

Forming 4-methylcatechol as the dominant bioavailable metabolite of intraruminal rutin inhibits *p*-cresol production in dairy cows

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SUPPLEMENTARY DATA

Table S1. Source of chemicals and reagents used in chemical analysis, LC-MS analysis, structural confirmation, and quantification.

Chemicals and reagents	Vendor
Acetonitrile (LC-MS grade), Formic acid (LC-MS grade), Hydrochloric acid, Water (LC-MS grade)	Fisher Scientific (Houston, TX)
Amino acid mixture (acidic), Amino acid mixture (basic), <i>tert</i> -Butyl Hydroxyquinone (tBHQ), Dansyl chloride (DC), Hippuric acid	Sigma-Aldrich (St. Louis, MO)
3,4-Dihydroxyphenylacetic acid (DHPAA), 2-Hydrazinoquinoline (HQ), Rutin, Triphenylphosphine (TPP)	Alfa Aesar (Tewksbury, MA)
2-2'-Dipyridyl disulfide (DPDS)	MP Biomedicals, LLC (Irvine, CA)
Fatty acids standards (C4-C22)	Nu-Chek Prep, Inc. (Elysian, MN)
Methanol (LC-MS grade)	Avantor performance materials (Radnor, PA)
4-Methylcatechol, Quercetin	Acros Organics (Fair Lawn, NJ)
3-O-methyl Quercetin/Isorhamnetin	Frontier Specialty Chemicals (Logan, UT)
4-O-methyl Quercetin/Tamarixetin	Cayman Chemical Company (Ann Arbor, MI)
Kaempferol	APExBIO (Houston, TX)

Table S2: Ingredient and nutrient content of the total mixed ration (TMR).

Component	Contribution
Ingredient, % of DM	
Corn silage	19.70
Corn, extra fine, rolled	10.60
Corn gluten	4.90
Alfalfa hay, chopped	5.00
Protein mix ¹	8.45
QLF commercial dairy mix ²	2.75
Cottonseed, Fuzzy	3.20
Megalac ³	0.40
Nutrient content, DM basis	
DM, %	55.8
Crude protein, %	16.4
Acid detergent fiber, %	17.3
Neutral detergent fiber, %	17.5
Total digestible nutrients, %	77.1
Net energy-lactation, Mcal/kg	1.72
Calcium, %	0.95
Phosphorus, %	0.41
Magnesium, %	0.37
Potassium, %	1.44
Sodium, %	0.50
Iron, ppm	276
Zinc, ppm	86.2
Copper, ppm	17.4
Manganese, ppm	66.5
Molybdenum, %	1.8
Sulfur, %	0.31
Chloride ion, %	0.58
Dietary cation anion difference ⁴ , mEq/kg	227
¹ Extra fine rolled corn, 29.72%; soybean meal 47% protein, 17.50%; canola meal, 12.50%; amino plus (AG Processing, Inc., Omaha, NB), 8.75%; blood meal, 6.25%; calcium carbonate, 5.50%; sodium bicarbonate, 5.00%, distillers grain, 5.00%; WR Elite Dairy Micro (Vita Plus Corporation, Madison, WI), 2.50%; potassium carbonate, 2.00%; UltraMet (Vita Plus Corporation, Madison, WI), 2.00%; sodium chloride, 2.00%; urea 46% N, 1.25%; Rumensin 90 (Elanco Animal Health, Greenfield, IN), 0.03%.	
² Molasses-based liquid supplement of soluble sugars. Quality Liquid Feeds, Dodgeville, WI.	
³ Arm & Hammer Animal Nutrition, Ewing, NJ.	
⁴ DCAD = (Na+K) - (Cl+S) according to Goff (2018).	

Table S3: LC-MS data acquisition condition in a 10-minute run.

