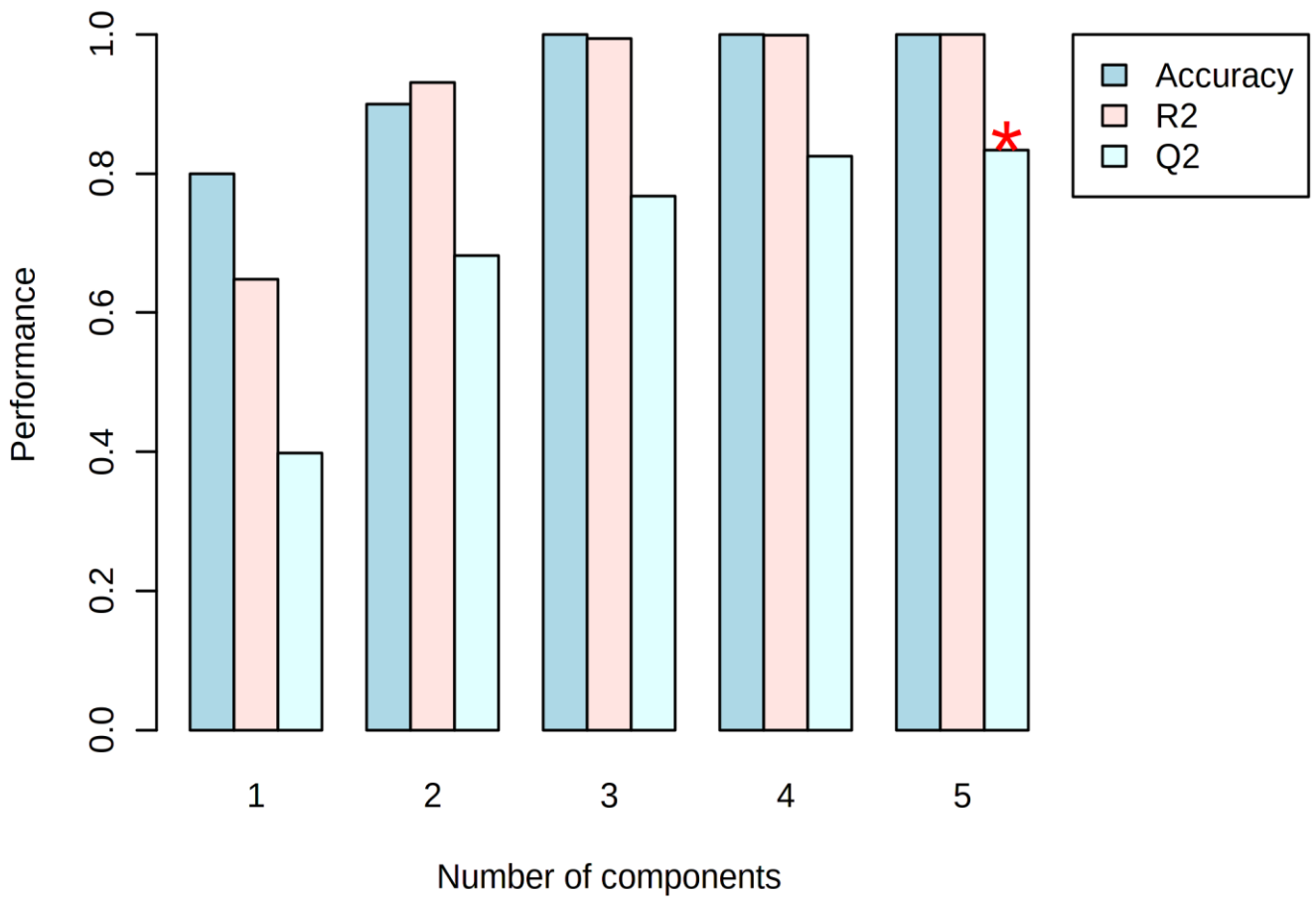
1= KW Alternative Feeds, Staffordshire, UK. 2= Kemin Europa, Belgium. 3= Volac. 4= KW Profeed Harper Dairy I/F vitamin and mineral premix. 5= Metabolisable protein if nitrogen supply is limiting. 6= Metabolisable protein if energy is limiting.

