Plant polyphenols: effects on rumen microbiota composition and methane emission

MARCELLO MELE, ARIANA BUCCIONI.

DIPARTIMENTO SCIENZE AGRARIE, ALIMENTARI. AGRO-AMBIENTALI – UNIVERSITÀ DI PISA

DIPARTIMENTO DI SCIENZE DELLE PRODUZIONI AGRO-ALIMENTARI E DELL'AMBIENTE – UNIVERSITÀ DI FIRENZE

OUTLINE

- Plant polyphenols and their role in ruminant nutrition
- Plant polyphenols and methane emissions: in vitro and in vivo studies
- Rumen microbiota associated to methane emission
- Plant polyphenols and microbiota composition
- Conclusions

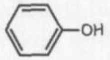
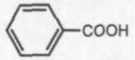
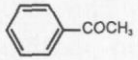
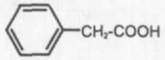
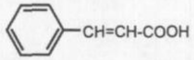
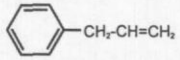
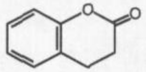
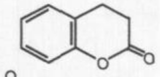
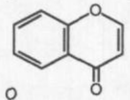
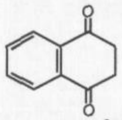
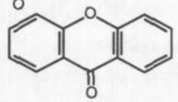
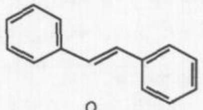
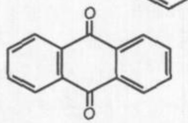
Plant polyphenols

- The occurrence of polyphenols (PP) in herbivores' diets is very common, especially for grazing animals.
- A grazing cow could consume up to 500 g per day of PP (Fraisse et al., 2017)
- In dry areas of the Mediterranean, sheep and goats browse tree leaves rich in tannins such as acacia (Acacia cyanophylla) or the argan tree (Argania spinosa) or are fed local marginal feeding resources rich in tannins, such as carob (Ceratonia siliqua).
- Agro-industry by-products may contain considerable amounts of plant secondary metabolites, including different kind of PP, such as tannins or flavonoids.

Plant Polyphenols

- Plant polyphenols (PP) are a wide class of plants' secondary metabolites with a phenolic moiety, bearing at least one hydroxyl substituent.
- The PP can range from simple phenolics (e.g. ellagic and gallic acids), to dimeric or oligomeric compounds (e.g., procyanidins, lignans) or to polymeric compounds with high molecular weight (e.g. tannins).

Table 1. Main Classes of Polyphenolic Compounds

Class	Basic Skeleton	Basic Structure
Simple phenols	C_6	
Benzoquinones	C_6	
Phenolic acids	C_6-C_1	
Acetophenones	C_6-C_2	
Phenylacetic acids	C_6-C_2	
Hydroxycinnamic acids	C_6-C_3	
Phenylpropenes	C_6-C_3	
Coumarins, isocoumarins	C_6-C_3	 
Chromones	C_6-C_3	
Naftoquinones	C_6-C_4	
Xanthones	C_6-C_1-C_6	
Stilbenes	C_6-C_2-C_6	
Anthraquinones	C_6-C_2-C_6	
Flavonoids	C_6-C_3-C_6	
Lignans, neolignans	$(C_6\text{-}C_3)_2$	
Lignins	$(C_6\text{-}C_3)_n$	

Plant Polyphenols

- Polyphenols are classified as flavonoids, the most common group of phenolics, or as non-flavonoids.
- Flavonoids are in general based on two aromatic rings linked by a bridge constituted by three-carbon, with a C_6-C_3-C_6 basic structure.

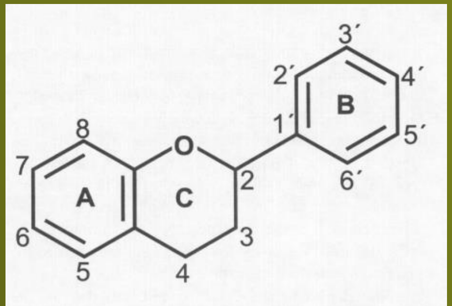
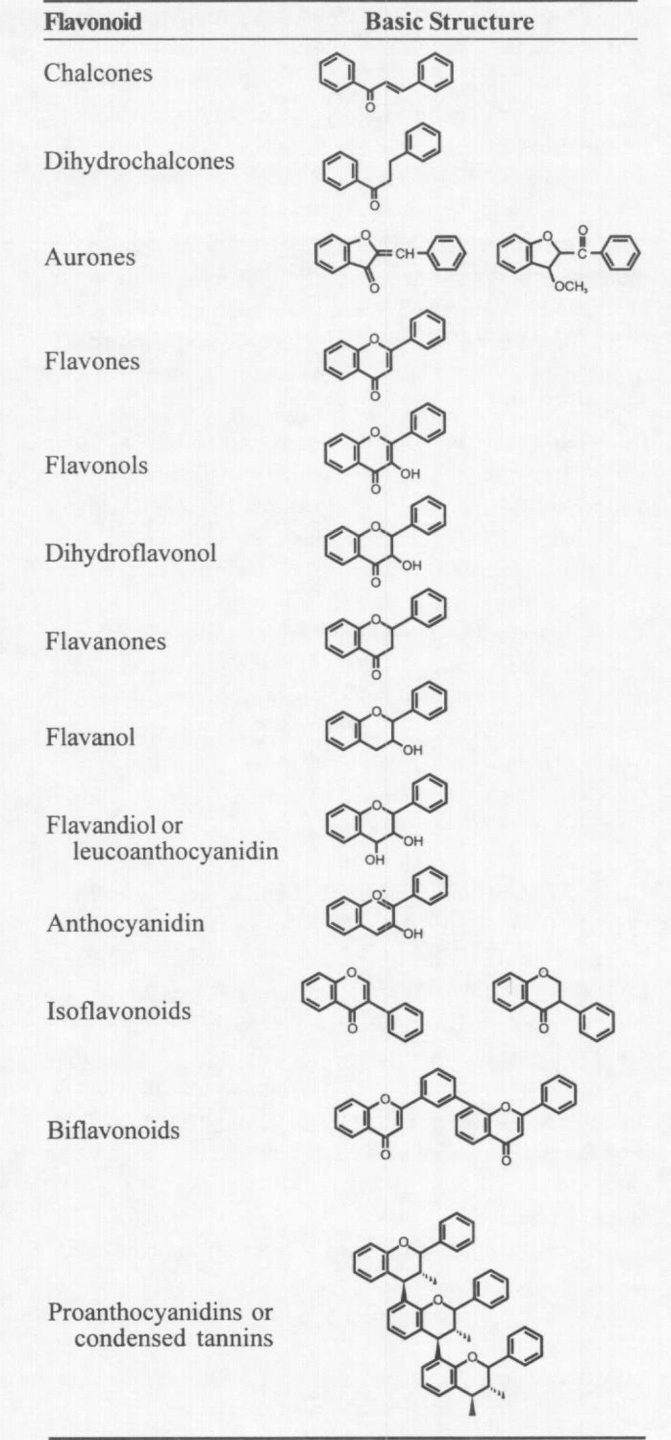


