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Optimal gut function in monogastric livestock

^{1}H-NMR metabolomics reveals alterations in the metabolism of ascarid-infected laying hens

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Abstract

1H-NMR metabolomics reveals alterations in the metabolism of hens infected with ascarids and concurrent histomonosis infection

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Gastrointestinal infections with nematode species including Ascaridia galli and Heterakis gallinarum are associated with impaired performance in chickens, particularly in high-performing breeds. Furthermore, H. gallinarum is a vector of Histomonas meleagridis which causes blackhead disease in poultry. Here, we provide the first mechanistic insight into the alteration of chicken plasma and liver metabolome due to ascarid infections with concurrent histomonosis. 1H nuclear magnetic resonance (1H-NMR) metabolomics was applied to explore the variation in the metabolite profiles of the liver (N=105) and plasma (N=108) from laying hens experimentally infected at 24 weeks old with A. galli and H. gallinarum in comparison to uninfected chickens at 2, 4, 6, 10, 14 and 18 weeks post infection (wpi). A total of 31 and 54 metabolites were quantified in plasma and aqueous liver extracts, respectively. Data analysis showed no significant difference ($P > 0.05$) in any of the 54 liver metabolites between the two groups. In contrast, 20 plasma metabolites showed significantly elevated concentrations in the infected samples ($P < 0.05$). Alterations of plasma metabolites occurred in wpi 2, 6 and 10, covering the pre-patent period of worm infections. Plasma metabolites with the highest variation at these time points included glutamate, succinate, trimethylamine-N-oxide, myo-inositol, acetate, and glucose. Pathway analysis suggested that infection induced changes in 1) phenylalanine, tyrosine, and tryptophan biosynthesis; 2) alanine, aspartate and glutamate metabolism; and 3) arginine and proline metabolism.

In conclusion, 1H-NMR metabolomics revealed significant alterations in the plasma metabolome of hens infected with ascarids. The alterations were dependent on the presence and patency of infections which caused an upregulation of key metabolic pathways.

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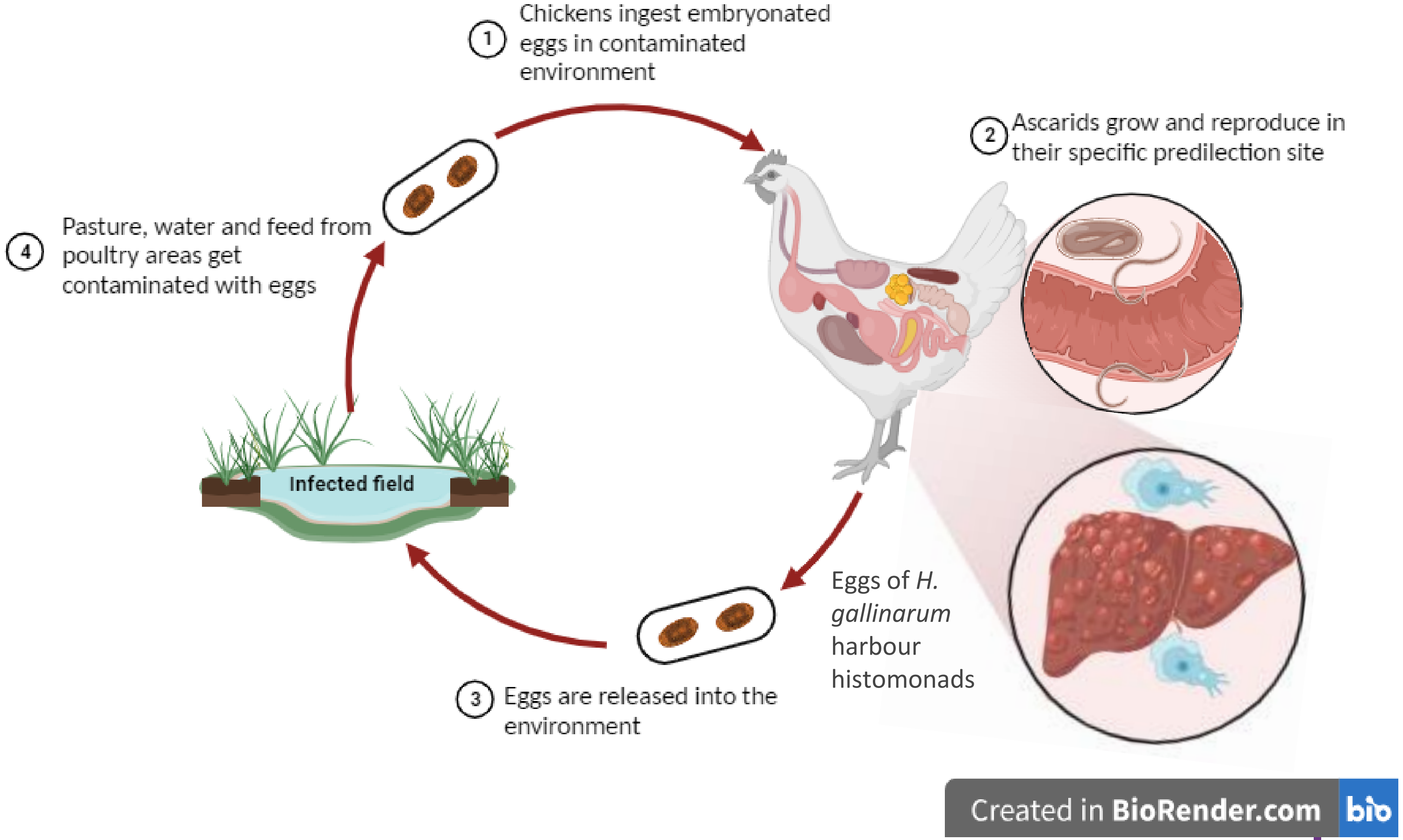
Background