Target compounds	Column type	Mobile phase	MS detection mode
Amino acids (dansylated)	BEH C18	A: 0.1% formic acid in H ₂ O B: 0.1% formic acid in ACN	Positive
Fatty acids (HQ derivatization)	BEH C18	A: 2 mM NH ₄ OAc in water with 0.05% CH ₃ COOH B: 2 mM NH ₄ OAc in 95% ACN and 5% H ₂ O with 0.05% CH ₃ COOH	Positive
Hydrophilic metabolites	BEH Amide	A: 0.1% formic acid in H ₂ O B: 0.1% formic acid in ACN	Positive and negative
Rutin and quercetin metabolites	BEH C18	A: 0.1% formic acid in H ₂ O B: Methanol	Negative

Figure S1. Scores plot of plasma samples after intraruminal rutin administration.

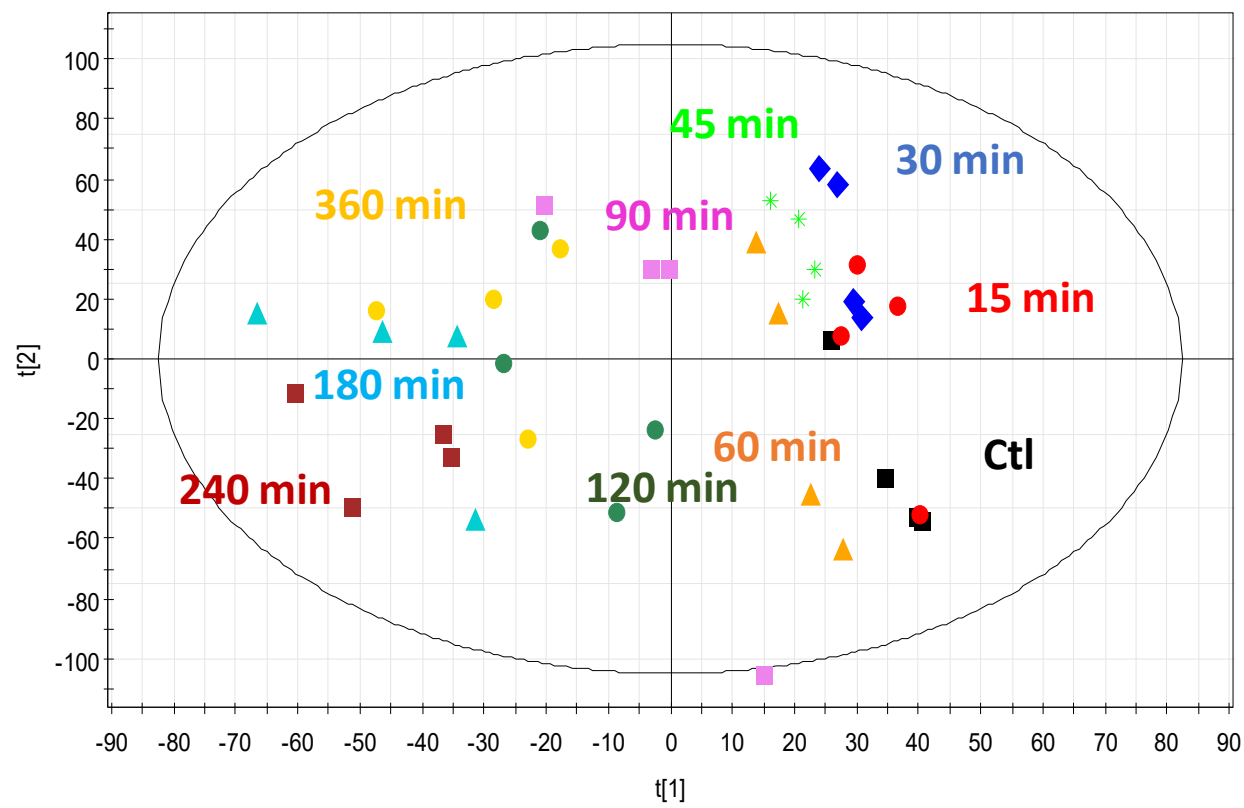


Figure S2. Identification of ruminal degradation of rutin in rumen fluid. **(A)** Extracted chromatography of rutin, quercetin, and DHPAA at 30 min, 1 h and 2 h after intraruminal rutin administration. **(B)** MS/MS fragmentation of DHPAA standard, and **(C)** 4-methylcatechol standard.