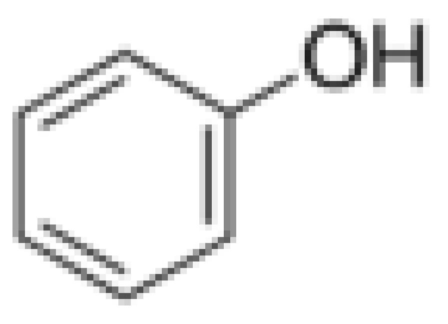
Figure 1. Basic structure and numbering system of flavonoids.

- According to the degree of oxidation of the heterocycle structure, the kind of sugar residue, and the degree of polymerization, flavonoids can be classified in flavonols, flavones, isoflavones, flavan-3-ols, flavanones, and anthocyanidins (Bravo, 1998).

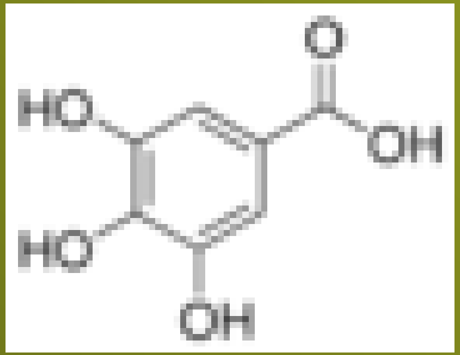


Plant Polyphenols

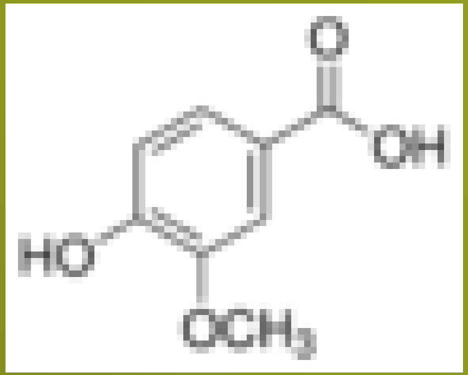
- Among nonflavonoid compounds, there are phenolic acids, hydrolyzable tannins (HT), and stilbenes.
- Phenolic acids derive from benzoic acid and hydroxycinnamic acid and they can be simple phenols (C6 structure, such as phenol or thymol) or with a C6-C1 structure, such as gallic or vanillic acid (Bravo, 1998).



Phenol



Gallic acid



Vanillic acid

Tannins, a special class of plant polyphenols

Non-flavonoids

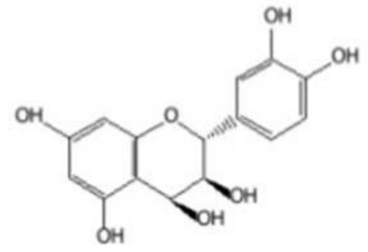
Hydrolisable tannins

Gallotannins

Glucose esterified to gallic acid

Ellagitannins

Glucose esterified to both gallic acid and ellagic acid



flavan-3,4-diol

Flavonoids

Condensed tannins

The polymerization of flavan-3-ols leads to the formation of condensed tannins. The degree of polymerization of condensed tannins can account for 50 or more units with a molecular weight of up to 30,000 Da: **catechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, proanthocyanidins, theaflavins and thearubigins.**

Polyphenols and ruminant nutrition

Data on the effects of dietary PP on ruminant nutrition and performance have been extensively reviewed in the past, especially in the case of tannins (both CT and HT), by considering either the addition of purified substances or the natural PP present in forages and concentrate feed ingredients

	Production	Quality	Environmental impact
Dairy cattle	= ↑	= ↑	↓
Beef cattle	↑	↑	↓
Dairy ewes	=	↑	↓