Supplementary Table 3 False discovery rate (FDR)-adjusted two-way ANOVA of metabolites which demonstrated VIP scores > 1.0 when differentiating between MAP infected and control cattle in early and mid-lactation.

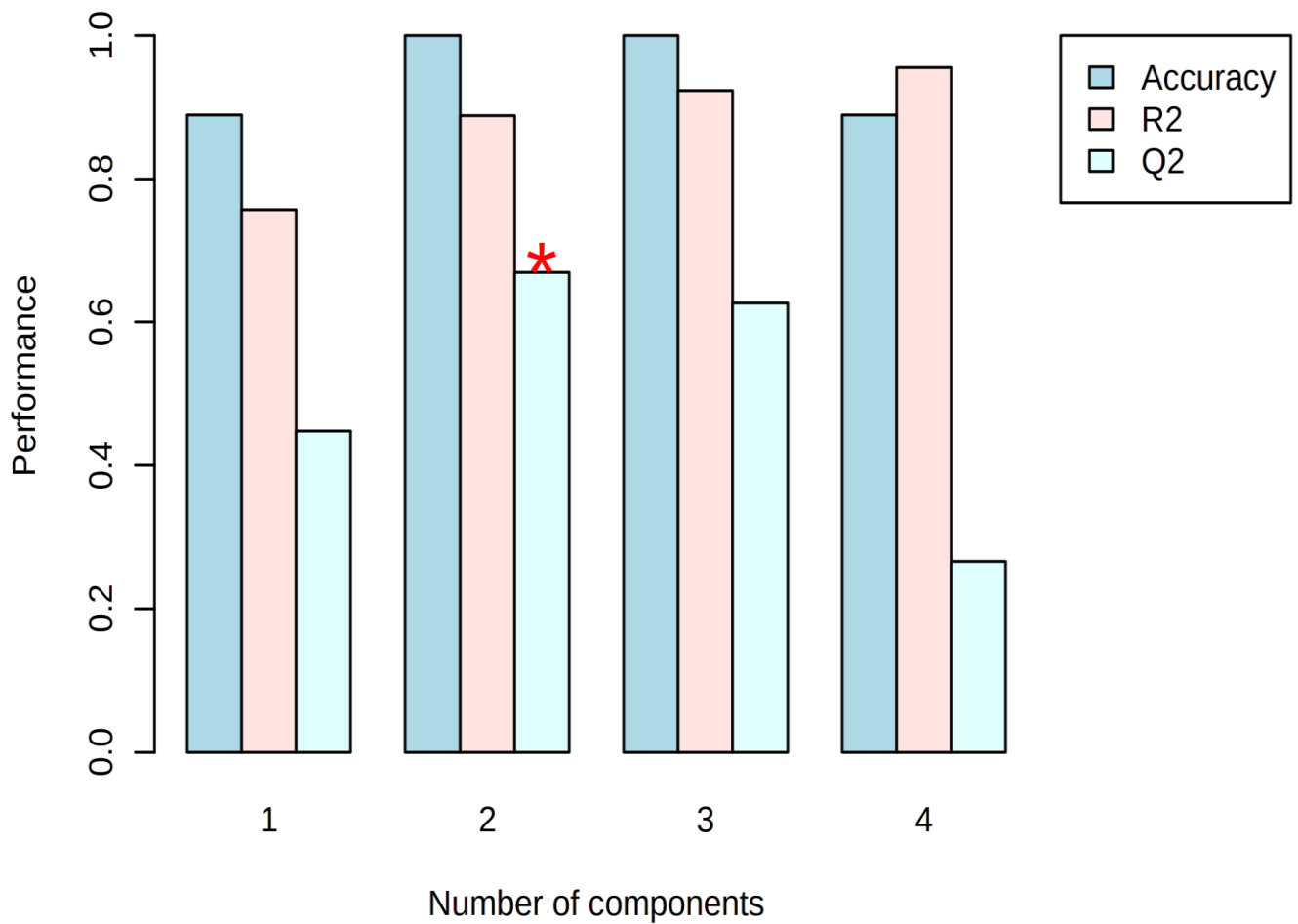
| Metabolite | p-values | | |
|------------------------|-----------|------------|-------------|
| | Lactation | MAP Status | Interaction |
| 2-Butenoic acid | 0.000 | 0.460 | 0.035 |
| Glycerol 1-acetate | 0.002 | 0.025 | 0.897 |
| Galactonic acid | 0.154 | 0.014 | 0.897 |
| 2-Methylpentanoic acid | 0.910 | 0.130 | 0.897 |
| 6-Methylheptanoic acid | 0.910 | 0.130 | 0.897 |
| Glutamic acid | 0.910 | 0.130 | 0.897 |

