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Rutin and Quercetin Biotransformation in Ruminants Revealed by Molecular Networking

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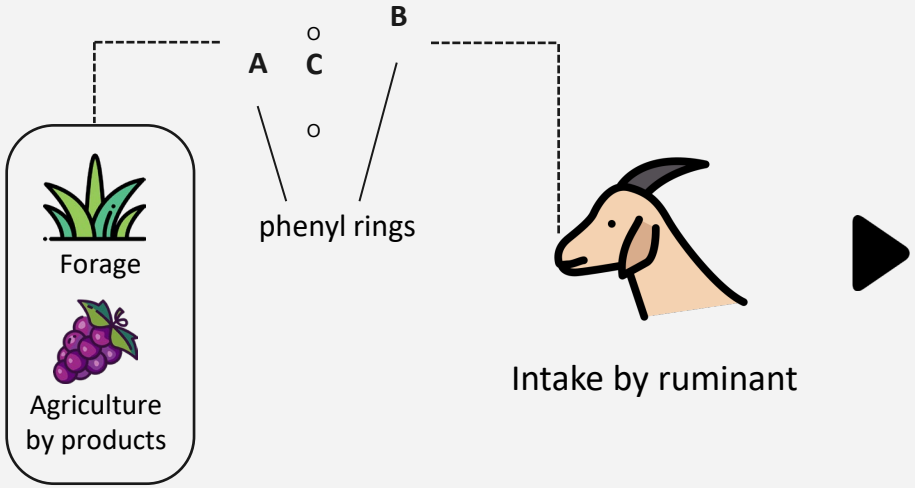
01

Introduction

Flavonoid and its benefits on livestock



OUTCOMES

- **Increase antioxidant**
- **Increase meat quality**
- **Increase production**

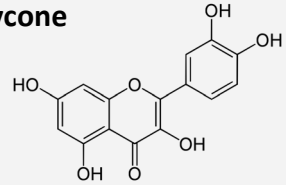
(North et al., 2019)

Flavonoids is a plant secondary metabolites (polyphenol group) commonly found in plants

Introduction

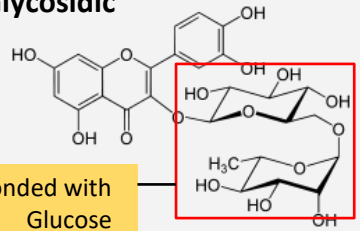
THE TYPES OF FLAVONOID

Aglycone



Quercetin

Glycosidic

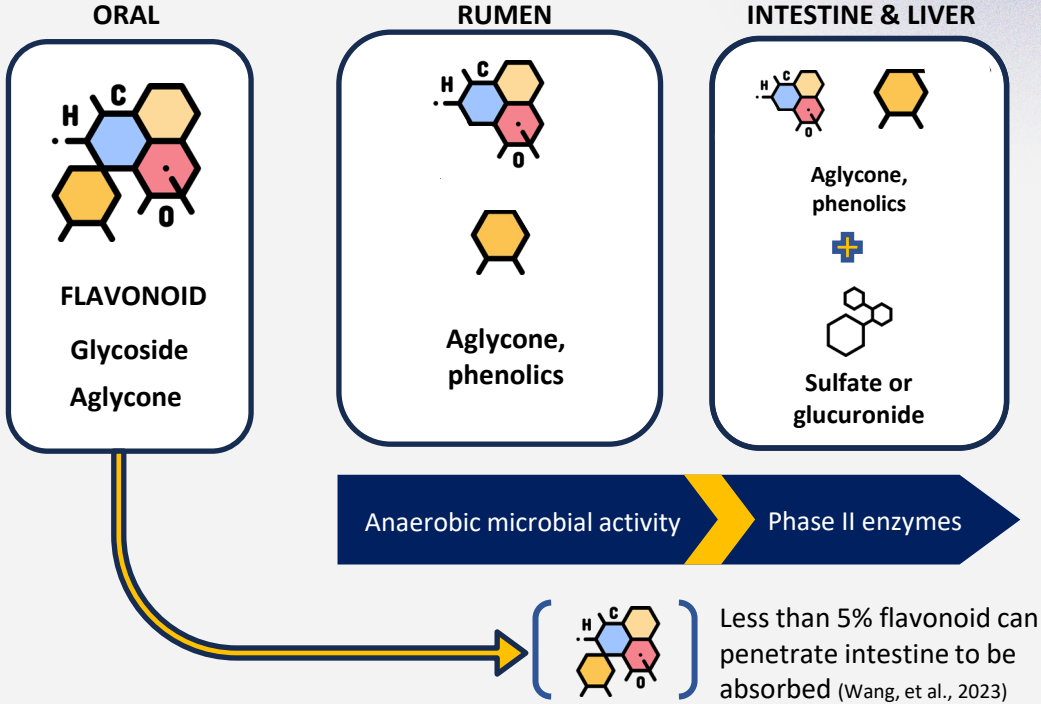


Bonded with Glucose monomers

Rutin

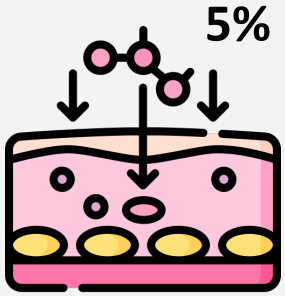
Found **six times** higher in fresh forage

FLAVONOID PATHWAY IN RUMINANT



Less than 5% flavonoid can penetrate intestine to be absorbed (Wang, et al., 2023)