- **Production: the main effects refer to protein bypass; effects of intestinal parasites**
- **Quality: enhancement of milk protein; decreasing milk urea, increasing polyunsaturated fatty acids**
- **Environmental impact: reduction of N excretion; reduction of ammonia emission from manure**

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Meta-
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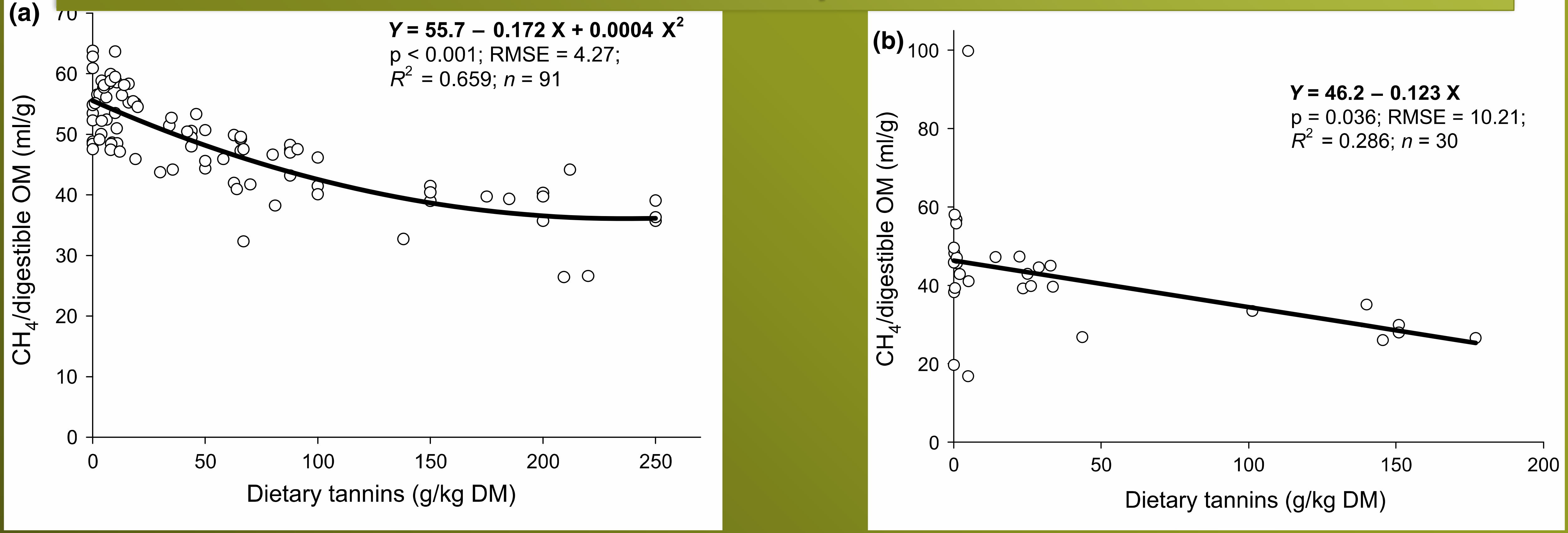
exper

A. Jayan

It is interesting to note that the variation in CH_4 production/digestible OM in vivo was very high at low levels of dietary tannins of <20g/kg DM, whereas variability clearly decreased with increasing tannin concentrations. This might explain why experiments using low levels of tannins led to inconsistent results in terms of effects on CH_4 emissions.



Relationships between dietary tannins (g/kg dry matter) and CH_4/digestible organic matter (ml/g) from (a) in vitro batch culture and (b) in vivo experiments.

Effect of different type of polyphenols on methane production: in vitro studies

J. Dairy Sci. 102:3781–3804
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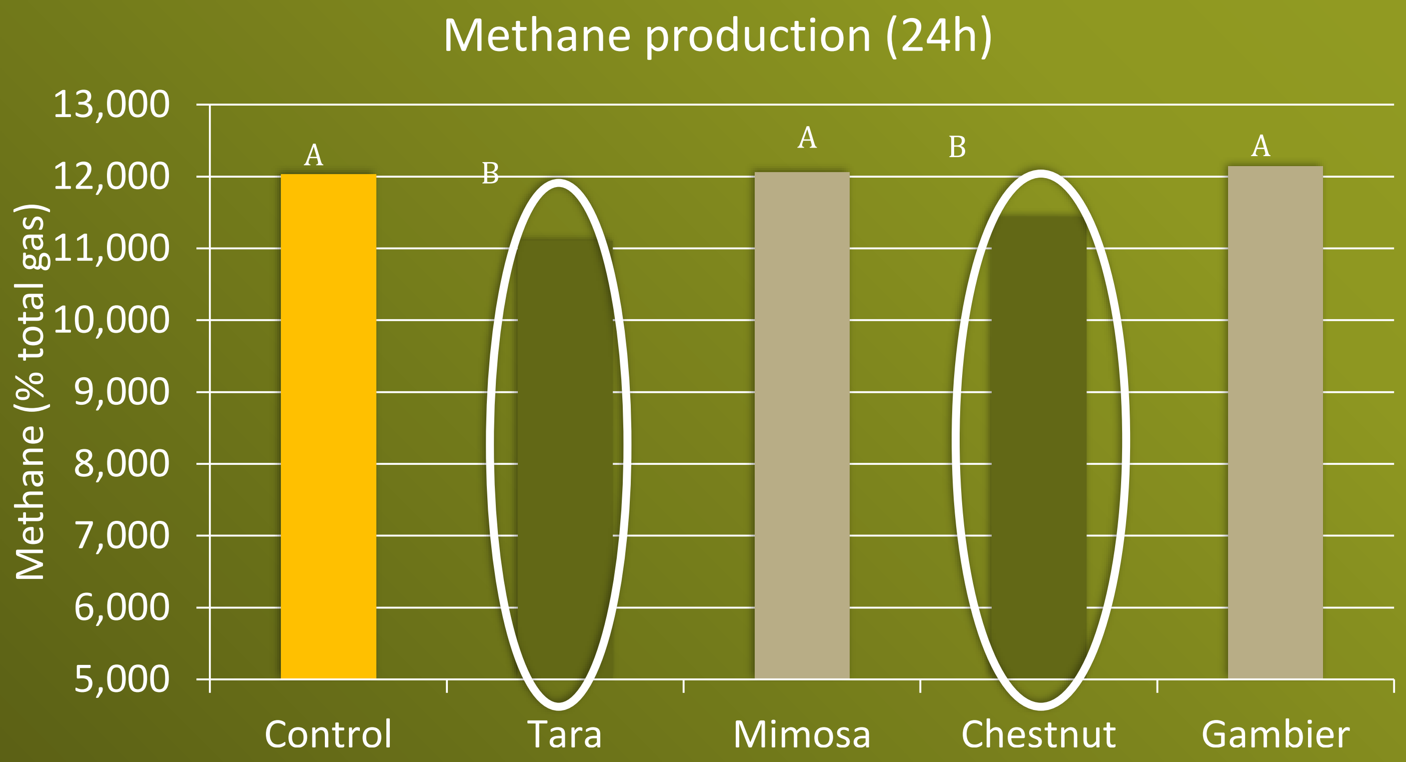
Invited review: Plant polyphenols and rumen microbiota responsible for fatty acid biohydrogenation, fiber digestion, and methane emission: Experimental evidence and methodological approaches

