

Exploring Kaempferol 3-O-Glucoside as a Potential Feed Additive: *In vitro* Assessment for Mitigating Methane Emissions in Livestock Nutrition

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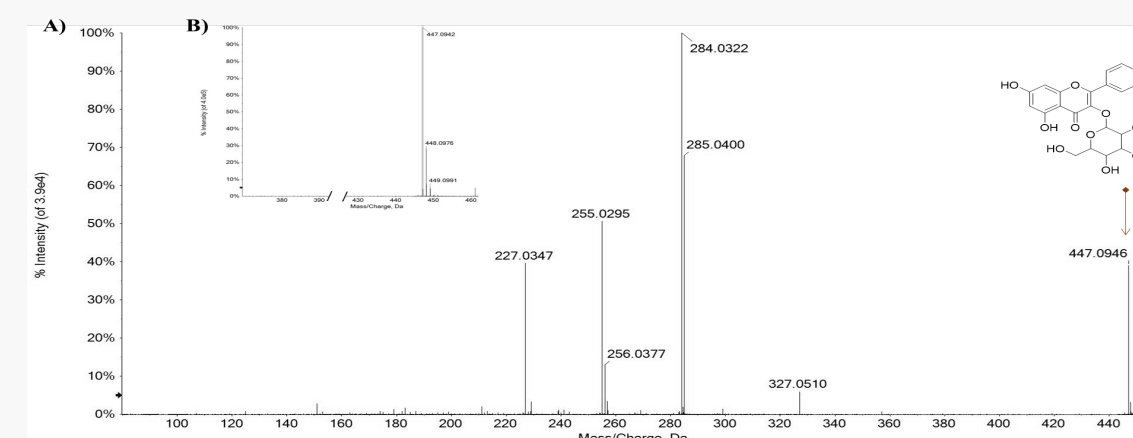
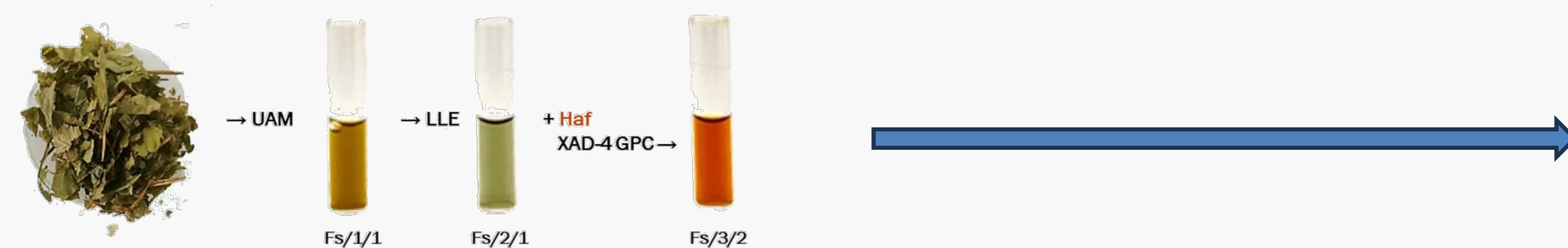
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Introduction

- Improving livestock animal health and performances
- Reduce methane emission
- Plants, dried or in the form of their extracts, or also pure specialised compounds (e.g., phenols, flavonoids tannins, saponins, etc.) could have effects on ruminal fermentation
- Identification of extracts, or pure compounds, in the unexplored plant materials, is becoming a new challenge in the animal husbandry sector



- ✓ An alcoholic extract (2.3 g), namely Fs/3/2, obtained by ultrasound assisted maceration from beech leaves of *Fagus sylvatica* L. was chromatographed .
- ✓ Astragalin was the most abundant in the studied polyphenol fraction.
- ✓ The compound was identified as kaempferol 3-O-glucoside, bioactive constituent of various medicinal plants.
- ✓ Slow down reactive oxygen species production, and to modulate inflammation.



Aim



To evaluate by *in vitro* gas production technique the supplementation of pure kaempferol 3-O-glucoside, a metabolite abundant in the leaves of *Fagus sylvatica* L.