Supplementary Table 4 Milk yield and composition for MAP infected and control cattle within early and mid-lactation.

| Item | MAP Status | | S. E | P-value |
|---------------------------------|------------|--------|------|---------|
| | Pos | Neg | | |
| Early Lactation | | | | |
| Milk Yield, kg/d | 37.8 | 37.4 | 5.19 | 0.942 |
| Fat, g/kg | 44.2 | 43.8 | 2.47 | 0.890 |
| Protein, g/kg | 31.63 | 31.5 | 0.86 | 0.924 |
| Lactose, g/kg | 44.7 | 45.1 | 0.99 | 0.752 |
| Fat Yield, kg/d | 1.66 | 1.62 | 0.17 | 0.790 |
| Protein Yield, kg/d | 1.2 | 1.16 | 0.14 | 0.765 |
| Lactose Yield kg/d | 1.69 | 1.68 | 0.22 | 0.969 |
| MUN, mg/dL | 14.3 | 17.1 | 2.40 | 0.307 |
| Milk SCC, log ¹⁰ /mL | 1.94 | 1.44 | 0.48 | 0.354 |
| Mid Lactation | | | | |
| Milk Yield, kg/d | 35.1 | 37.3 | 6.20 | 0.749 |
| Fat, g/kg | 38.5 | 45.7 | 4.27 | 0.190 |
| Protein, g/kg | 32.2 | 32.9 | 1.26 | 0.602 |
| Lactose, g/kg | 44.8 | 45.6 | 0.25 | 0.054 |
| Fat Yield, kg/d | 1.36 | 1.66 | 0.15 | 0.128 |
| Protein Yield, kg/d | 1.14 | 1.21 | 0.16 | 0.689 |
| Lactose Yield kg/d | 1.58 | 1.70 | 0.29 | 0.697 |
| MUN, mg/dL | 11.8 | 13.6 | 2.16 | 0.462 |
| Milk SCC, log ¹⁰ /mL | 1.9715 | 1.5243 | 0.31 | 0.239 |