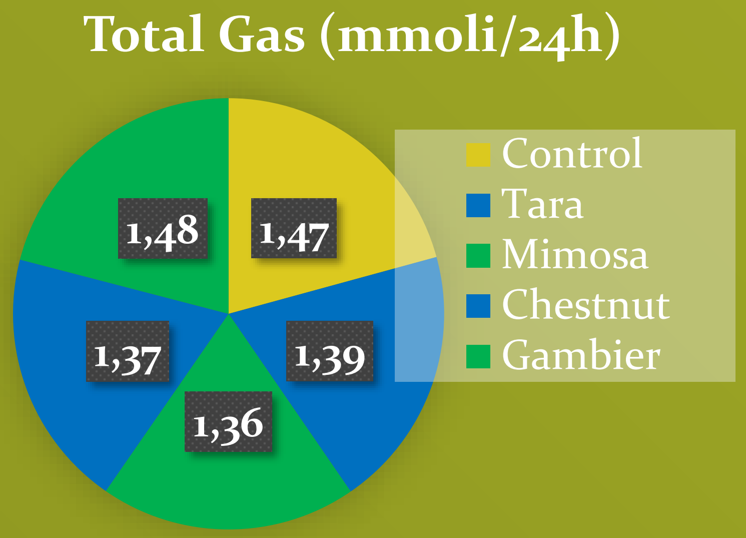
V. Vasta,[1] M. Daghio,[2] A. Cappucci,[3] A. Buccioni,[2] A. Serra,[3] C. Viti,[2] and M. Mele[3,4]*

Polyphenol source	Dose*	Δ methane**	Reference
Lotus corniculatus (CT)	10%	-29%	Tavendale et al. 2005
Pure flavonoids ***	4.5%	- 8.1 to -38%	Oskoueian et al., 2013
Pure flavonoids from citrus	0.02%	-18.7 to -26.5%	Seradj et al., 2014
Chestnut (HT); Sumach (HT)	7.8%	-7.9%; -14.4%	Jayanegara et al., 2015
Quebracho (CT); Mimosa CT)	7.8%	-8.4%; -6.3%	Jayanegara et al., 2015
Mix of poliphenols from Papaya	6%	-20% to -29%	Jafari et al., 2016
Mix of poliphenols from Palm oil leaf	2.5 to 10%	-11.% to 19.6%	Aiman-Zakaria et al., 2017
Chestnut (HT); Sumach (HT)	10%	-64%; -29%	Wischer et al., 2013
Quercus valonea (CT), Vitis vinifera (CT)	10%	-35%; -23.5%	Wischer et al., 2013

* Percentage of dry matter incubated
** Compared to the control treatment within the study
*** flavone, myricetin, naringin, catechin, rutin, quercetin, and kaempferol

HT vs CT on methane production: an in vitro study





Hydrolysable tannins are able to reduce the methane production by nearly -10%.