Flavonoid supplementation issue on-farm

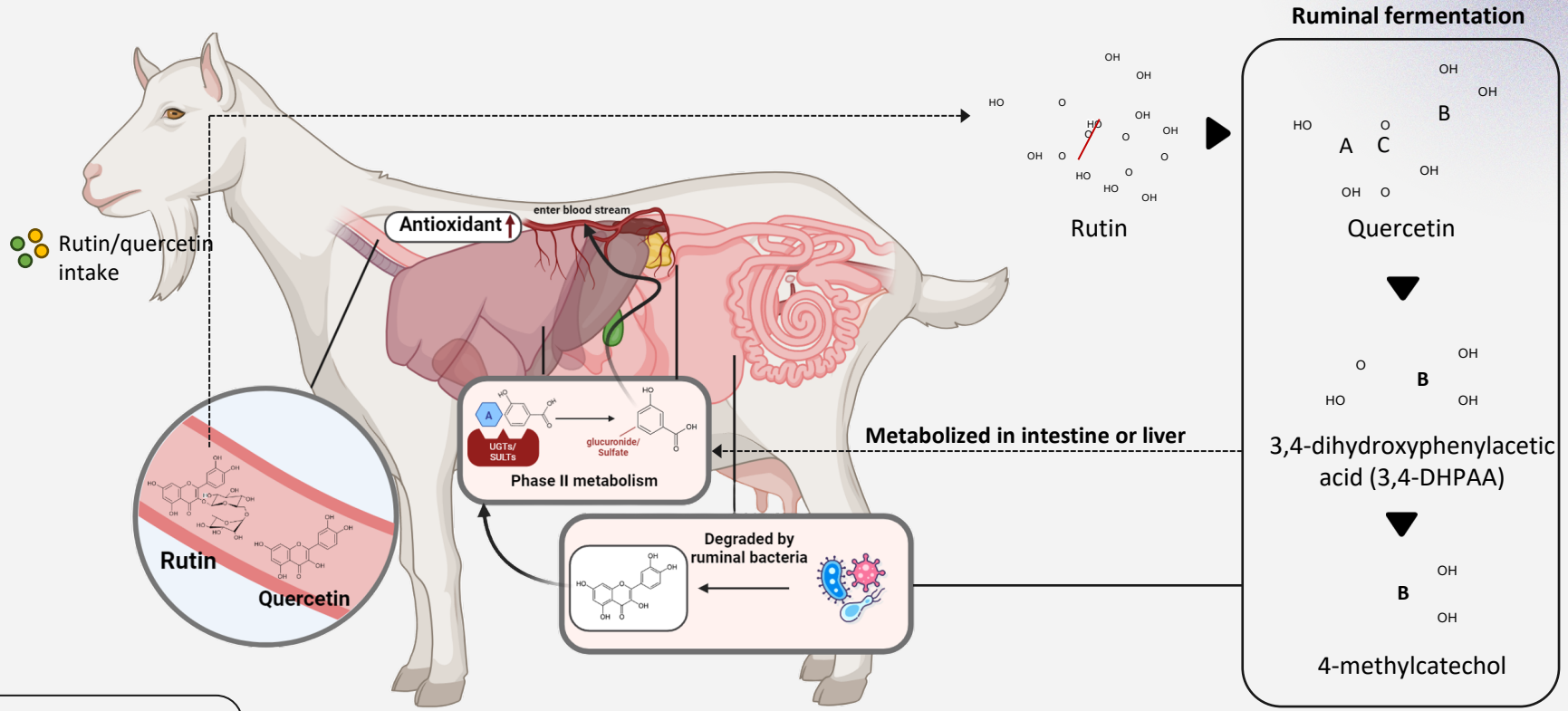


Since less than 5% flavonoid can penetrate the intestine to be absorbed,

The beneficial effects shown after flavonoid supplementation may not be directly caused by the flavonoid molecule but by **its metabolites.**

Therefore, exploring **the metabolic pathway of flavonoids is important** to observe the importance of flavonoid supplementation on-farm

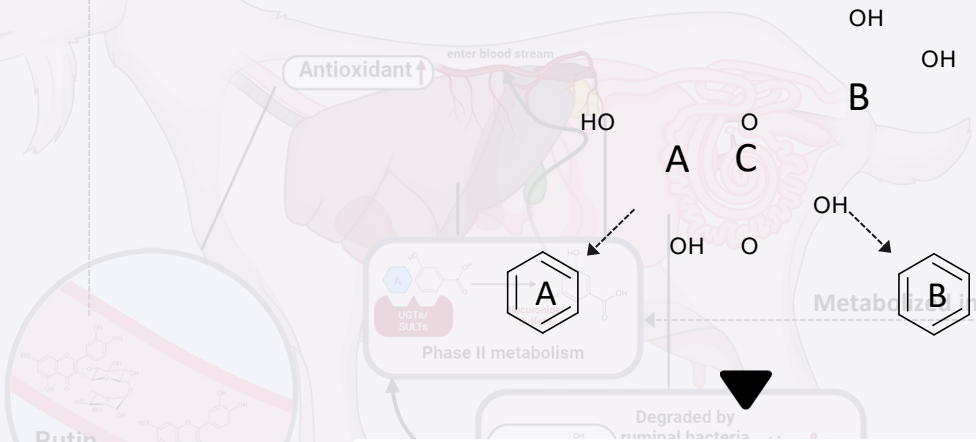
Rutin and Quercetin Metabolism in Ruminant



Berger, et al 2015; Guo, et al, 2022

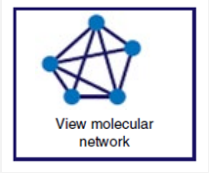
Rutin and Quercetin Metabolism in Ruminant

However, there is possible **pathway of quercetin** that has not established yet.

