Impact of dietary tannins on rumen microbiota of bovines


Buenos Aires, Argentina
• Intestinal clostridial diseases: basic and applied research

• Developing strategies to prevent/control diseases

• Some years ago changed the focus: bacteria to surrounding environment

• Antibiotics misuse increase the problems

• Microbiota is key
Tannins are a complex group of polyphenolic compounds widely distributed in the plant kingdom (they are common both in Gymnosperms and Angiosperms)
Tannins are found in:

- leaf
- bud and stems
- root
- seed

They are often found in **growth areas** of plants like secondary phloem and xylem and layer between cortex and epidermis.
**Tannins** are produced by chloroplast derived organelle, the **tannosome**.

Tannosomes condense **tannins** in chlorophyllous organs, providing defenses against herbivores and pathogens, and **protection** against UV radiation.
Chemical classification

Based on identity of phenolic nuclei involved and the way they are joined
Hydrolysable tannins: hydrolyzed by enzymes or acids

Precursors: Phenolic acid (gallic acid, ellagic acid)
Condensed tannins

Precursors: derivatives of Flavonoid, catechin, flavonol-3-4-diol
An ester linkage there is between phenolic acid and glucose sugar.
Complex tannins

These tannins are mixtures of both, hydrolizable and condensed tannins

Examples: Tea, Quercus, Castanea.
Tannins extracted from chestnut wood (Castanea sativa) are an example of hydrolysable tannins.

whereas tannins extracted from quebracho (Schinopsis lorentzii) are an example of condensed tannins.
Tannins have ability to precipitate different molecules, particularly proteins.
Tannins are very common in animal and human diets
Tannins in foods

The best known human dietary source of tannins are tea and wine.
Many other foods contain tannins.
Everybody eat tannins!!

Also the World’s Champion Beef Eaters, Argentineans...

Total amount of polyphenols contained in Yerba Mate is higher than the ones found in green or black tea.
Also tannins are naturally present in soil and waters
Tannins negatively affect an animal’s feed intake, feed digestibility, and efficiency of production.

An old fashion and misunderstood concept
However, it is now known that lower concentrations of tannins may be beneficial.
In ruminants:

Low/moderate concentration of tannins in diets increase digestibility and **efficiency of utilization of nutrients**.

The attribute of tannins to **precipitate proteins** allow bypass of ruminal digestion and enhance **protein availability** at small intestine level.

**Steers in commercial feedlot (n=300)**

![Bar chart showing weight gained (as % of initial weight) for Monensin and Tannins, with P < 0.05 difference.]
Inclusion of hydrolyzable and condensed tannins increased dry matter intake, average daily gain and final body weight of steers during the finishing feedlot phase.

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<th>Publicación</th>
<th>Referencia</th>
<th>Cant. de animales</th>
<th>Rep/tx</th>
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<th>Fuente de Proteína</th>
<th>Fuente de Granos</th>
<th>PC %</th>
<th>Nem Mcal/kg</th>
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Tannins improve ADG and Feed conversion
However, the ruminal bypass cannot entirely explain the performance improvement associated with tannins addition in feed.
Known mechanisms

Unknown mechanisms

Ruminal by-pass of proteins

Decrease in the ruminal degradation of urea

Decrease in the ruminal degradation of starch and prevention of ruminal acidosis

Modulation of the peristaltic activity in the gastrointestinal smooth muscle, increasing the exposition time to enzymes.

Selection favorable to beneficial bacterial in the ruminal microbiota

Natural antioxidant activity.

Modulation of the inflammatory processes taking place in the gastric mucosa.
Idea:

Tannins improvement of metabolic and energy extraction functions in ruminants could be also associated with changes in ruminal microbiota.
Therefore, we studied the effect of tannins on rumen bacterial populations, exploring the relationship between rumen microbiome composition and physiological parameters of the rumen.

chestnut and quebracho
Experimental design

• Fistulated Holstein steers (n=6).

• After 36 day of adaptation period, tannin were or not added to diet.

• Ruminal samples were taken at -2, 0 and 12 days after tannins supplementation.
Grain-based diet

From 60% alfalfa bale, 40% concentrate <80% ground corn grain and 20% soybean meal) to high starch (19% alfalfa bale, 81% concentrate).

Tannins at a concentration of 2 g per kg of feed

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<th>Ingredients</th>
<th>% of DM</th>
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<td>Soybean meal</td>
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<td>Trace mineral and vitamins</td>
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<td>Tannins blend</td>
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<th>Composition analysis</th>
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<td>CP</td>
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<td>RDP</td>
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<td>Total Calcium</td>
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<td>NEm</td>
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<tr>
<td>NEg</td>
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DM: dry matter; CP: crude protein, RDP: rumen degradable protein; ME: metabolizable energy; NEm: net energy for maintenance. NEg: net energy for gain.
Samples were collected in sterile containers (200 mL including solid and liquid material), frozen in liquid nitrogen, freeze-dried overnight and DNA extracted by affinity columns.
Sequenced in Illumina MiSeq

1. Attach adapters to create sequencing library
2. Apply to flowcell
3. Cluster generation by solid phase PCR (bridge amplification)
4. Sequencing by synthesis with reversible terminators

[Diagram showing the process]
Dietary tannins alter ruminal richness

Bacterial populations within rumen microbiome were homogenized by tannins treatment (richness of individual animals were more “similar”)

(a)

number of OTUs

- Control: 1960
- Tannins: 1928

p = 0.05
Richness of microbiome gene content could be linked with higher feed efficiency in cattle.

Lower dispersion of microbiome richness would explain part of tannin increased efficiency?

$P < 0.01$

Coefficient of Variation in percentage of weight gained by feedlot cattle with high-starch diet (n=300).