Tannin extract (4% of DM)

- Mimosa (CT; *Acacia dealbata*)
- Gambier (CT; *Unicaria gambier*)
- Tara (HT; *Caesalpinia spinosa*)
- Chestnut (HT; *Castanea sativa*)

Mele et al., unpublished

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Polyphenol source	Dose*	∆ methane**	specie	Reference
Chestnut (HT)	1 to 3%	-10.8 to -25.4%	Sheep	Liu et al., 2011
Mimosa (CT)	12.7%	-30%	Sheep	Abdalla et al., 2011
Mix of polyphenols from grape marc (CT)	2.4%	-22.6%	Dairy cow	Moate et al., 2014
Pure resveratrol	0.02%	-16%	Sheep	Ma et al., 2015
Flavonoids from mulberry leaf	0.13%	-11%	Sheep	Ma et al., 2017
Pure tannic acid	0.6 to 2.6%	-11 to -30%	Beef cattle	Yang et al., 2017

* Percentage of dry matter intake
** Compared to the control treatment within the study
*** flavone, myricetin, naringin, catechin, rutin, quercetin, and kaempferol

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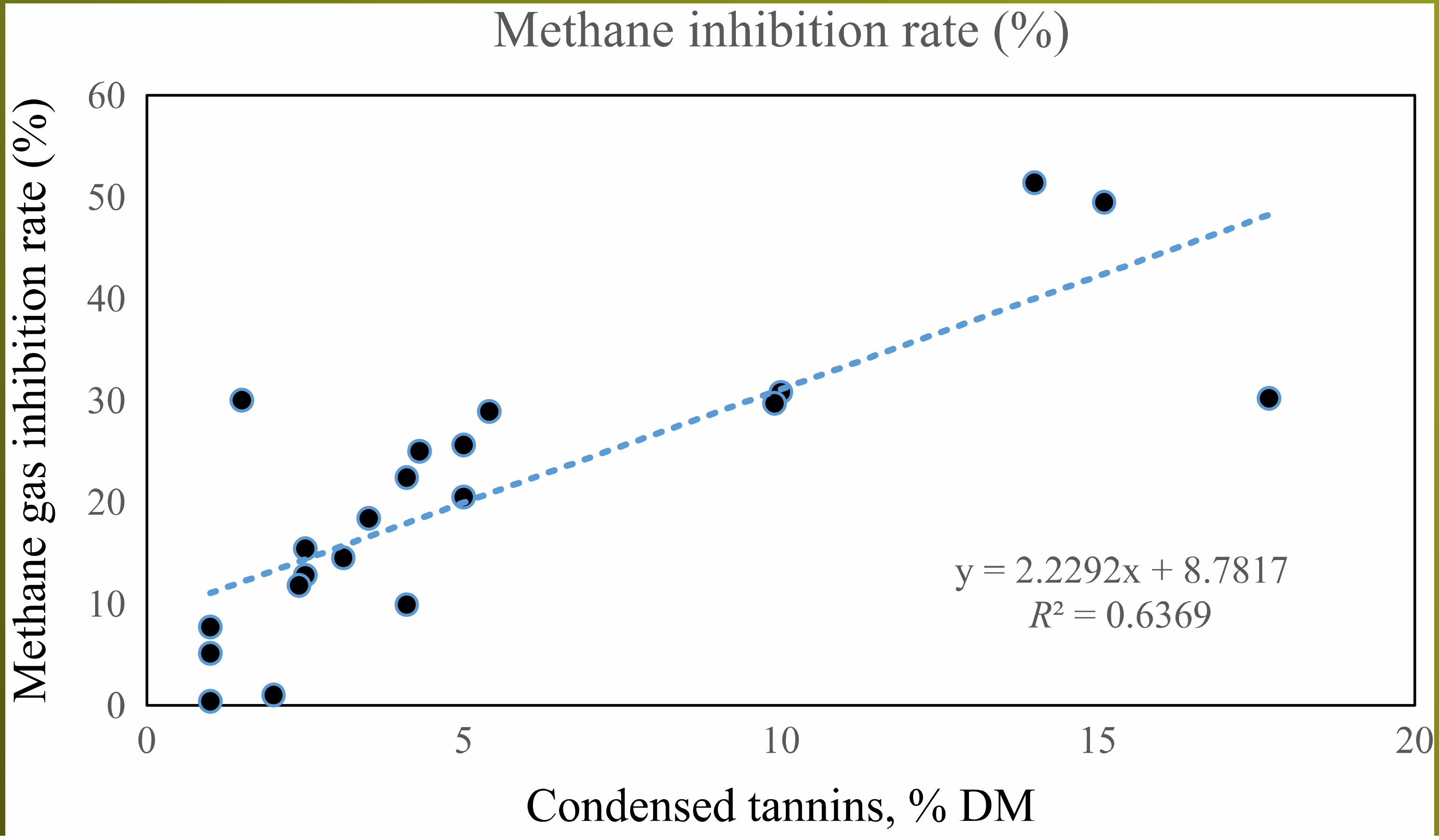
REVIEW ARTICLE

WILEY Journal of Animal Physiology and Animal Nutrition

Comparative aspects of plant tannins on digestive physiology, nutrition and microbial community changes in sheep and goats: A review

Byeng Ryel Min[1,2] | Sandra Solaiman[1]



The methane gas emission response to action of CT or CT-containing forages depends upon the concentration of CT. The variability of the response is reduced when CT content is higher than 2%

Inhibition of methane production by dietary polyphenols: proposed mechanisms.

- Large part of the studies deals with tannins.
- Tannins may bound structural polysaccharides such as cellulose, hemicelluloses and pectin slowing their fermentation.
- Tannins may also bound enzymes of the microbial cell.
- Several PP may interact with rumen microbiota and are considered antimicrobial compounds, inhibiting some ruminal microorganisms.

Rumen microbiota associated to methane emission

Microbiota – The microorganisms present in a defined environment (Bacteria, Archaea, Fungi, Protists, Phage, ...).

Microbiome – The entire habitat, including microbiota, their genomes, and the surrounding environment (the host).