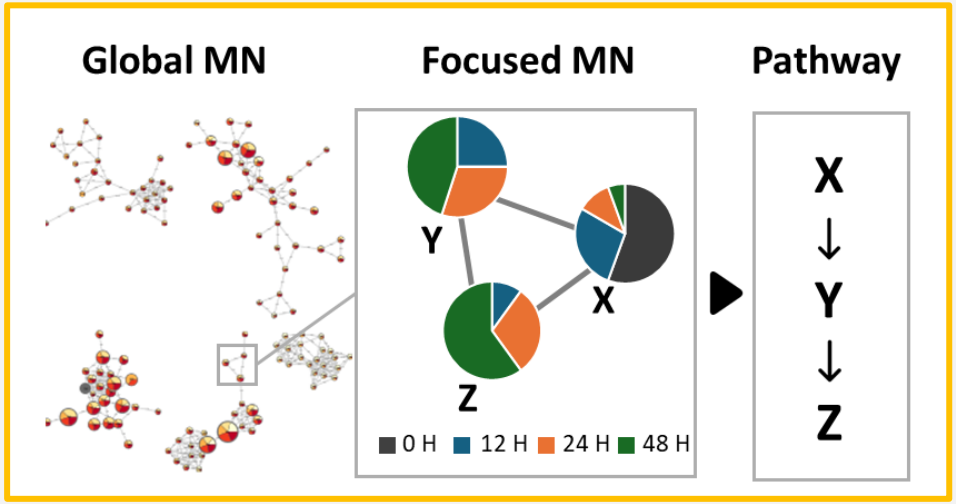


Thus, to reveal the remaining metabolites, we use a **molecular networking approach.**

What Is **Molecular Networking?**



Molecular networking is an approach to creating a network within metabolites according to their structure similarities.



Purposes of Study

Exploring quercetin and rutin metabolites degraded by microbes in the rumen

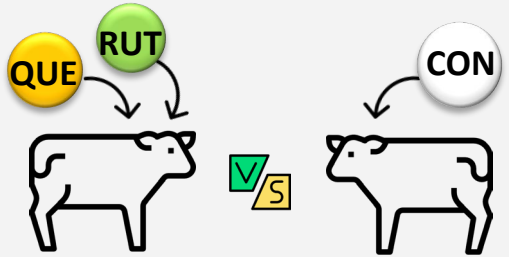
Exploring major quercetin and rutin metabolites post-rumen in blood

02

Materials & **Methods**

Material & **Methods**

in-vivo experiment



3 x 5 crossover design

0, 1, 4, 6, 8, and 22 hours

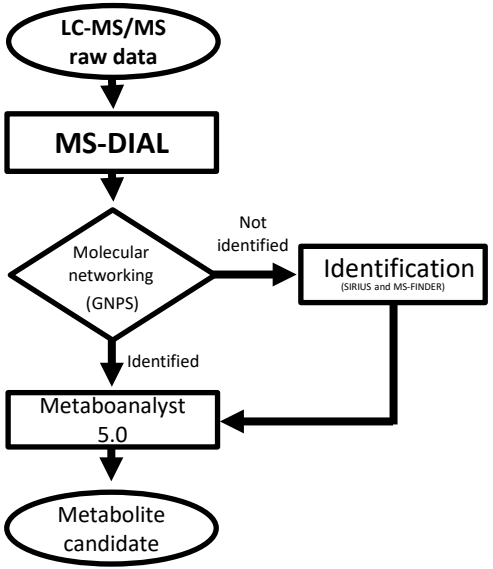
Plasma

Rumen Fluid

UPLC-QTOF-MS/MS acquisition

Raw data

Metabolomics and data analysis



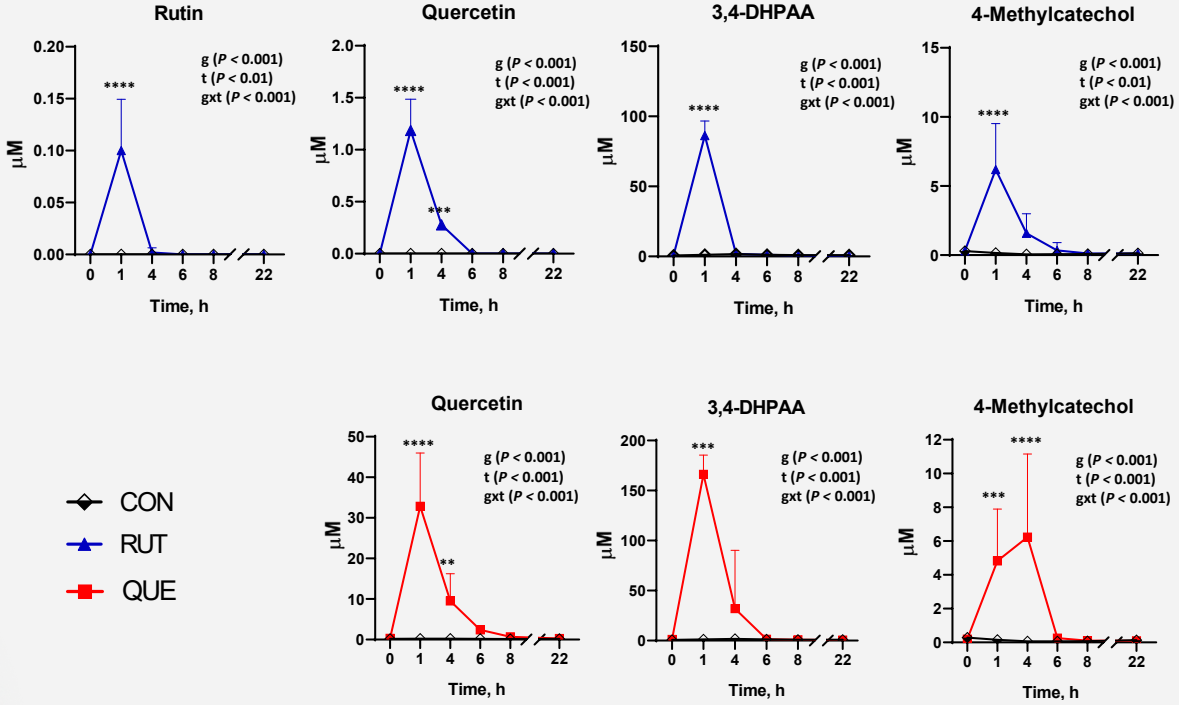
Univariate data

Quantify using Quanlynx® and statistically analyzed with *lmerTest* Package on R studio