- Gastrointestinal nematode infections are common in chickens with negative effects[1,2,3]

Ascarid infection with direct life cycle



① Chickens ingest embryonated eggs in contaminated environment

② Ascarids grow and reproduce in their specific predilection site

③ Eggs are released into the environment

④ Pasture, water and feed from poultry areas get contaminated with eggs

Eggs of *H. gallinarum* harbour histomonads

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(1) Jansson et al., 2010, *Avian Pathology*
(2) Sharma et al., 2019, *Poultry Science*
(3) Stehr et al., 2019, *Veterinary Parasitology*

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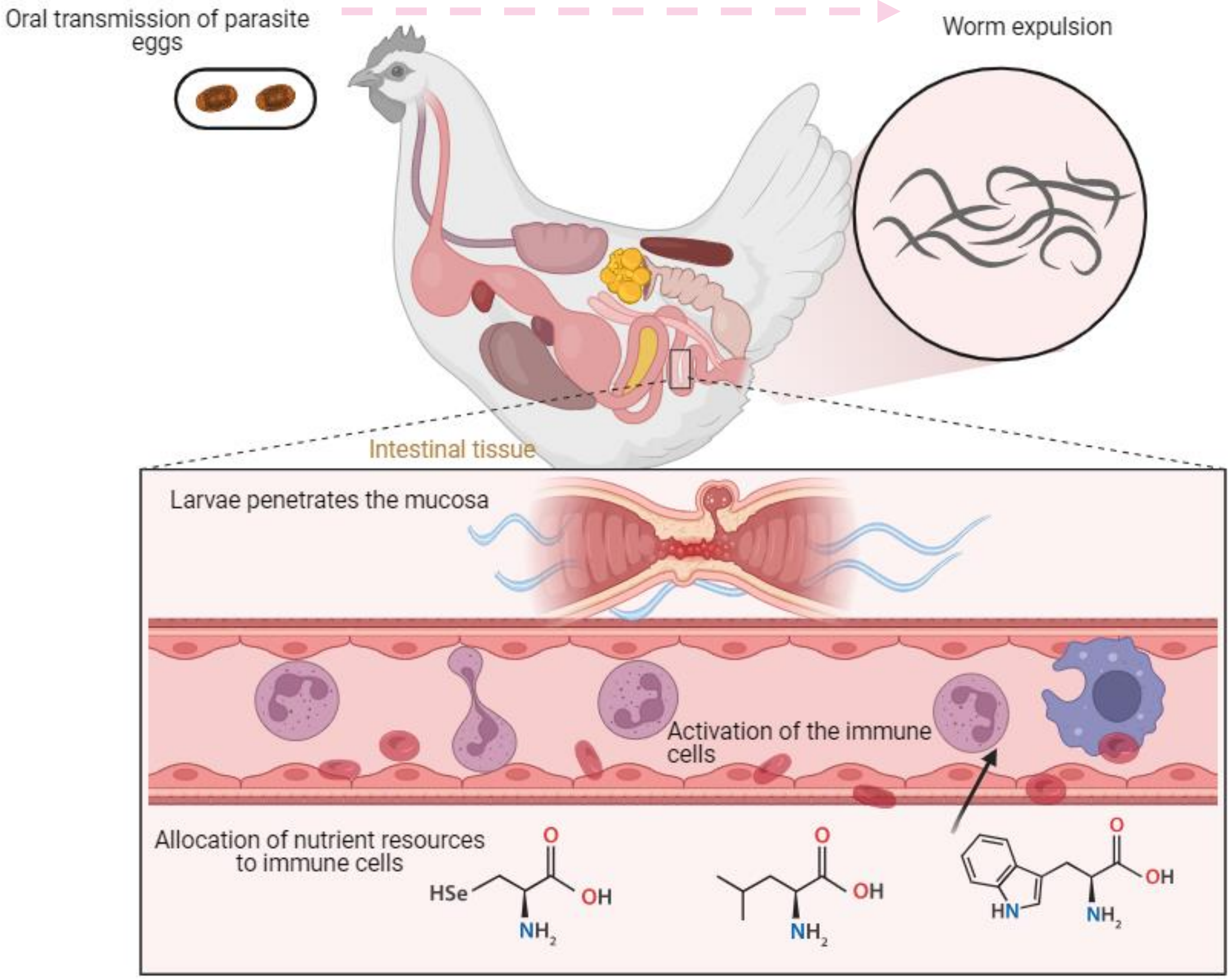
Background

- Birds can initiate an immune response leading to effective expulsion of worms[1]



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(1) Stehr et al., 2018, *Int. Journal of Parasitology*