Bacteria and Archaea	10^{10} - 10^{12}
Protozoa	10^{5} - 10^{6}
Fungi	10^{3} - 10^{4}

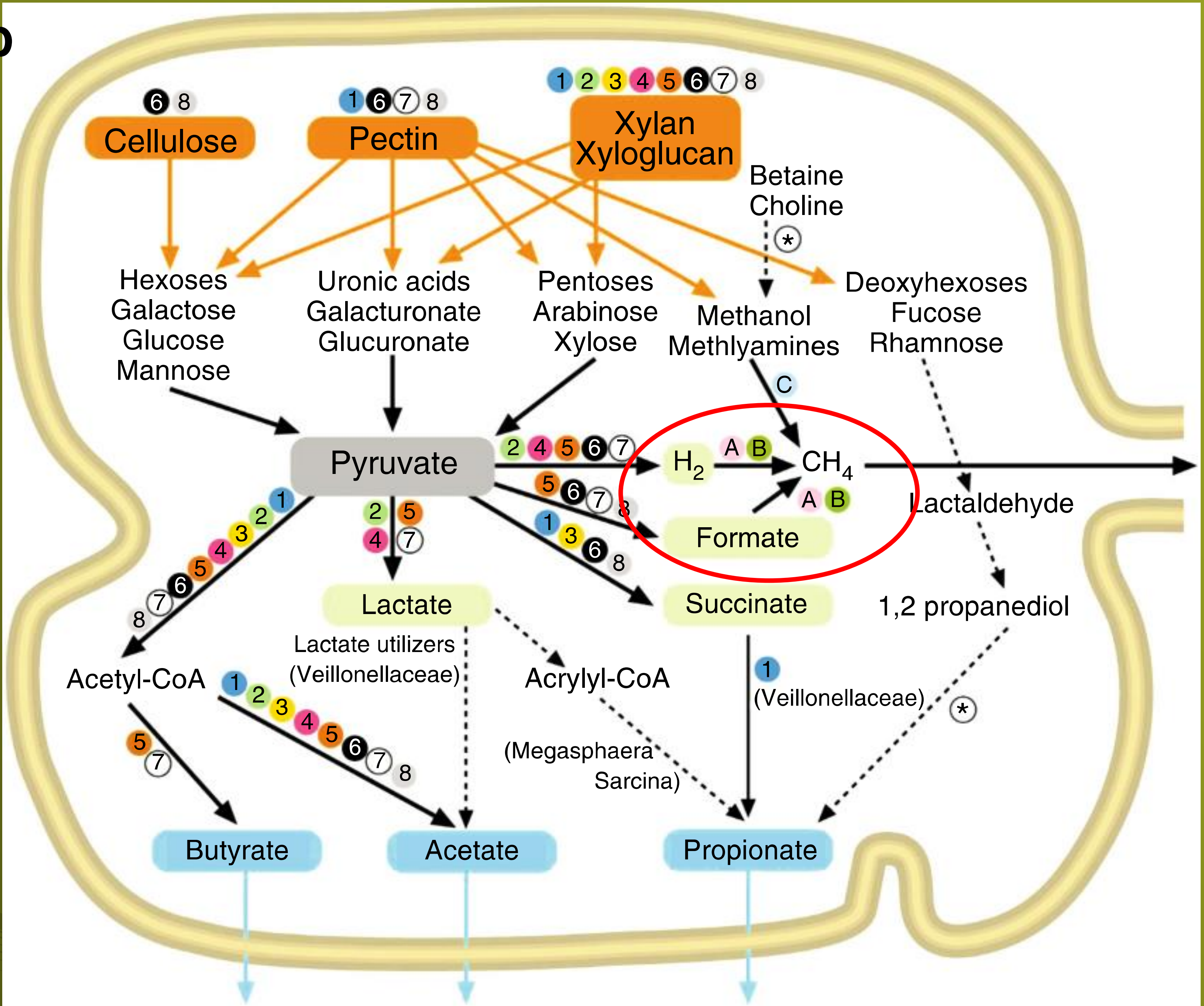
Environment

Anaerobic

High availability of H_2

pH 6.2-6.7

b



Bacterial group	Abundance %	Prevalence %
1 *Prevotella*	22	100
2 Clostridiales	15.3	100
3 Bacteroidales	8.4	100
4 Ruminococcaceae	7.9	100
5 Lachnospiraceae	6.3	100
6 *Ruminococcus*	3.6	100
7 *Butyrivibrio*	3.4	100
8 *Fibrobacter*	2.9	93
	Total 69.8	
Archaeal group		
A *M. gottschalkii*	46.9	100
B *M. ruminantium*	27.1	99
C *Methanomassiliicoccales* group 12 sp.	6.5	87
	Total 80.5	

Two main routes for methanogenesis:

- The hydrogenotrophic pathway converts H2 and CO2 produced by the protozoa, bacteria and fungi to CH4
- methyl groups, such as those present in methyl-amines and methanol.

Seshadri et al., Nature Biotech. , 36, 4; 2018

Rumen Archaea

- The ruminal methanogens can be either free-living, or associated with protozoa or fungi to improve the exposure to H_2. Approximately 9–25% of ruminal methanogens are associated with protozoa (Newbold et al., 1995), and contribute to nearly 37% of the methane production from ruminants (Finlay et al., 1994).
- Rumen methanogenic archaeal diversity is restricted to four orders and is highly conserved across ruminant species (Henderson et al., 2014).
- The most common hydrogenotrophic archaea are from the genus Methanobrevibacter, that includes Methanobrevibacter gottschalkii and Methanobrevibacter ruminantium.
- Methylotrophs archaea are less abundant and they include Methanosarcinales, Methanosphaera, Methanomassiliicoccaceae.
- The composition of the archaeal community rather than its size may have greater significance with regard to methane emissions (Tapio et al., 2017).