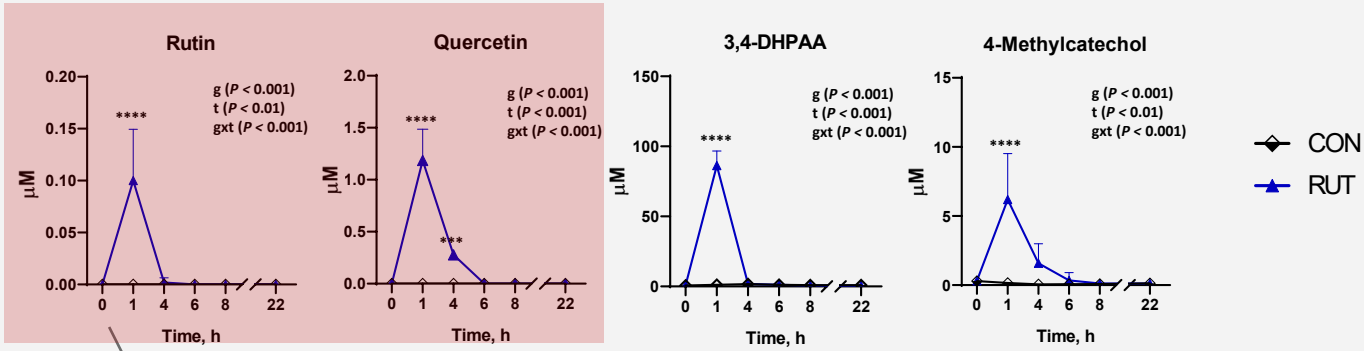
03

Results & **Discussion**

Rutin and quercetin and their metabolites' concentration in the rumen

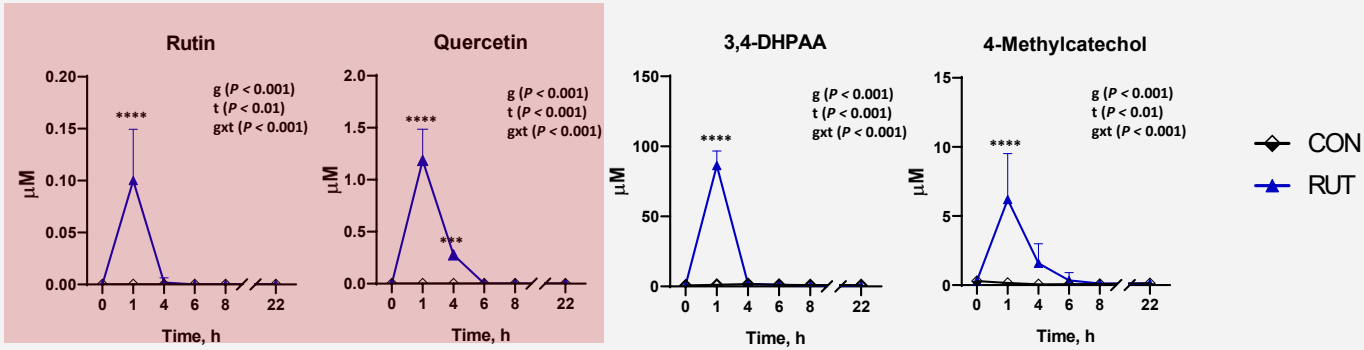


Rutin and quercetin and their metabolites' concentration in the rumen



The rutin is rapidly transformed into quercetin in less than 1 h

Rutin and quercetin and their metabolites' concentration in the rumen

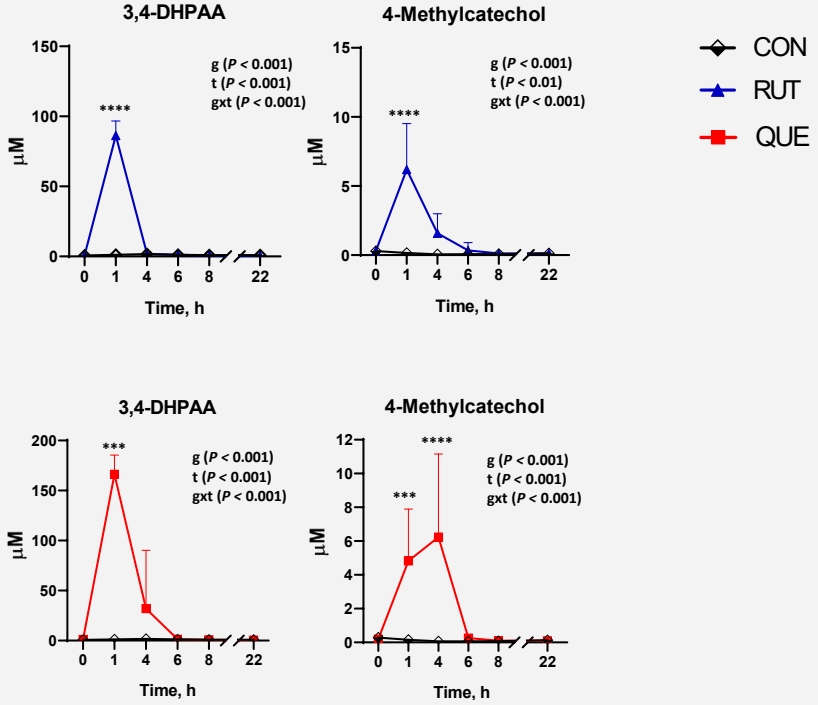


The transformation of rutin to **quercetin→3,4-DHPAA** occurred once rutin entered the rumen, even though the former metabolite was not completely metabolized.

