

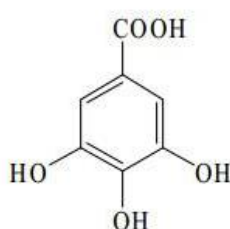
Anti-nutrition and biodegradation of vegetable tannin in feed



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Tannin is a kind of polyphenol compound with molecular weight of 500-3000, which exists widely in plants. Tannin can be divided into hydrolyzed tannin and condensed tannin according to their different structures. Tannin is active in chemical properties, especially tannin in feed is easy to combine with protein, which makes it have anti-nutritional effect. The results show that the molecular weight of tannin is the decisive factor for the combination of tannin and protein and its bacteriostasis. Polyphenols with molecular weight less than 500 can hardly make protein denature and precipitate. Based on this principle, tannin with high molecular weight can be degraded into small molecular compounds by the action of tannin degrading bacteria, thus reducing or eliminating the anti-nutritional effect of tannin.

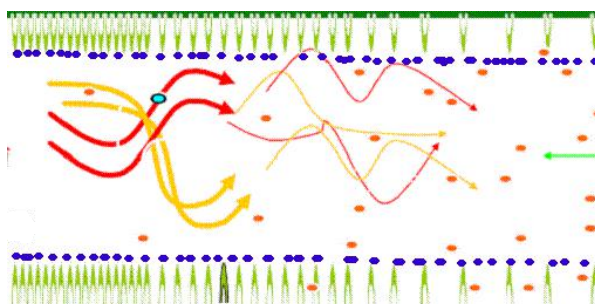
Gallic acid



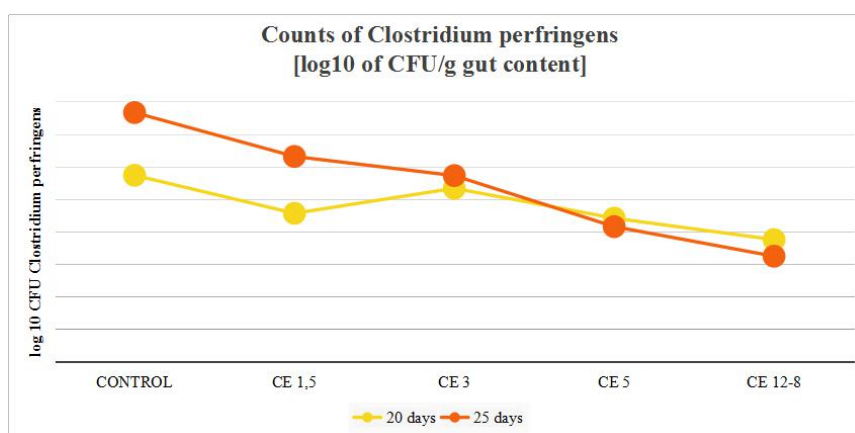
1 Antinutritional properties of tannin

The anti-nutritional effect of tannin can be considered as the result of the comprehensive action of many factors. Tannin and protein combine into indigestible molecular complex, which reduces the effective utilization of nitrogen by intestinal microorganisms. Tannin combines with macromolecular substances (such as polysaccharide, cellulose, etc.) on the cell surface, which reduces the permeability of cell wall or cell membrane, and makes the nutrients in cells difficult to be dissolved and utilized. Tannin combines with oral saliva protein, which produces bad astringency and reduces the feed intake of animals. Tannin combines with enzymes secreted by microorganisms in animal digestive tract, which makes its activity lose, slows down the digestion speed of feed, prolongs

the gastric emptying time and reduces food intake. The broad-spectrum bacteriostasis of tannins to intestinal microbes reduces or loses the digestive ability of animals to feed containing tannins.



Hydrolyzed tannin and condensed tannin can inhibit the growth of most microorganisms. At the concentration of 25mg/L, the inhibitory rates of condensed tannins on *Butyrivibrio fibrisolvens* and *Streptococcus bovis* were 48% and 92%, respectively. At the concentration of 25mg/L, tannin can inhibit endoglucanase secreted by *Fibrobacter succinogenes* S85, and at the concentration of 400mg/L, it can inactivate endoglucanase. The lethal concentrations(mg/L) of tannin to *Cellvibrio fulvus*, *Clostridium cellulosolves*, *Sporocytophaga myxococcides* and *Bacillus subtilis* were 15, 15, 45 and 75 respectively, and the lethal concentrations(mg/L) of tannic acid to these four bacteria were 12, 10, 45 and 30 respectively.