Hypothesis

- There is a possible interchange in allocation of metabolic resources between defense and performance in the infected host[1,2]
- Identification of infection-induced alterations in metabolic status over time can provide insight into potential patho-physiological changes

Objective

- To study the metabolomic alterations in plasma and liver associated with worm development

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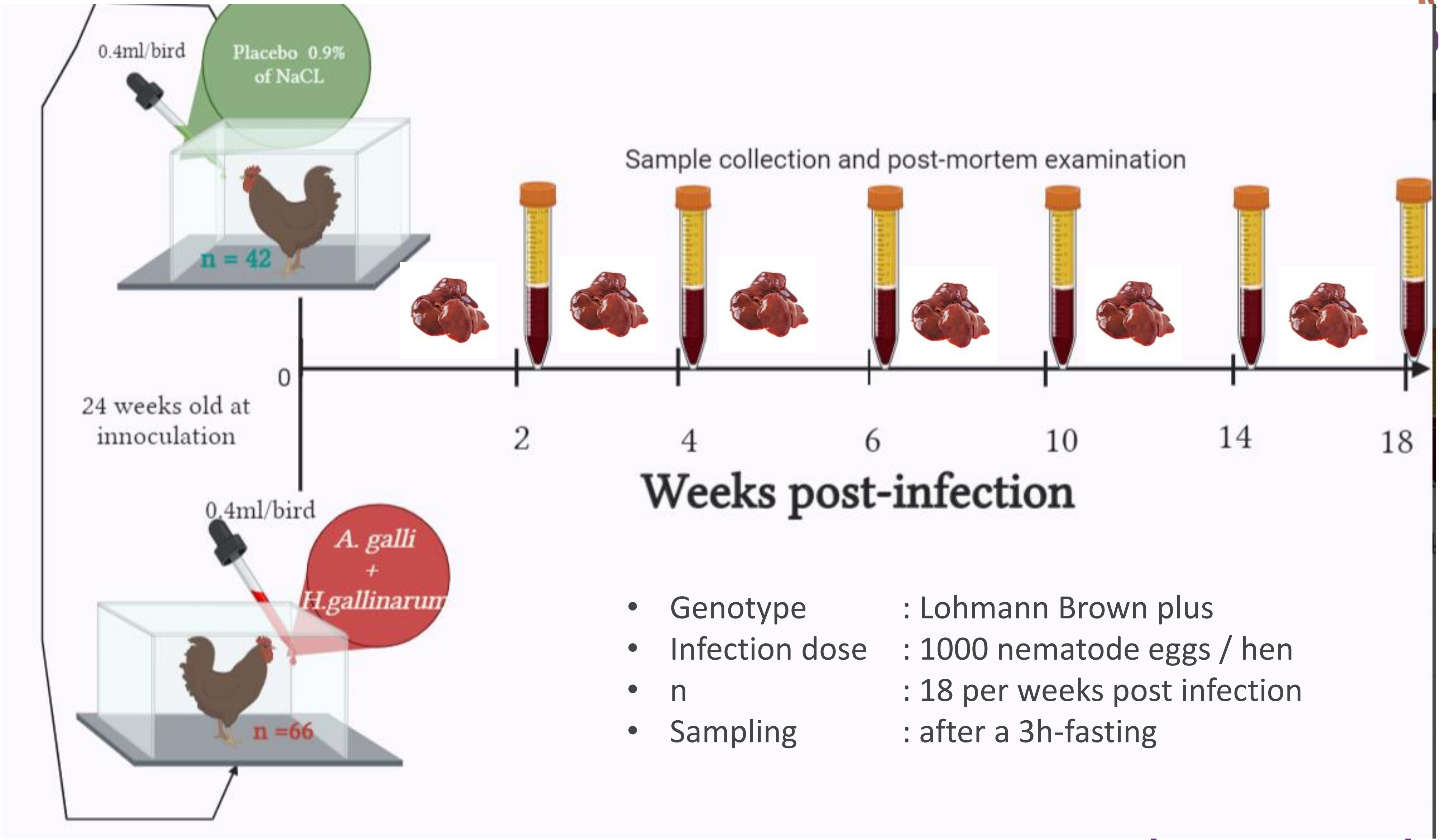
(1) Stehr et al., 2019, Veterinary Parasitology
(2) Most et al., 2011 *Functional Ecology*

Experimental design



- Genotype : Lohmann Brown plus
- Infection dose : 1000 nematode eggs / hen
- n : 18 per weeks post infection
- Sampling : after a 3h-fasting