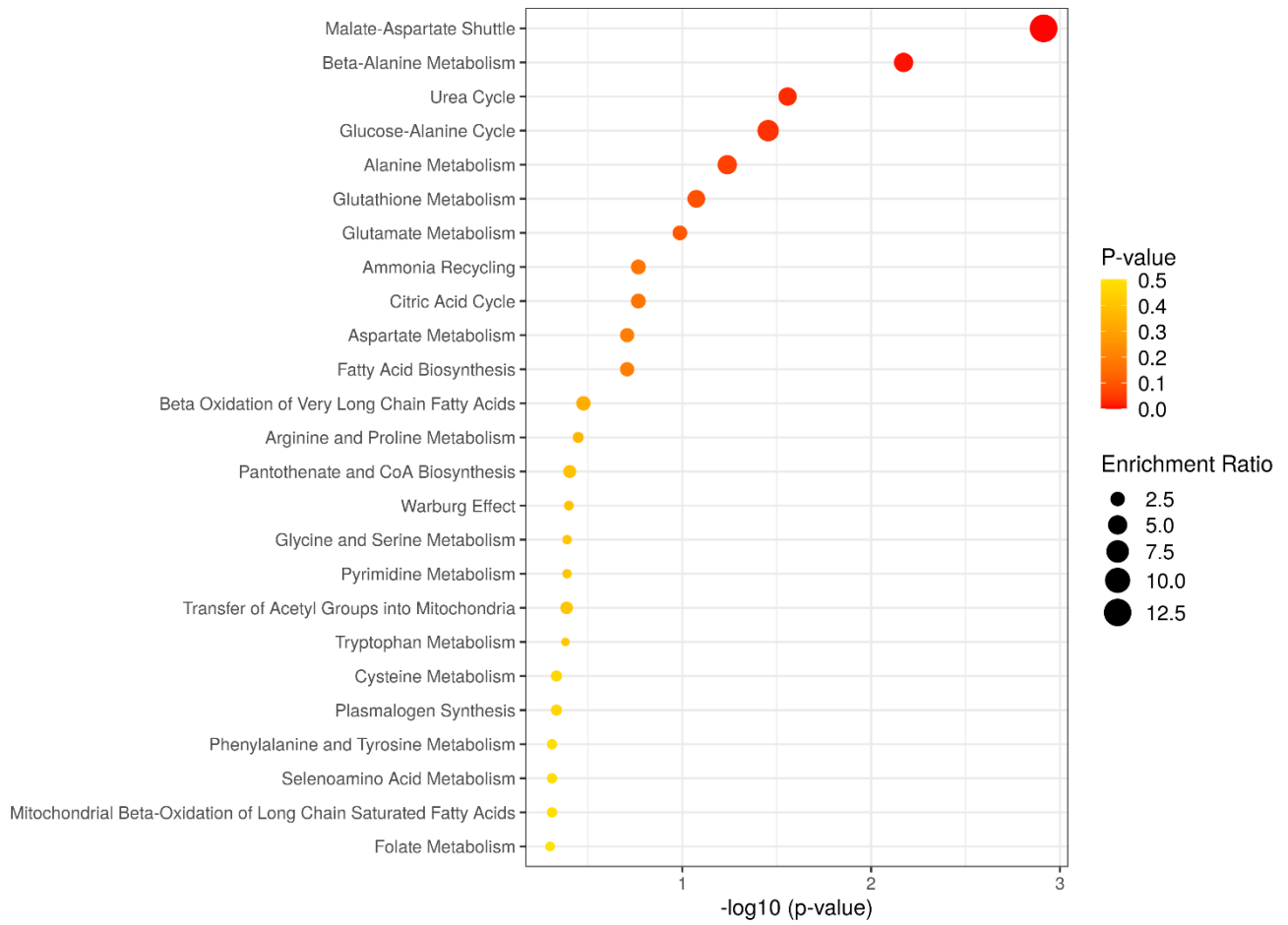


Supplementary Fig. 1 Cross validation of the PLS-DA for MAP-infected and control cattle in early lactation. Blue bars indicate the accuracy of the model, pink bars (R2, variations) indicate the goodness of fit, and light-blue bars (Q2, prediction of the model) indicate the goodness of prediction. Good predictions with a high Q2 value are marked by *. This was achieved when five components were considered. Minus Q2 results would indicate that the model had no predictive power.