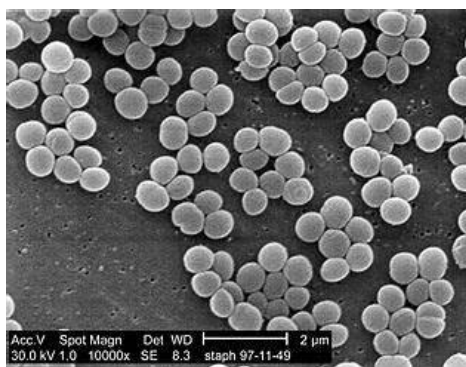


There are many ways to eliminate the anti-nutritional effect of tannin. Some herbivores secrete proline-rich salivary proteins (PRPs).

Polyethylene glycol (PEG) and polyvinylpyrrolidone (PVP) are added into the feed, which have strong affinity with tannin and can be preferentially combined with tannin. Thereby reducing the combination probability of tannin with protein or enzyme. Even the protein or enzyme which has formed the complex can be replaced, which is very beneficial to overcome the anti-nutritional property of tannin. However, research work in recent years shows that degradation of tannin in feed by microorganism is an effective way to solve its anti-nutritional effect.

2 Microorganism capable of degrading tannin

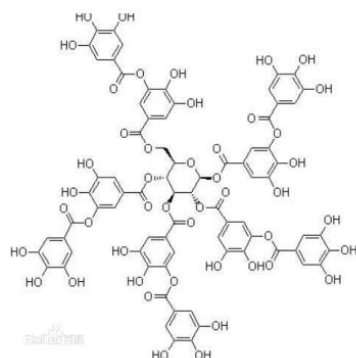
In view of the anti-nutritional effect of tannin, many scholars have isolated and bred a variety of tannin-resistant or tannin-degrading bacteria from different animals. Nelson isolated anaerobic diplococcus which can degrade hydrolyzed tannin from goat gastric juice fed with *Lysimachia christinae* with tannin up to 17%. Under anaerobic conditions, the strain can degrade tannic acid with a concentration of 30g/L into pyrocatechol in 3-4 hours, and the tolerance concentration to tannic acid can reach 70 g/L. This is the first report of anaerobic microorganism which can rapidly degrade tannic acid. In addition, the bacteria which can degrade tannin were isolated from mammalian feces by Nemoto, from cattle stomach by Tsai, from koalas by Osawa, from wild goats by Brooker and from sewage sludge by Field.



Gamble et al. found that some fungi (*Ceriporiopsis subvermispora*, *Cyathus stercoreus*) can effectively degrade condensed tannin, reduce its content by 70% and 47% respectively, and effectively remove other phenols.

3 Biodegradation mechanism of tannin

Microbial degradation of tannins is essentially due to tannase secreted by microorganisms, which transforms tannins into substances and energy needed by microbial growth. Hydrolyzed tannins and condensed tannins are combined with amino groups and carboxyl groups of protein molecules by a large number of phenolic hydroxyl groups, alcoholic hydroxyl groups and carboxyl groups, etc. through multi-point hydrogen bonding or hydrophobic bonding. It is generally believed that the biodegradation of tannin can be divided into two stages. Firstly, the intermolecular bonds in tannin-protein complex are broken by enzyme; Then tannin in free state is further degraded by tannase. Because of the different chemical structures of hydrolyzed tannin and condensed tannin, the degree of difficulty of biodegradation is different. Hydrolyzed tannin is an ester with a polyol as the core and several cool nucleic acids, and the molecular lactone bond is easy to hydrolyze under the action of acid, alkali and enzyme. At present, it is considered that hydrolyzed tannins are easily degraded by various microorganisms, especially the degradation of tannin is easier, followed by ellagic tannins. This is because tannase produced by microorganisms belongs to phenolic hydrolase in chemical nature, which can act on ester bonds in tannin molecules to degrade polyacid (or cyanuric acid) and glucose, and microorganisms can continue to secrete other enzymes (such as polyphenol oxidase, etc.), and further degrade them into small molecules such as aromatic fatty acids.



Condensed tannins are polymers of flavanols (flavanol-3-monoacid or flavanol-3,4-diol), and the units are connected by C-C bonds. Under the acidic condition of animal digestive tract, the linkage between flavanol monomers is easy to break to form a highly electrophilic center with carbonium ion, and it is easy to form covalent bond with nucleophilic groups (-NH₂, -SH, etc.) in protein molecule, which makes the degradation of condensed tannin in the first stage much more difficult than hydrolyzed tannin. Moreover, the molecular structure of condensed tannin itself is much more complicated than that of hydrolyzed tannin, which mainly shows that C-2, C-3 on the C ring of flavanol are chiral carbon atoms, which can form various stereoisomers. The units can be connected at 46 and 48 positions to form various polymers with different molecular weights. According to the specificity and high specificity of enzyme catalysis, in order to effectively degrade condensed tannins with different structures, it is necessary to have rich enzyme systems, and the C-C bond of condensed tannins is well protected due to large steric hindrance, which is the reason why condensed tannins are more difficult to degrade than hydrolyzed tannins in the later stage of biodegradation.