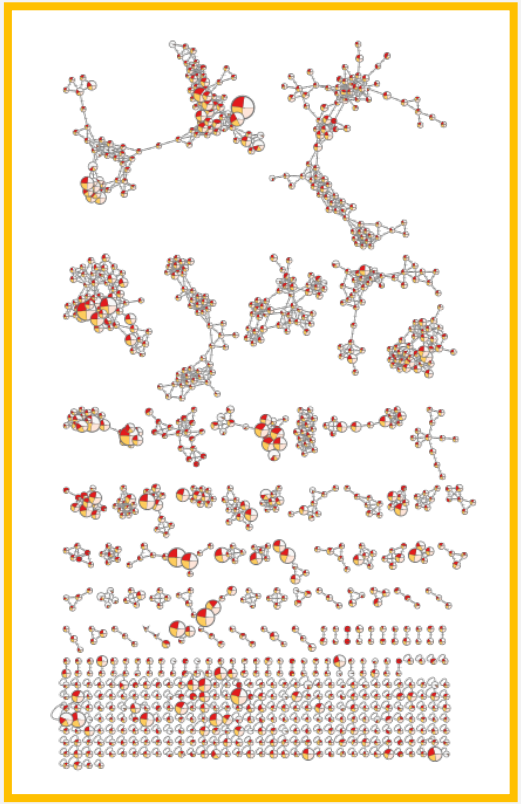
the concentration of the measured metabolites increased in the order of **rutin < quercetin < 3,4-DHPAA** at 1 h

Rutin and quercetin and their metabolites' concentration in the rumen

In contrast, 4-Methylcatechol concentration is **lower than** the maximum concentration of 3,4-DHPAA, assuming there were other metabolites beside 4-Methylcatechol produced from 3,4-DHPAA in the rumen.



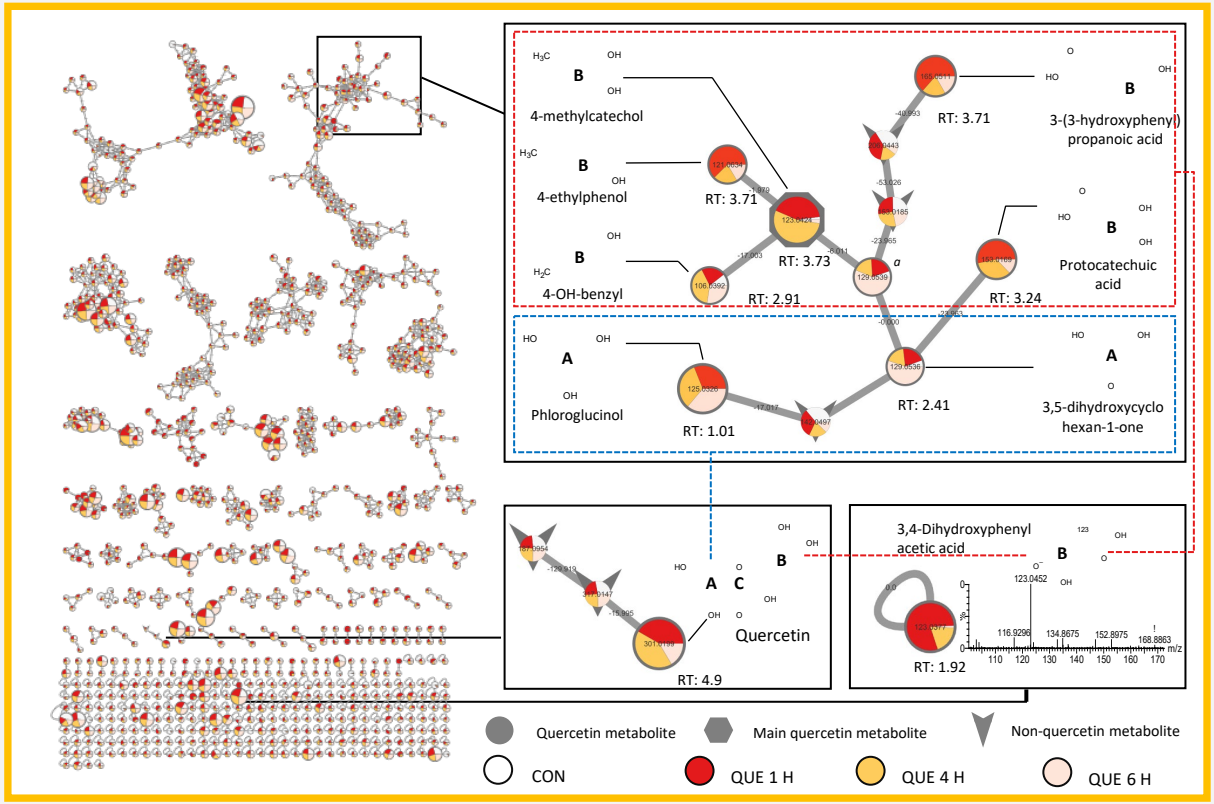
Ruminal quercetin metabolites exploration



Whole **molecular networking** of ruminal fluid obtained was analyzed by LC-MS/MS

It showed the similarity of connected features/metabolites.