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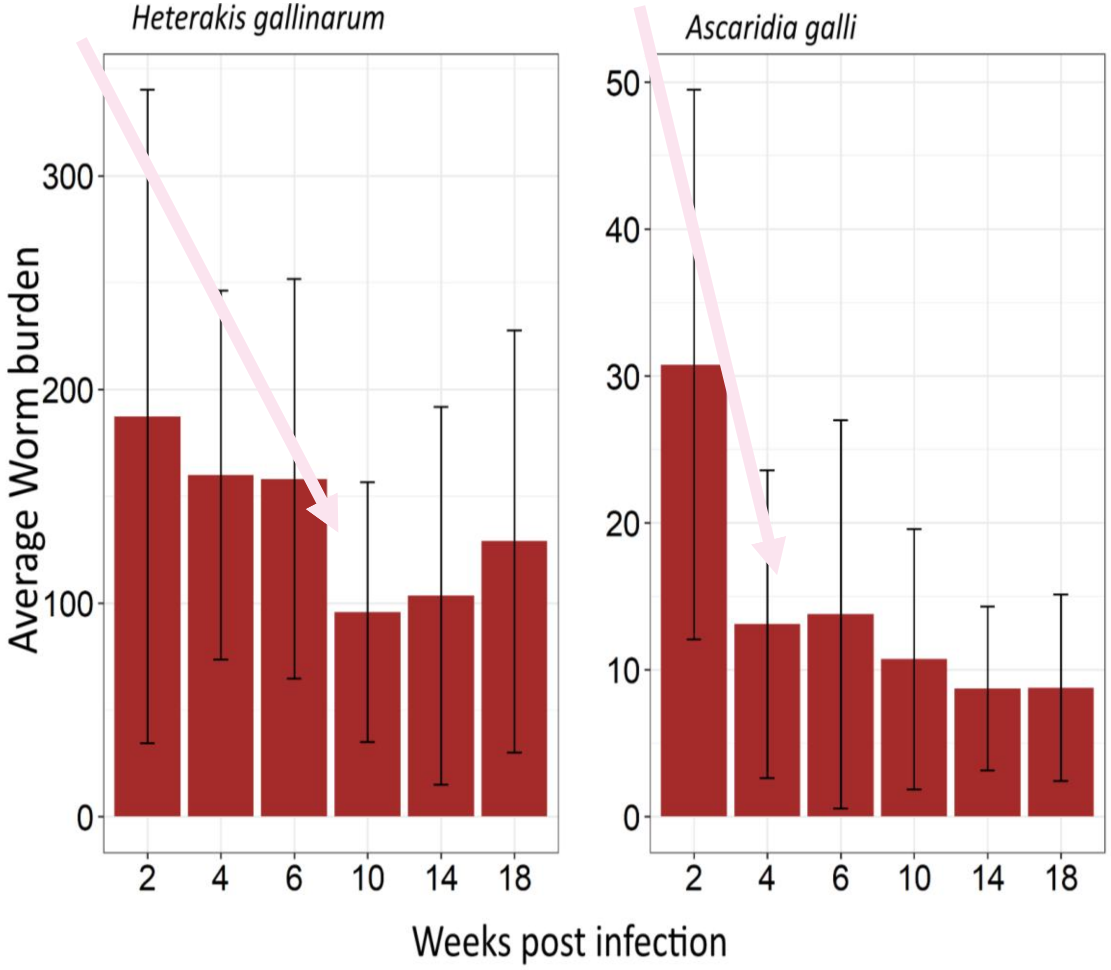
Worm recovery from infected chickens



Heterakis gallinarum

Ascaridia galli

- All infected hens harbored worms throughout the experimental period
- Worm expulsion was highest during early phase of infection

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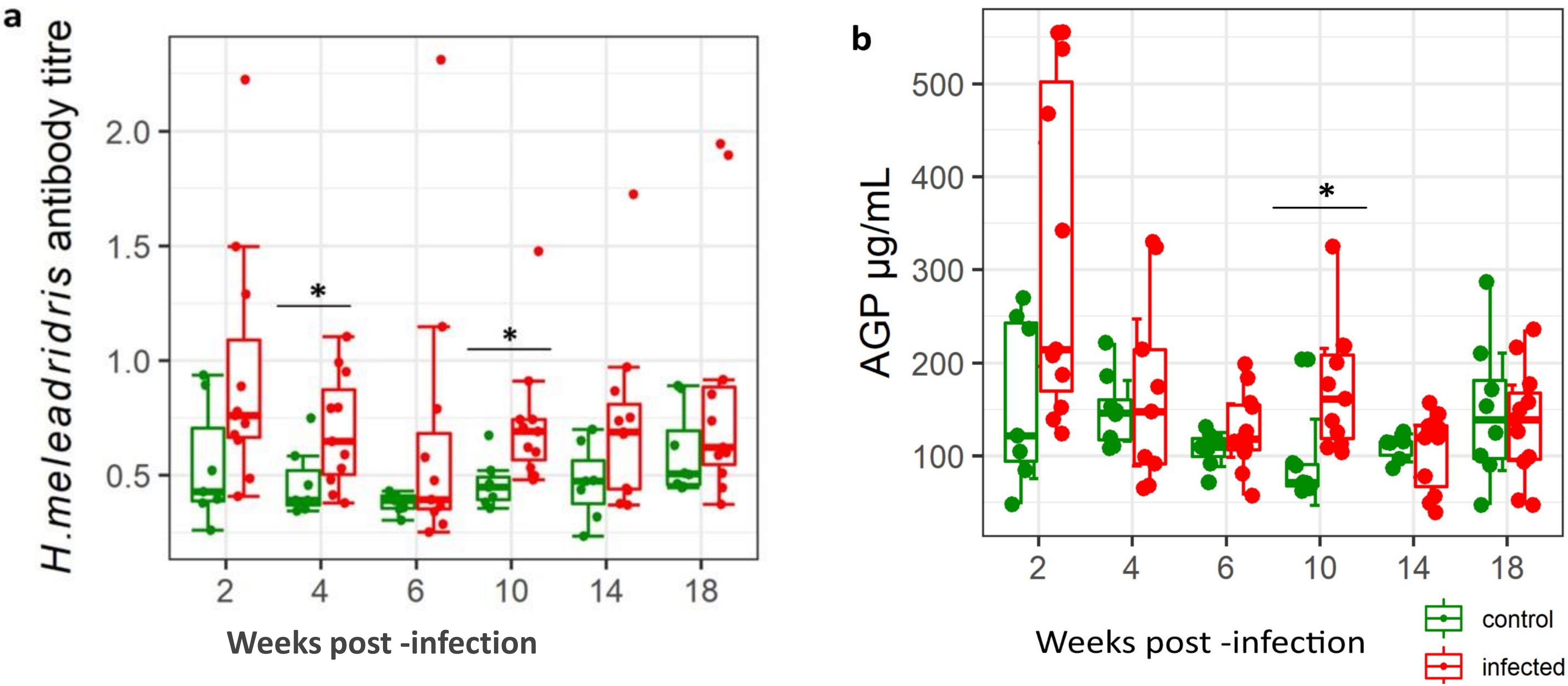
Involvement of histomonosis in the infections