Rumen bacteria

- The most abundant members of rumen microbiota, more than 450 taxa have been reported
- The diversity of bacterial species in the rumen is estimated to be approximately 7,000 species. The bacterial sequences were assigned to 5,271 operation taxonomic units, which represented 19 existing phyla.
- Most represented phyla in rumen content are Bacteroidetes, Firmicutes, Proteobacteria, accounting for more than 80% of total bacteria.
- More than 90% of the Firmicutes sequences were related to the class Clostridia and Lachnospiraceae, Ruminococcaceae, and Veillonellaceae were the largest families.
- In the Bacteroidetes phylum, the majority of sequences were assigned to class Bacteroidia, and Prevotella.

Rumen bacteria associated to methane production

Several fibrolytic bacteria, such as cellulolytic Ruminococcus and Eubacterium spp (Firmicutes), are net H_2 producers.
However, other cellulolytic bacteria, such those of genus Fibrobacter, do not produce H_2 and Bacteroidetes are net H_2 utilizers.

Different 'ruminotypes' associated with variations in methane production has been identified in sheep (Kittelmenn et al., 2014):

- Low CH_4 ruminotype is characterised by high relative abundances of the propionate-producing Quinella ovalis or by higher abundances of lactate- and succinate-producing Fibrobacter spp., Kandleria vitulina, Olsenella spp., Prevotella bryantii, and Sharpea azabuensis. High relative abundances of Proteobacteria, with the dominant family Succinivibrionaceae, are associated to low emitting animals.
- High CH_4 ruminotype is characterised by higher relative abundances of species belonging to Ruminococcus, other Ruminococcaceae, Lachnospiraceae, Catabacteriaceae, Coprococcus, other Clostridiales, Prevotella, other Bacteroidales, and Alphaproteobacteria.

Rumen protozoa

Protozoa play an active role in fibre, carbohydrates, proteins and lipid digestion.

- Ciliate protozoa communities have been classified into four types: the A-type; B-type; O-type; K-type
- Protozoa of the genera *Entodinium (B-tyhpe)* and *Epidinium* (O-type) are dominant in the rumen microbial consortium.
- Protozoa are important candidates associated with methane production, because their abilities of abundant H2 production in their hydrogenosomes, their ability to host epi- or endo-symbiotic methanogens and to protect them from the toxicity of oxygen.
- Removal of protozoa could correlate to up to 11% of methane reduction, although the total methanogen abundance was not decreased significantly (Newbold, 2015).

Effect of polyphenols on rumen microbiota

- Polyphenols might have a toxic effect on some rumen microbes, by altering the permeability of the membranes and by inhibiting the enzyme activity of the ruminal microorganisms.
- The toxic effect is strongly dependent by the dose and the nature of tannins and also by the bacteria specie: gram-positive are usually more sensitive to PP than gram-negative bacteria (Smith and Mackie, 2004).
- As general effect, PP are able to reduce both methanogens and protozoa populations, but differences have been reported according to the nature of PP and to the dose adopted.
- CT have a direct inhibitory effect on hemicellulases, endoglucanase and proteolytic enzymes of several rumen microbes such as *Fibrobacter succinogenes*, *Butyrivibrio fibrisolvens*, *Ruminobacter amylophilus* and *Streptococcus bovis*. Conversely, *Prevotella ruminicola* is able to counteract the negative effect of tannins by producing protective extracellular material.

Tannins and rumen microbiota: effect on fibre digestion and methane production

- The reduction of fibre digestion could contribute to the lower methane production in the rumen of tannin-fed animals.
- This explanation could be applied to CT that are usually associated to a reduction of fibre digestion.
- As far as HT, literature evidences suggest a direct effect on methane emission, by acting through inhibition of the growth and/or activity of methanogens and/or hydrogen-producing microbes.
- Early studies on the rumen degradation of HT, reported that specific rumen bacteria, such as *Eubacterium oxidoreducens*, can degrade metabolites of HT (gallate, pyrogallol, phloroglucinol) to acetate and butyrate, by using H_2 and formate, suggesting a potential role of this pathway in the reduction of methane production (Krumholz and Bryant, 1986).

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Comparative aspects of plant tannins on digestive physiology, nutrition and microbial community changes in sheep and goats: A review