Ruminal quercetin metabolites exploration

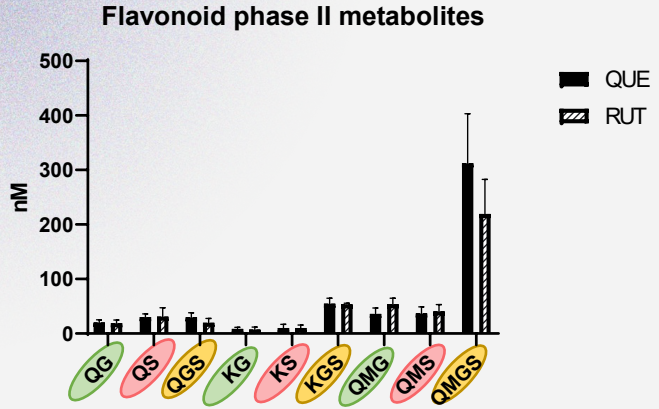


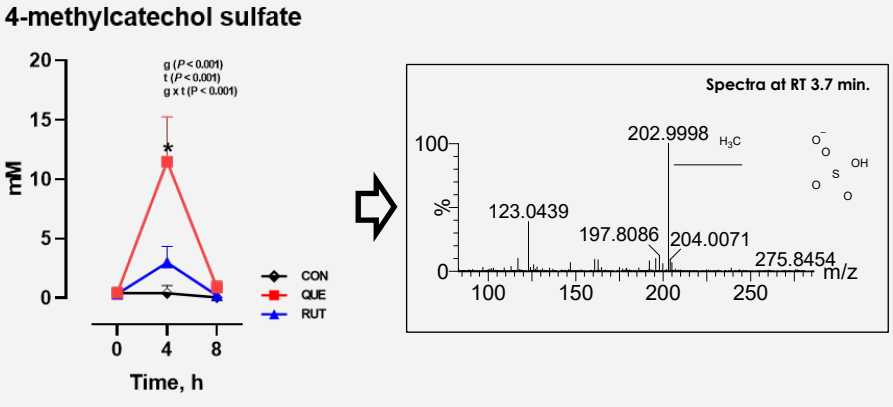
Quercetin metabolite candidates produced by ruminal microbial degradation obtained from untargeted metabolomics

Metabolites	RT	ion (m/z)	Formula	AUC	P value	Log2 (FC)
4-Methylcatechol (4-MC)	3.32	123.0403	$C_7H_8O_2$	1.00	2.28E-08	-4.76
3,4-dihydroxyphenylacetate (3,4-DHPPA; fraction)	1.92	123.0377	$C_8H_8O_4$	0.98	1.20E-04	-5.32
4-OH-benzyl	2.91	106.0392	C_7H_7O	0.94	8.91E-05	-1.59
Phloroglucinol (PG)	1.01	125.0326	$C_6H_6O_3$	0.85	1.13E-02	-0.61
3,5-dihydroxycyclo-hexan-1-one (3,5-DHDC)	2.41	129.0536	$C_6H_{10}O_3$	0.8	1.71E-01	-1.70
3-(3-hydroxyphenyl)propanoic acid (3-HPPA)	3.71	165.0511	$C_9H_{10}O_4$	0.79	1.90E-02	-1.16
4-ethylphenol	3.71	121.0634	$C_8H_{10}O$	0.76	1.72E-02	-1.14
Protochatechuic acid (PCA)	3.24	153.0169	$C_7H_6O_4$	0.71	5.53E-02	-0.81

Abbreviations: AUC = area under curve, FC = fold change, RT = retention time. A negative fold change shows that the corresponding metabolite in the QUE group is log2(FC value) times greater than that in the CON group.

Quercetin and rutin conjugates in animal plasma





Abbreviation: **KG** = kaempferol glucuronide; **KGS** = kaempferol glucuronide sulfate; **KS** = kaempferol sulfate; **QG** = quercetin glucuronide; **QGS** = quercetin glucuronide sulfate; **QMG** = isorhamnetin glucuronide; **QMGS** = isorhamnetin glucuronide sulfate; **QMS** = isorhamnetin sulfate.