- About 65% of experimentally ascarid-infected birds were positive histomonas infection
- The increased AGP in plasma suggests tissue inflammation

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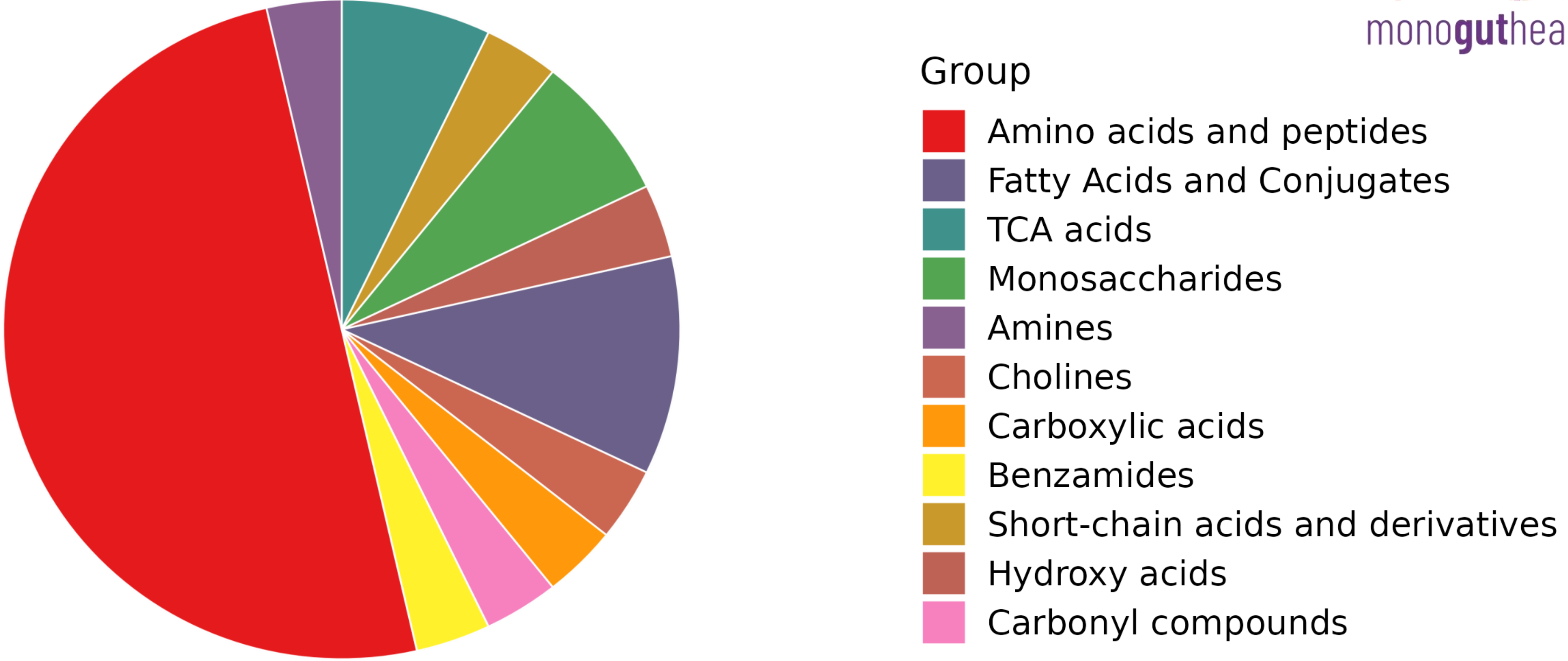
***AGP** – Alpha-1-acid glycoprotein*

Compounds from NMR spectroscopy



- A total of 31 and 54 compounds were identified in the plasma and liver respectively
- Amino acids were the most abundant compounds