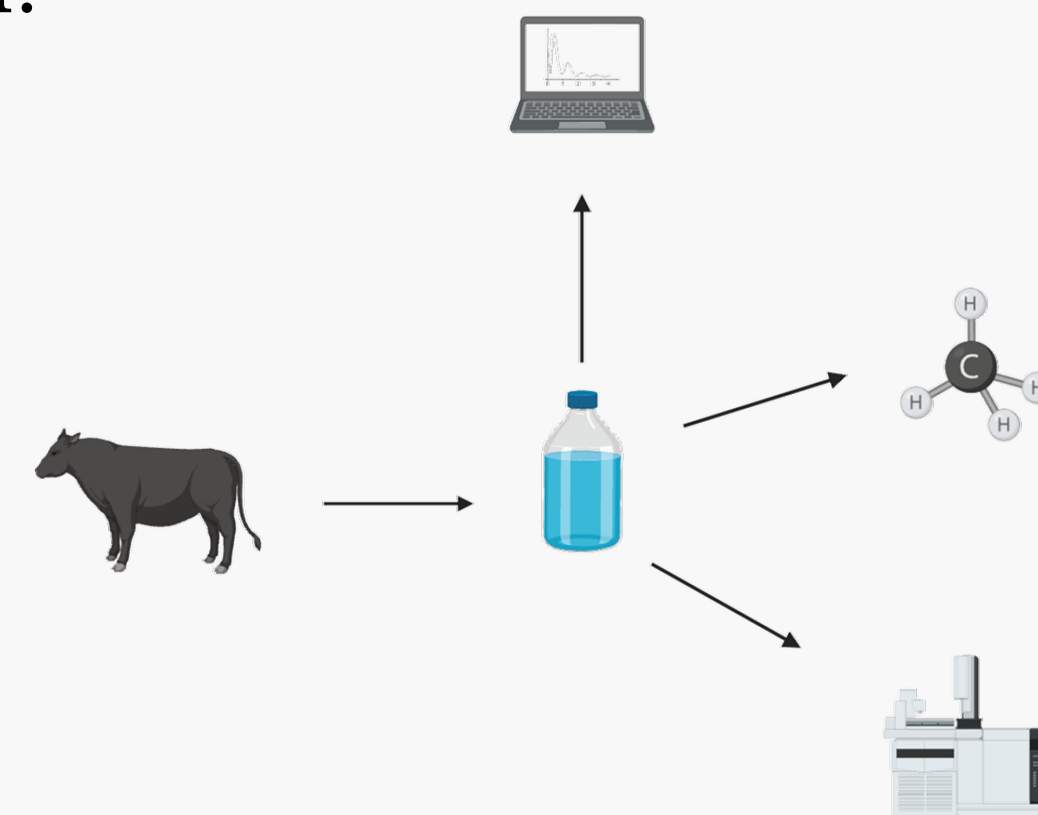


1. In vitro gas production

- ✓ The pure compound was incubated at 0 (control), 5, 50 and 500 μM dose levels with a standard diet at 39 °C under anaerobic conditions for 24 and 120h.
- ✓ Rumen liquor collected at slaughterhouse from four Italian Mediterranean buffaloes.
- ✓ The gas produced was related to incubated OM (OMCV, ml/g)
- ✓ The organic matter degradability (OMD, %) was assessed.
- ✓ Maximum fermentation kinetics (R_{max} , mL/h and T_{max} , h) were estimated
- ✓ pH was measured after 120h of incubation
- ✓ Volatile fatty acids (VFA) were assessed by gas-cromatography.

2. In vitro methane production

- For each dose of compound, two flasks of four, were stopped at 24 h to measure the methane (CH_4)
- The gas-phase from each flask was sampled (3 ml) in duplicate with a gastight syringe and injected into a gas chromatograph.
- The methane production was expressed related to related to degraded OM (dCH_4 , ml/g)
- The organic matter degradability (OMD, %) was assessed
- Volatile fatty acids were determined



3. Metabolomic analysis of kaempferol ruminal fluid

□ *Kaempferol* 500 μM treated ruminal fluid (24h and 120h) was analysed through UHPLC-ESI-QqTOF-MS/MS.

4. Statical analysis

❖ Shapiro-Wilk test, for normally distributed data, has been performed.

❖ The polynomial contrasts (0, 5, 50, and 500 μM).