UGT — UDP-glucuronosyltransferase

SULT — Sulfotransferases

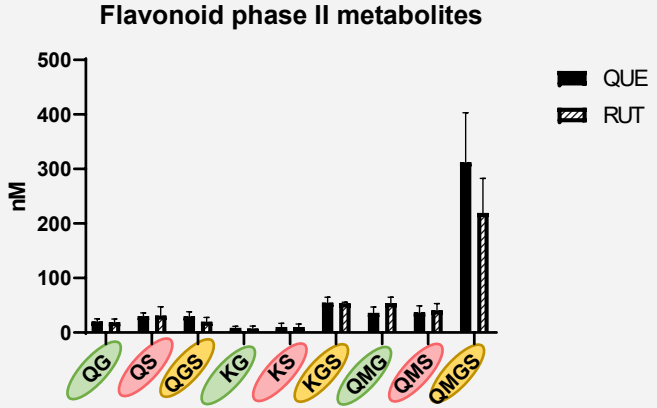
COMT — Catechol-O-methyltransferases

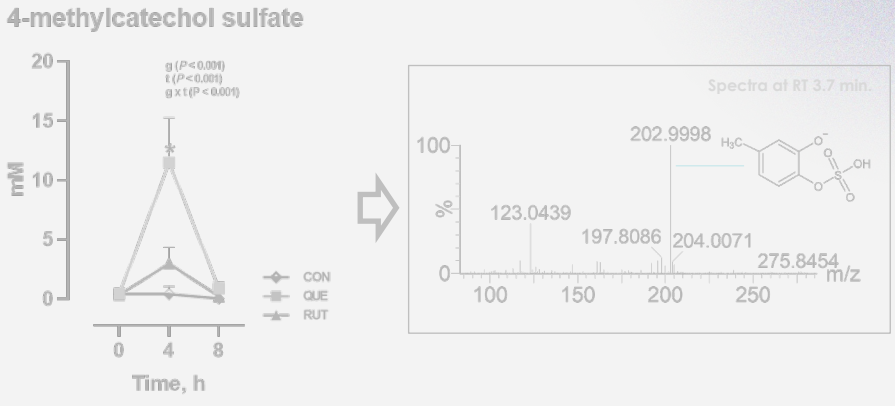
→ Glucuronide conjugates

→ Sulfate conjugates

→ Quercetin methylation to quercetin

Quercetin and rutin conjugates in animal plasma





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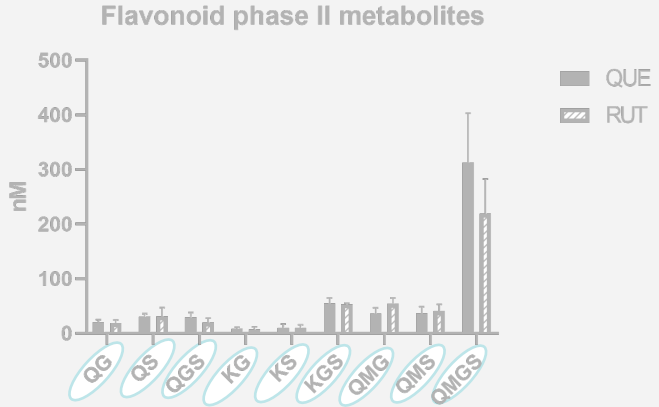
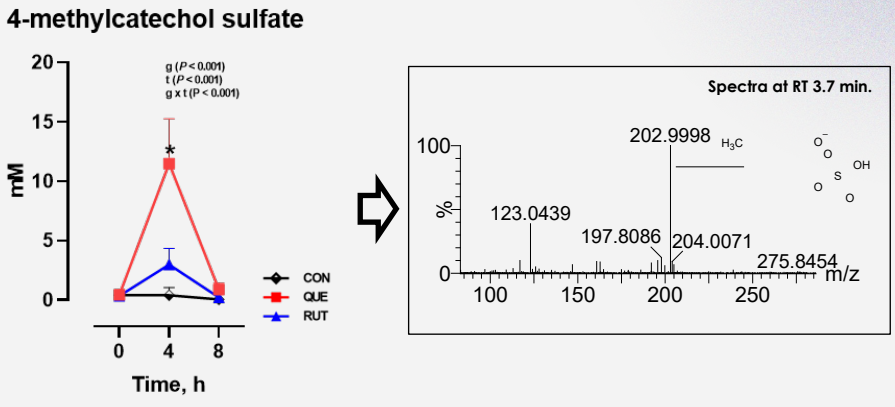
- In general, the majority of flavonol metabolites were **isorhamnetin glucuronide sulfatase**. The same finding was also found in rats fed with quercetin glucosides (Tanaka et al., 2019).
- Overall, kaempferol and isorhamnetin mixed conjugates are significantly higher than their respective glucuronide or sulfate conjugates since mixed conjugates are excreted slower, leading to blood accumulation (Tanaka et al., 2018).