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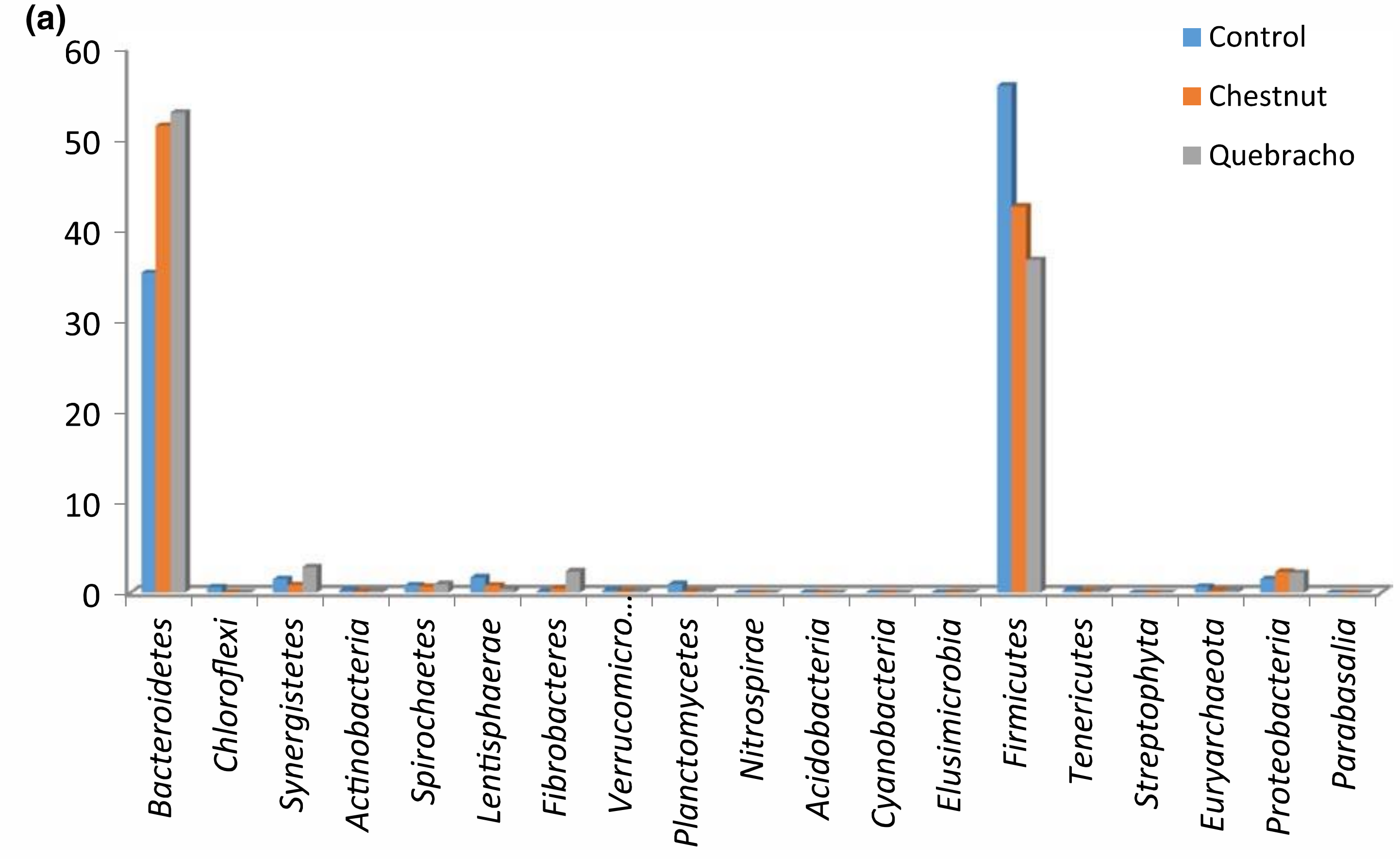
Effect of tannins (blend of HT and CT at 0.2%) on the ratio of phyla Firmicutes and Bacteroidetes in rumen microbiota in dairy goats.

Tannins have a direct influence on the composition of the rumen microbiota by selectively inhibiting gram-positive firmicutes bacteria and by enhancing the gram-negative bacteroidetes bacteria.



Polyphenols and rumen microbiota: effect on fibre digestion and methane production

	CH_4	Total methanogens	Methanobrevibacter spp	Protozoa	Fibrobacter succinogenes	Ruminococcus flavefaciens	VFA
Hydrolysable tannins	↓↓	↓	↓	↓	=↓	=↓	=↓
Condensed tannins	↓	↓	↓	↓	↓↓	↓↓	↓↓

- Hydrolysable tannins had a greater effect in reducing methane emission with less adverse effect on digestibility of dietary fibre than those of condensed tannins (Jayanegara et al, 2015; Costa et al., 2018; Mannelli et al., 2019).
- In some cases, HT also promoted the relative abundance of H_2 consuming bacteria (Mir et al., 2014).

Polyphenols and rumen microbiota: effect on fibre digestion and methane production

	Methane	Total methanogens	Methanobrevibacter spp	Protozoa	Total bacteria	Megasphaera elsdenii	Acetate:propionate
Pure flavonoids	↓↓	↓	↓↓	=↓	=	↑↑	↓

- Flavonoids have been suggested to indirectly reduce ruminal methanogenesis, acting as H_2 sinks by enhancing the concentration of the lactate consuming Megasphaera elsdenii. Moreover, a direct inhibitory effect of flavonoids on Methanosarcina spp. has been reported (Oskoueian et al., 2013; Seradj et al., 2014).

Conclusions

- Although some contrasting results are present in literature, overall, dietary PP are able to modulate the rumen microbiota composition by negatively affecting some species of fibrolytic bacteria, Hydrogenotrophic methanogens and ciliate protozoa.
- The effects of CT on cellulolytic bacteria, particularly on Gram-positive strains, and protozoa are also associated with the reduction of both fibre degradability and, indirectly, with methane emission.
- However, some authors have suggested that a direct interaction between some specific PP (such as hydrolysable tannins and citrus flavonoids) and methanogens microbes is also possible, without affecting fibre degradation.
- Tannins suppress methanogenesis by reducing methanogenic populations either directly or by reducing the protozoal population.
- Doses of tannins (especially CT) lower than 2% of DMI usually led to inconsistent results about methane reduction.

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