Tab. 1. *In vitro* methane production and main volatile fatty acids values obtained after 24 h of incubation.

Items		0	5	50	500	Polynomial contrast (p-value)			MSE
	Units					Linear	Quadratic	Cubic	
OMD	%	48.5	46.3	49.1	46.4	0.2659	0.4256	0.0011	0.17
OMCV	ml/g	108	108	103	99.5	0.0004	0.0572	0.0572	0.87
VFA	mmol/l	48.3	46.8	44.4	45.5	0.0016	0.0201	0.5743	0.23
dCH ₄	ml/g	27.8	25.5	25.2	28.6	0.7653	0.0032	0.2677	1.25

OMD_{24h}: organic matter degradability after 24 h of incubation; OMVC: cumulative volume of gas related to incubate organic matter; VFA_{24h} total fatty acids after 24h of incubation; dCH₄: methane production related to degraded organic matter. MSE: mean square error.

Tab. 2. cumulative gas production, organic matter degradability and fermentation kinetics parameters of different doses after 120h of incubation.

Items		0	5	50	500	Polynomial contrast (p-value)			MSE
	Units					Linear	Quadratic	Cubic	
OMD	%	72.7	71.8	72.3	73.3	0.2201	0.0637	0.7950	0.27
OMCV	ml/g	262	275	283	285	0.0003	0.0099	0.0041	3.30
T _{max}	h	5.07	4.13	4.38	5.70	0.3478	0.0435	0.6213	0.31
R _{max}	m/l	8.23	9.64	9.41	8.69	0.7296	0.0256	0.3251	0.19

0: control; 5: 5 µM concentration; 50: 50 µM concentration; 500: 500 µM concentration; OMD: organic matter degradability; OMVC: cumulative volume of gas related to incubate organic matter; R_{max}: maximum fermentation rate; T_{max}: time at which R_{max} occurs. MSE: mean square error.

Tab. 3. Effects of kaempferol 3-*O*-glucoside at different doses (0: control; 5: 5 μ M concentration; 50: 50 μ M concentration; 500: 500 μ M concentration) on fermentation end-products after 120 h of incubation.

Items		0	5	50	500	Polynomial contrast (p-value)			MSE
	Units					Linear	Quadratic	Cubic	
pH		6.60	6.55	6.59	6.66	0.3854	0.3445	0.8551	0.005
VFA	mmol/L	80.8	74.4	73.6	75.5	0.0113	0.0036	0.0297	0.94
Acetate		53.4	51.2	50.7	53.4	0.0097	0.0598	0.0428	0.30
Propionate		15.3	13.7	13.6	13.5	0.0481	0.2420	0.1045	0.07
Iso-Butyrate		1.22	0.89	0.76	0.80	0.0004	<.0001	0.0012	6.5E-4
Butyrate		7.78	7.08	5.70	7.07	0.8020	0.0037	0.7060	0.04
Iso-Valerate		1.54	1.20	1.15	1.24	0.8145	0.6098	0.0595	0.003
Valerate		1.53	1.82	1.81	1.40	0.9237	0.7049	0.7821	0.02
BCFA	% VFA	2.99	2.47	2.29	2.36	0.0518	0.0606	0.0680	0.005
A/P		3.48	3.73	3.77	3.48	0.8339	0.8339	0.8339	0.03

VFA: total volatile fatty acid (acetate + propionate + butyrate + iso-butyrate + valerate + iso-valerate); BCFA: branched-chain fatty acid proportion (iso-butyrate + iso-valerate)/tVFA; A/P: acetate/propionate ratio. MSE: mean square error.

The amount of incubated kaempferol 3-O-glucoside was completely absent in ruminal fluid after 24 and 120 h of incubation.

The percentages of produced aglycone, compared to the amount of *kaempferol* at 24h of incubation was 23.0%.

The aglycone kaempferol was absent at 120 h of incubation.