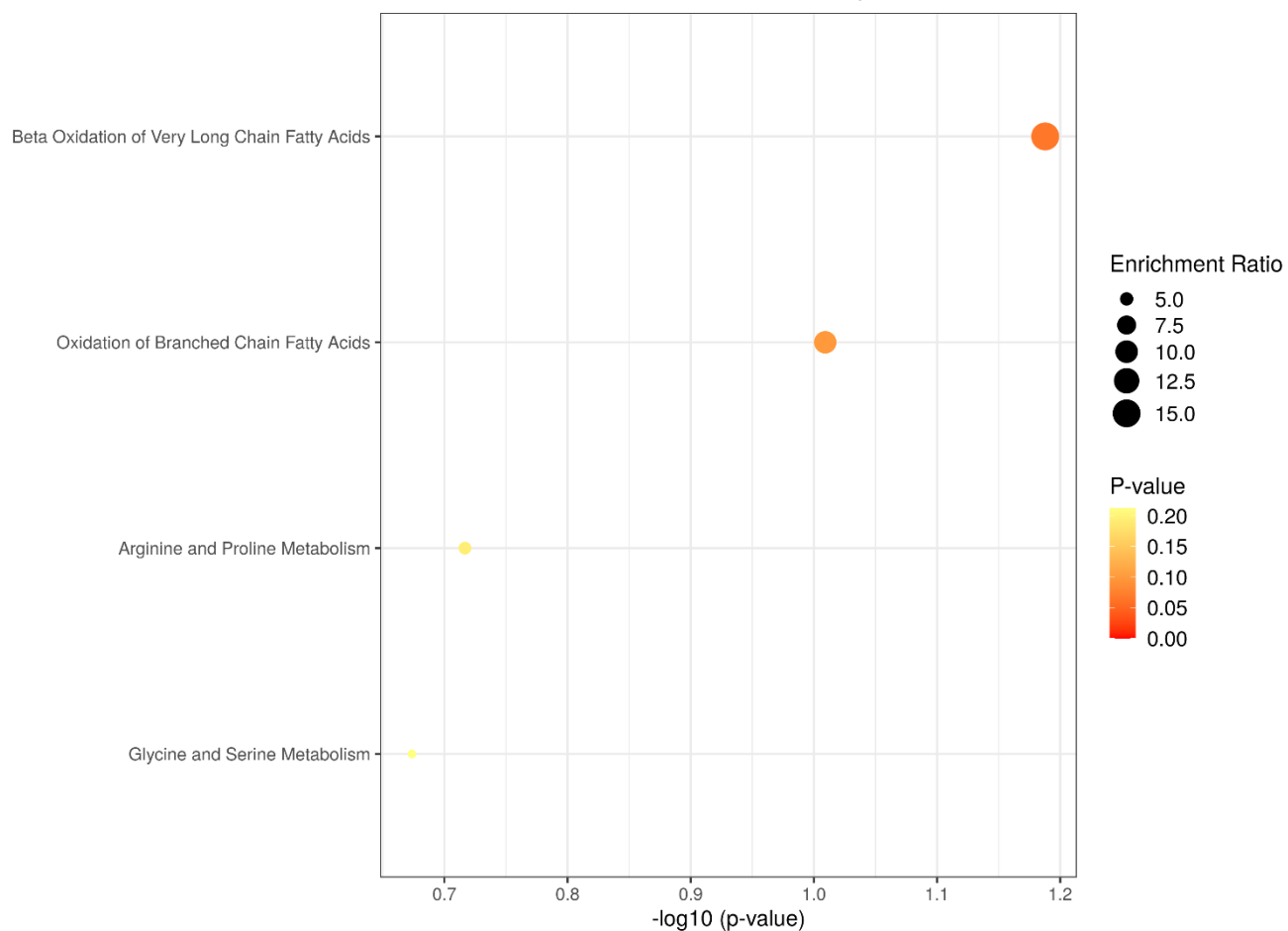
Supplementary Fig. 2 Cross validation of the PLS-DA for MAP-infected and control cattle in mid-lactation. Blue bars indicate the accuracy of the model, pink bars (R2, variations) indicate the goodness of fit, and light-blue bars (Q2, prediction of the model) indicate the goodness of prediction. Good predictions with a high Q2 value are marked by *. This was achieved when two components were considered. Minus Q2 results would indicate that the model had no predictive power.