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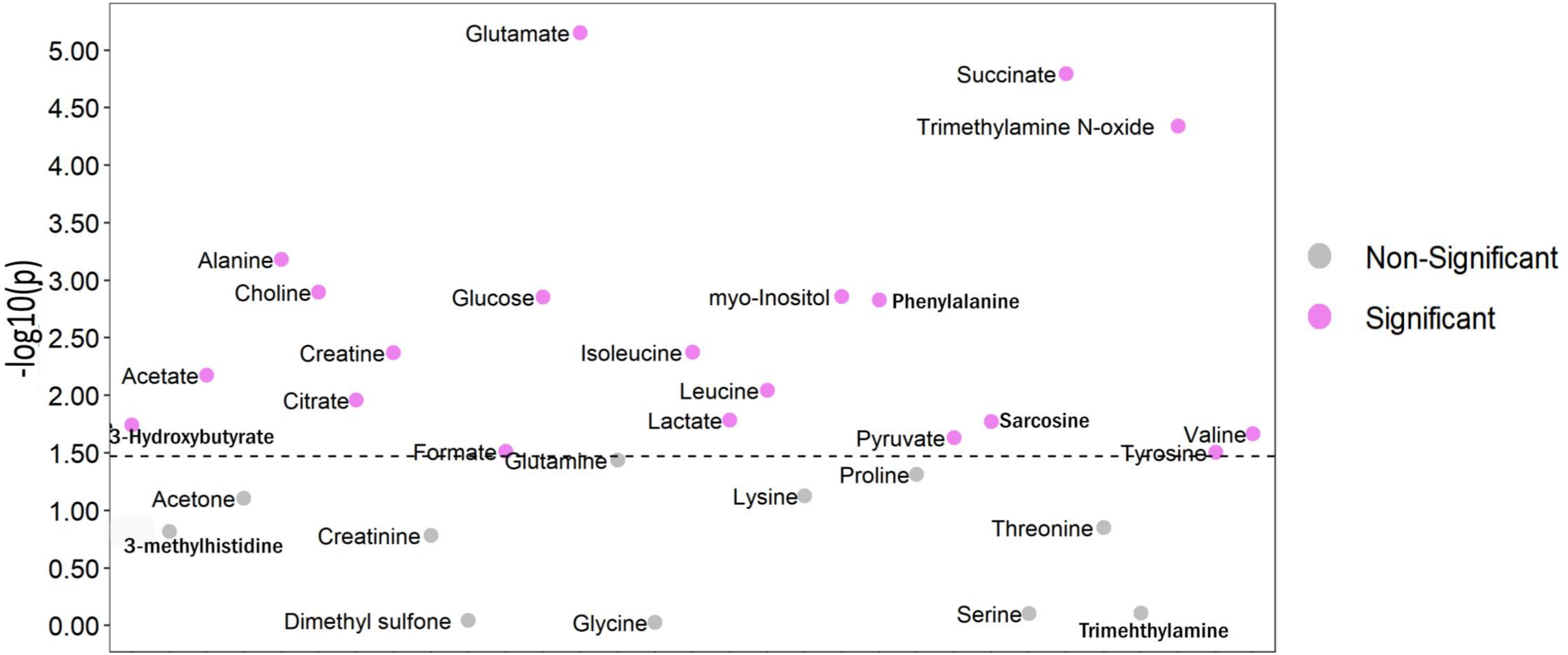
Infection-induced alterations in liver and plasma metabolites

- None of the liver metabolites was significantly altered by the infections
- Twenty plasma metabolites were significantly different between infected and non-infected hens