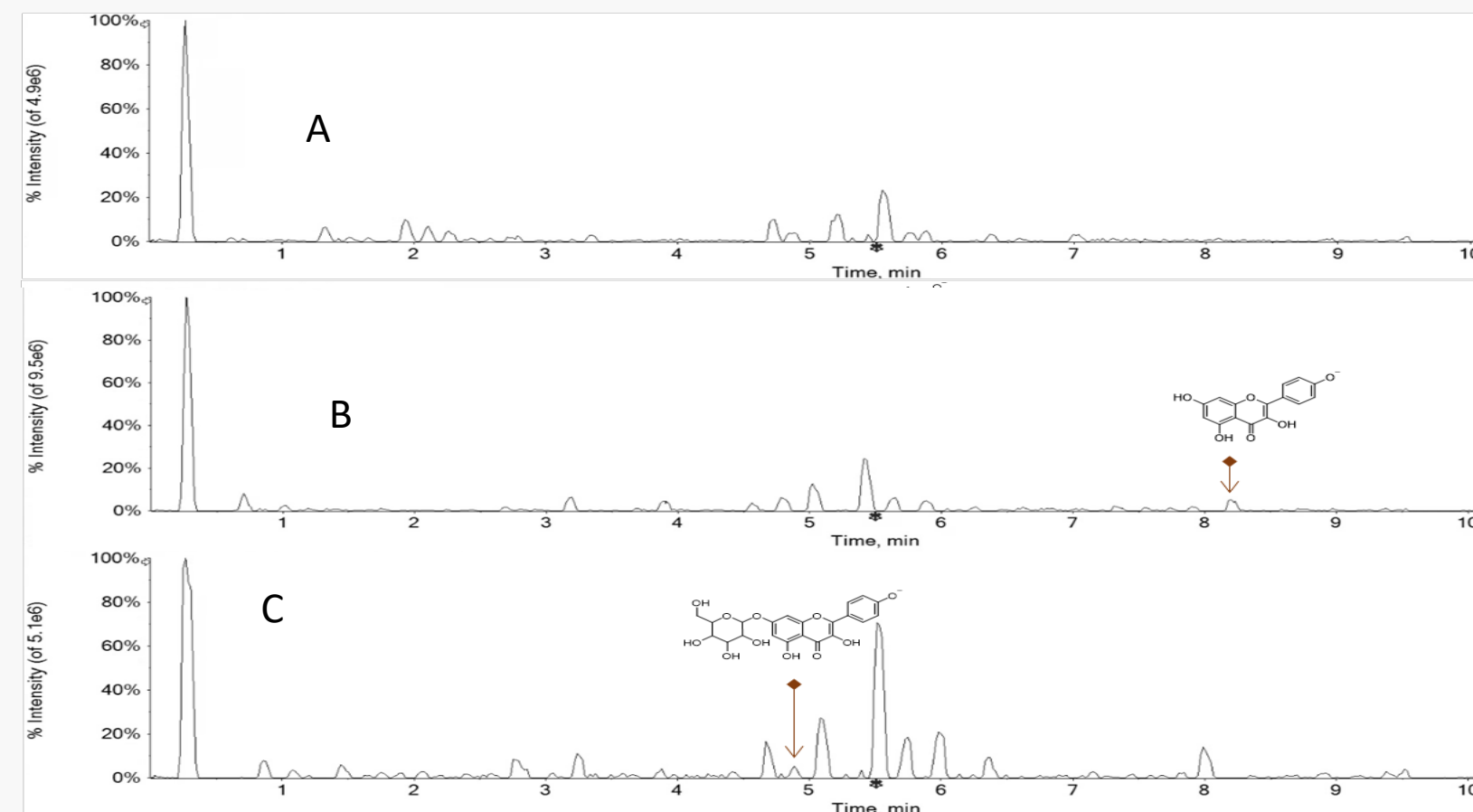


Fig.1. TIC (Total Ion Chromatograms) of the ions tentatively identified as biotransformations mechanisms in the ruminal fluid at time of 24 (**A**) and 120 h (**B**) compared to a control (**C**).

Among the different test dose levels:

- 50 μ M is an optimal dose in terms of organic matter degradability (OMD), cumulative volume of gas (OMCV) and methane production after 24 h of incubation.
- Did not significantly modify organic matter degradability (OMD) after 120 h.
- The results suggest a change in the ruminal microbial population.
- Further studies are needed to demonstrate and deepen biotransformation mechanisms of plant secondary metabolites at ruminal fluid.

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**Thank you for your
attention**