Quercetin and rutin conjugates in animal plasma





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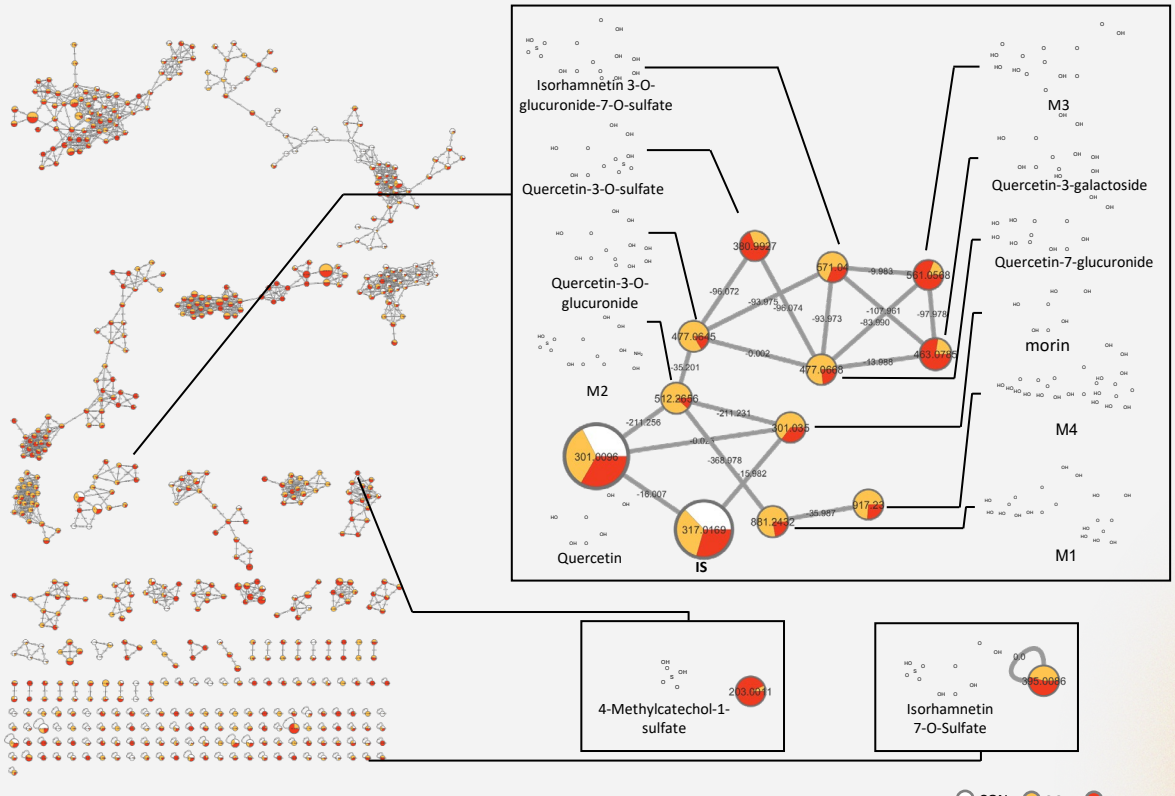
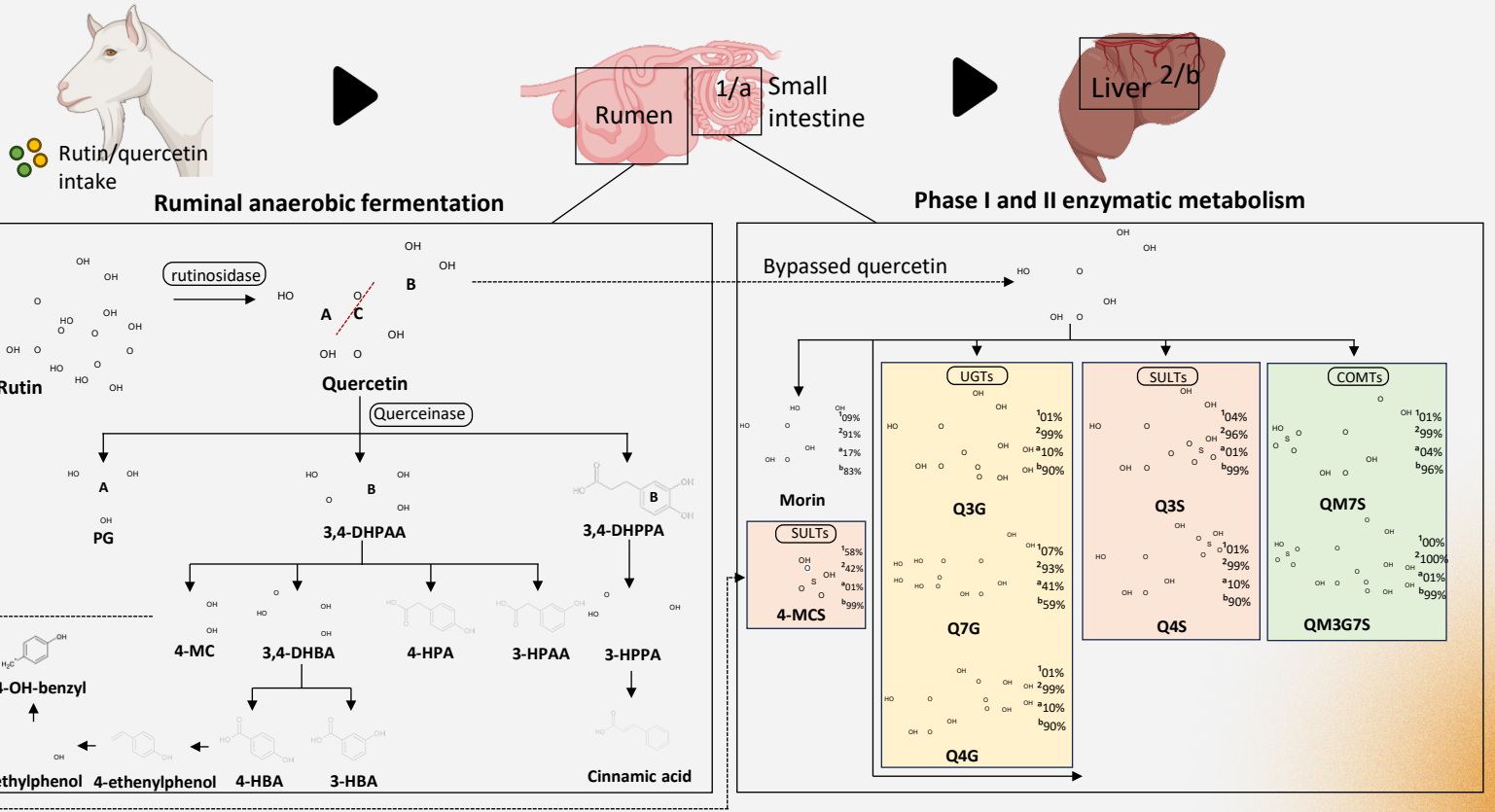
- Meanwhile, we found that 4-Methylcatechol sulfate was the major conjugate found in animal plasma. The same result was also reported by Guo, et al. (2022).
- Interestingly, the conjugation of 4-Methylcatechol was found only as the result of sulfation, not glucuronidation or mixed conjugation.

Quercetin and rutin conjugates using S9 fractions of liver and intestine of goat and cattle *in-vitro*



The proposed quercetin and rutin metabolism pathway



1: Small intestine S9 fraction of goat in-vitro result; **2:** Liver S9 fraction of goat in-vitro result; **a:** Small intestine S9 fraction of cattle in-vitro result; **b:** Liver S9 fraction of cattle in-vitro result

04

Conclusion

Conclusion

- **Phloroglucinol, 3,4-dihydroxyphenyl acetic acid, and 3,4-dihydroxyphenylpropanoic acid** were the first phenolics formed from quercetin molecule metabolism.
- Rutin or quercetin is mainly metabolized to **4-methylcatechol sulfate** and **isorhamnetin glucuronide sulfate** in ruminants.

Thank you!

Does anyone have any questions?

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