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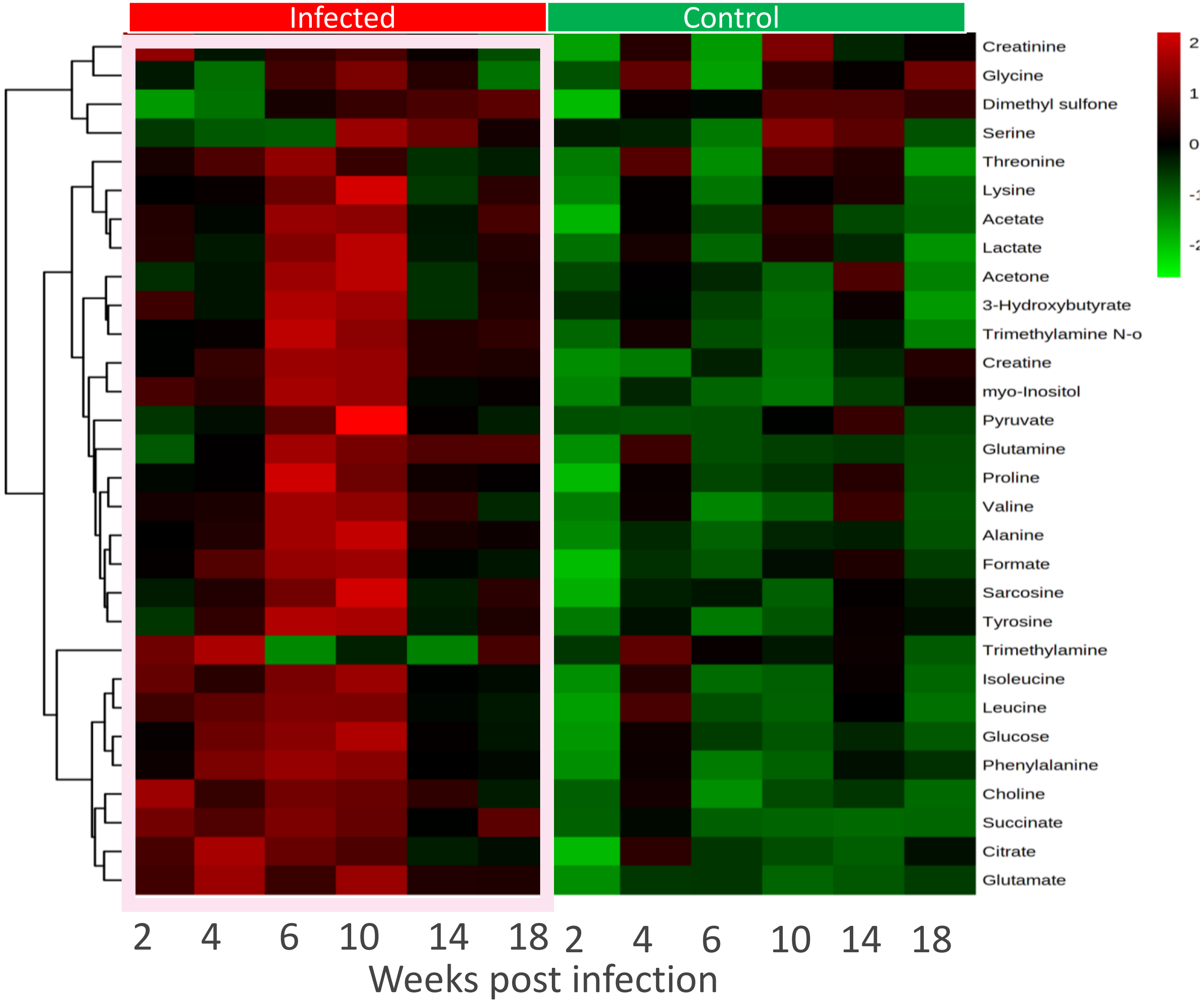
Plasma metabolites at different time of infection



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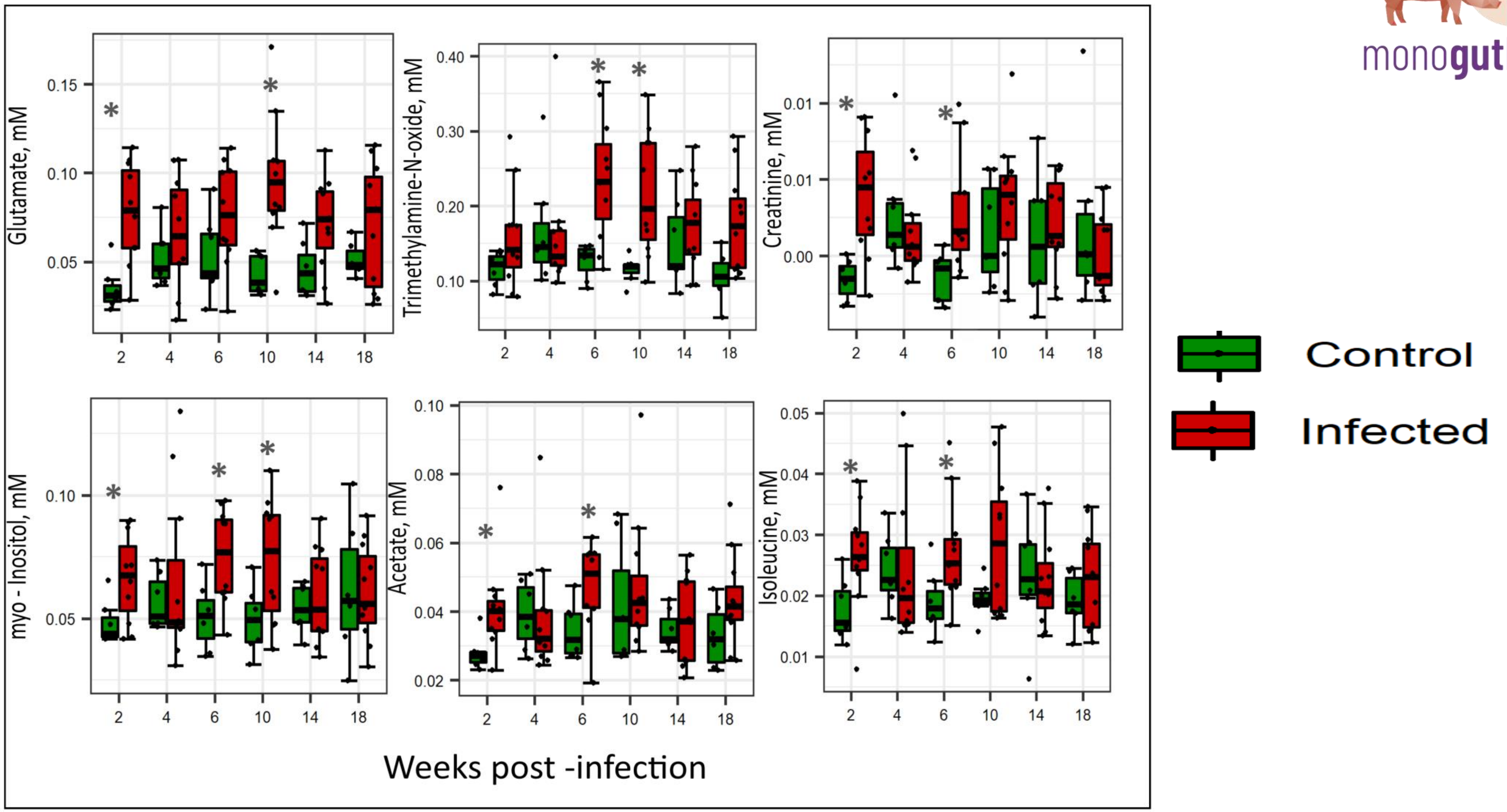
Time-dependent changes in selected plasma metabolites