Overview of Enriched Metabolite Sets (Top 25)



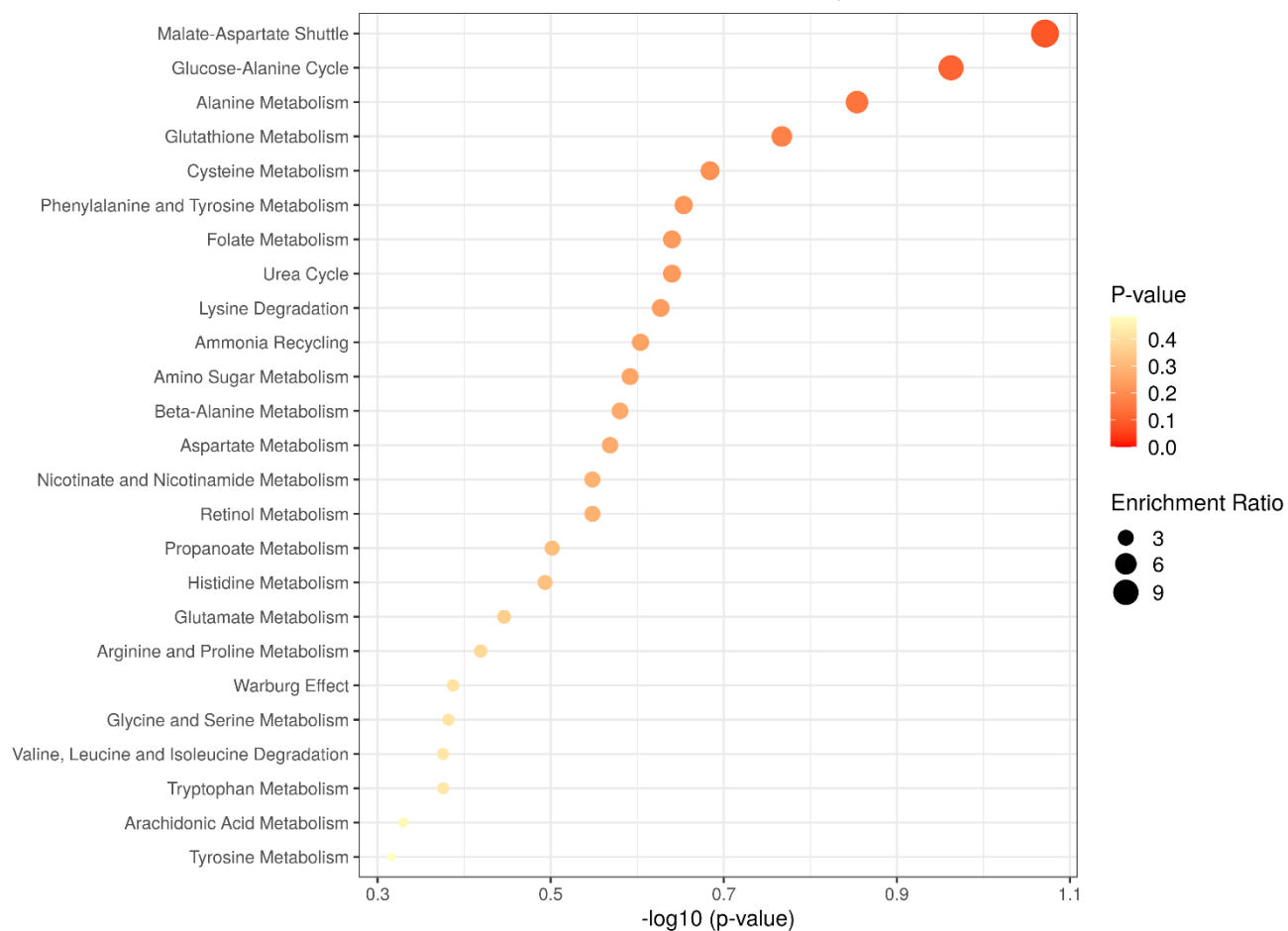
Supplementary Fig. 3 Significantly enriched pathways in naturally MAP infected cattle in early lactation in the negative ionisation mode.