Co-efficient of Variation in percentage of weight gained by feedlot cattle with high-starch diet (n=300).
Tannins stabilize bacterial diversity of the rumen (gradually approached to the “normal” area of the graph that concentrates most of the samples)

Principal coordinate analysis (PCoA) plot using unweighted UniFrac metric to visualize similarities in microbiota composition among animals and time points. Each color represents a single animal. Items shaped with triangles and squares correspond to pretreatment samples (T1) and samples taken at the end of tannins treatment (T5), respectively. Axes (PC1=36.2% and PC2=13.4%) account for 49.6% of total variation observed.
The ratio of *Firmicutes* to *Bacteroidetes* has been shown to affect energy harvesting and body fat accumulation in humans, mice, milking cows and steers.
Changes in Firmicutes

Within **Firmicutes** (35 vs 42% by tannins), the predominant class was **Clostridia** (90%).

Within Clostridia, Ruminococcaceae were the most abundant family (11.1% to 17.8% (p=0.009)), which explains almost 80% of the observed increase in Firmicutes.
A non-clostridia species, *Erysipelotrichi*, recently linked to beef cattle feed efficiency, was also increased.

*Veillonellaceae* family include members that are known to produce propionate as a major fermentation product (lower methane production): genus *Succiniclasticum* doubled their levels after tannins treatment (1.99 to 3.99%, p=0.08) and specializes in fermenting succinate to propionate.

Lipolytic genus *Anaerovibrio* was also favored by tannins (0.05 to 0.11%, p=0.01).

Genus *Selenomonas* was also found to be increased by tannins (0.05 to 0.12%, p=0.07) (some members of genera *Selenomonas* can break tannin–protein complexes and use tannins as energy source).
Among **Bacteroidetes** populations, the vast majority of the bacteria belonged to genus *Prevotella*.

*Prevotella* diminished in average from 21.9% to 16.5% of total microbiota after tannins addition to diet.

*Prevotella* abundance has been shown to be greater on the rumen microbiota of animals of beef cattle with low feed efficiency.
We hypothesize that the metabolic niche left by *Prevotella* would be occupied by more efficient energy-converting bacterial species, mainly belonging to phylum *Firmicutes*. 
Tannins are able to reduce fibre digestion.

Cellulolytic activity includes members of genera *Ruminococcus*, *Prevotella*, *Bacteroides*, *Fibrobacter*, *Eubacterium*, *Treponema* and *Butyrivibrio*.

Tannins would modulate ruminal cellulolytic activity mainly by selection of *Ruminococcus*, while inhibits other cellulolytic taxa.

*For example:* Treponema (1.21 to 0.41%) and Fibrobacter (0.10 to 0.005%) were significantly (p<0.05) affected by tannins.

Some fibrolytic bacteria of low abundance were enhanced by tannins: genus *Blautia* (p=0.01) and member of family *Eubacteriaceae*, genus *Anaerofustis* (p<0.05).
Inhibition of fibrolytic bacteria could contribute to decrease methane emissions in ruminants by reduction of H$_2$ production.

Our results suggest that tannins modulate ruminal cellulolytic activity mainly by selection of *Ruminococcus*, while inhibits other cellulolytic taxa.
Tannins lower the rate of microbial hydrolysis of starch–rich grains in the rumen.

The relative abundance of starch–fermenting bacteria was lightly favored by tannins addition in diet.

Streptococcus (0.45 to 1.20%), Bifidobacterium (0.69 to 1.16%) and Lactobacillus (0.83 to 1.44%). Genus Ruminobacter showed a large increase (0.005 to 0.07%, 14–fold increase).

Treponema (simple sugars) was significatively affected by tannins.
Tannins in feed reduced methane emission by 12%.

Methane production in the rumen represents a loss of 2–12% of gross energy.

Barbaro et al., 2007
Tannins are thought to directly inhibit methanogens, as well as indirectly limit methanogenesis through a reduction in hydrogen availability.

Although ruminal inhibition of methanogens has been observed with different tannins, the specific archaea affected may vary depending on tannins source and ruminants species.
Impact of tannins on physiological parameters of the rumen
Chestnut and quebracho tannins increase ruminal pH (this and others works)

Christensenellaceae has been reported by other author as a microbial marker for a balanced ruminal pH condition: we found no correlation between the abundance of family Christensenellaceae (or others) and pH ($r=0.51$)
Ureolytic bacteria in the rumen produce urease to hydrolyze urea to ammonia, which is subsequently used for the synthesis of amino acids and microbial protein.

Normally, the rate of urea hydrolysis exceeds the rate of ammonia utilization, which leads to poor efficiency of urea utilization and increases toxic ammonia concentrations in blood.
A previous study observed a drop in ruminal urease activity after addition of tannins to diet but suggested a direct interaction between tannins and urease enzyme as responsible for this inhibition.
Tannins reduce ammonia concentration.

The main ureolytic species belong to genera Succinivibrio, Treponema, Bacteroides, Butyrivibrio, Streptococcus and Bifidobacterium. Among these groups, the most abundant genera were Butyrivibrio and Treponema and were both negatively affected by tannins treatment (2.36 to 1.80% and 1.21 to 0.41%, respectively), as well as Succinivibrio (0.02 to 0.009%). Thus, the observed decline of ruminal UA may be related with the decrease of these urease–producing taxa.
Tannins modify the digestive processes not only by binding dietary protein but also through modulation of rumen microbiota and concomitant energy harvesting.
Tannins are so widely distributed in the plant kingdom that they were part of ruminant diets such as forage and sorghum since ever...
Do they have to be back?
Further research:

Tannins in a higher number of animals in different commercial productive conditions.

For how long and under which conditions remains the intestinal microbiota changes produced by tannins?
THANK YOU!