- Most changes in wpi 2, 6 and 10
- Overlapping with the pre-patent period of worms

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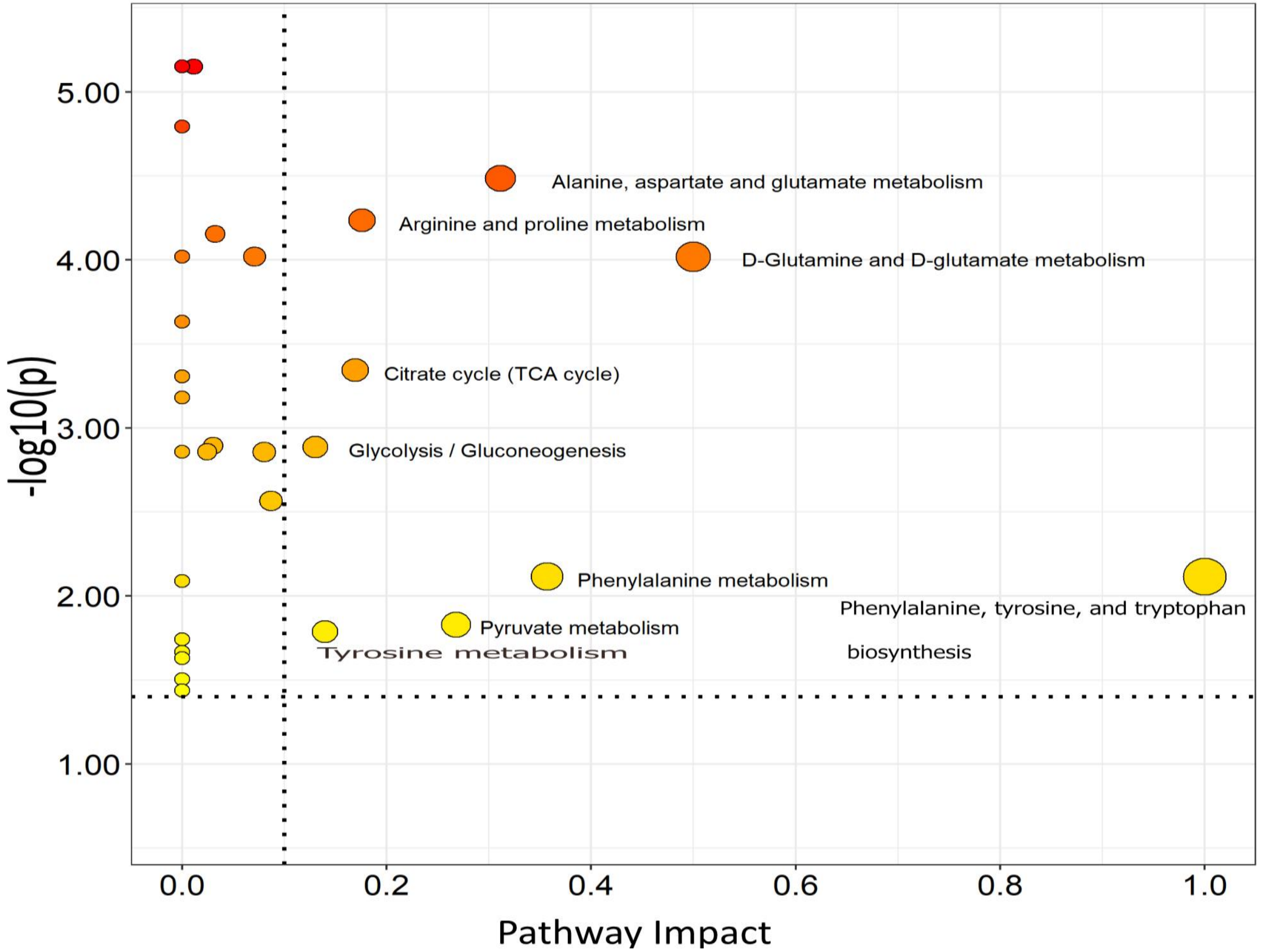
Alteration of metabolic pathways induced by infection



- Upregulation of important metabolic pathways in infected hens

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Conclusion

- Significant alterations in the plasma metabolome of nematode infected chickens
- The alterations were dependent on the presence and patency of infections

Outlook

- Integrating other omics technologies (e.g., genomics, transcriptomics, proteomics)
- Detailed analysis of intestinal tissues as the site of infection
- Candidate metabolites as infection proxy

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