Overview of Enriched Metabolite Sets (Top 25)



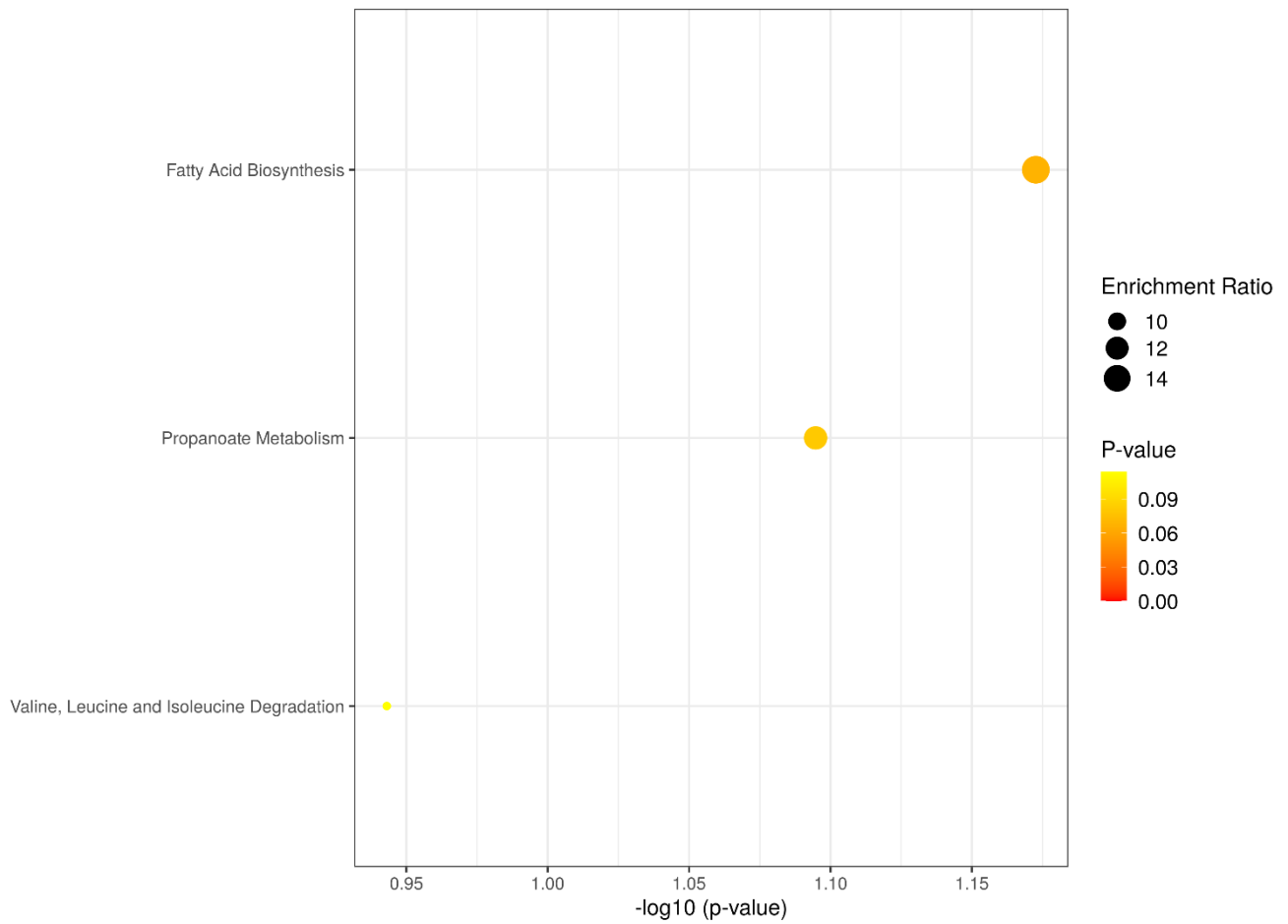
Supplementary Fig. 4 Significantly enriched pathways in naturally MAP infected cattle in early lactation in the positive ionisation mode.

Overview of Enriched Metabolite Sets (Top 25)

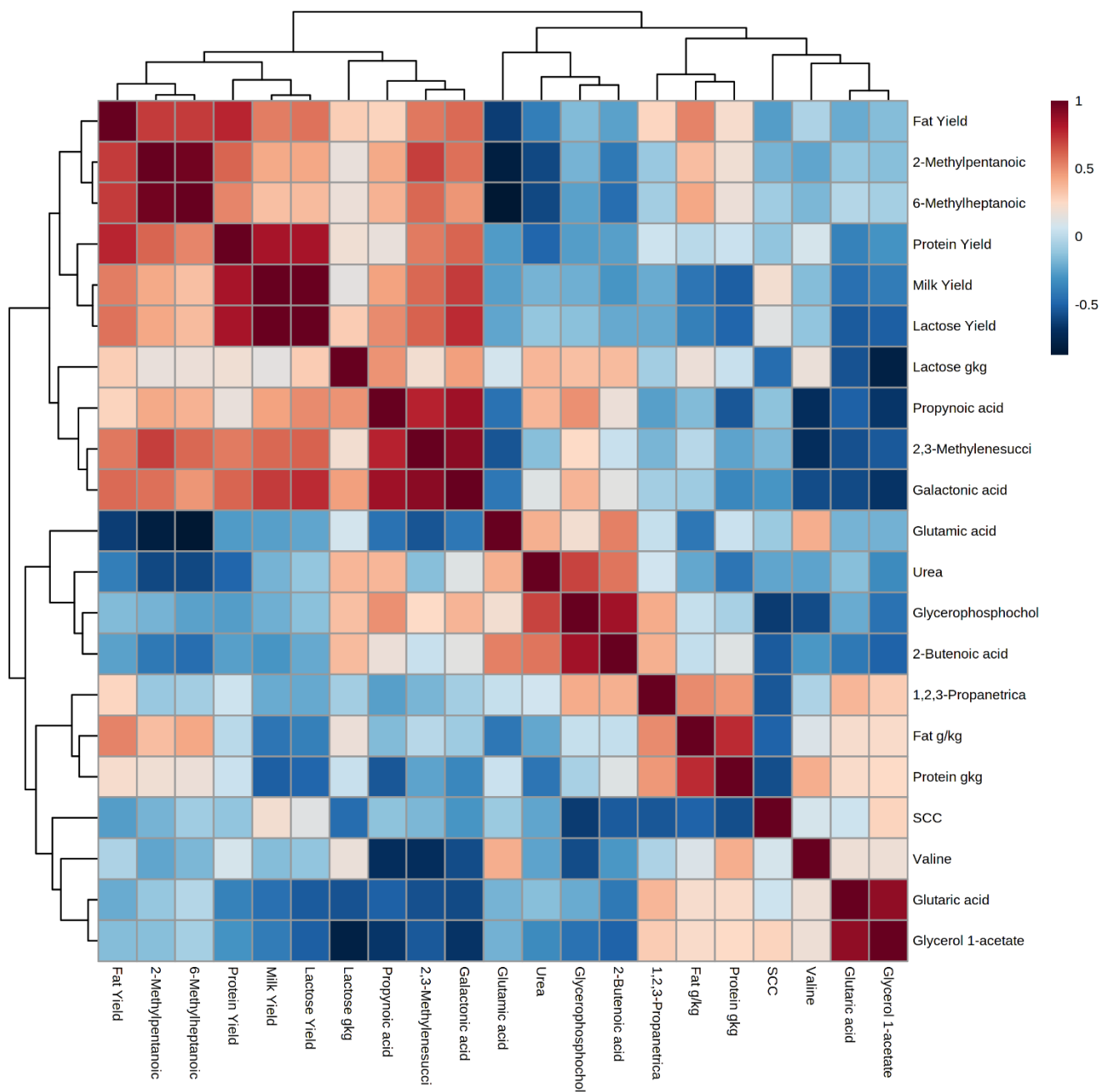


Supplementary Fig. 5 Significantly enriched pathways in naturally MAP infected cattle in mid-lactation in the negative ionisation mode.

Overview of Enriched Metabolite Sets (Top 25)



Supplementary Fig. 6 Significantly enriched pathways in naturally MAP infected cattle in mid-lactation in the positive ionisation mode.



Supplementary Fig. 7 A heatmap of the Pearson's correlation coefficients produced by comparing metabolites significantly affected by MAP infected and control cattle with milk composition in mid-lactation. Positive correlations are shown in red, negative correlations are shown in blue.