










Milk quality of ruminants fed with tannin-based diets

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Abstract

Tannins are found in various feeds and can produce numerous benefits to ruminants, such as preventing bloat, favoring a better use of dietary protein, and increasing microbial protein synthesis, according to their concentration, molecular weight, and structure. The tannins affect the digestibility of the protein because they form complexes with dietary proteins, improving the duodenal flow of proteins, some essential amino acids, and unsaturated fatty acids, modifying the profile of two fatty acids in meat and milk, and aging in rumen biohydrogenation. They provide faster growth rates of live weight, higher milk production, increased fertility, and a reduction in parasite load, improving the animal's well-being and health. The nutritional effects of two tannins may vary according to the type of tannin used, the level of inclusion in the diet, its chemical structure and molecular weight, the quantity ingested, the species or animal category involved, and the energy and protein balance of the diet.

Keywords: atherogenicity; fatty acids; nutritional quality; tannin.

Practical Application: Animal nutrition plays a fundamental role in the milk production system because in addition to being a determining tool for production, it influences the quality and is associated with a considerable share of the dairy herd.

1 INTRODUCTION

The maintenance and/or increase of milk production has been a challenge in the production of ruminants in arid and semi-arid regions. In this sense, the use of forage species or cultivars of high nutritive value as the main source of nutrients, associated with the use of rational management practices, makes it possible to increase animal productivity and make the dairy activity more competitive. Forages of high nutritional value, such as grasses and legumes of temperate climates, normally present high crude protein values (Waghorn et al., 1994). Therefore, most of the protein portion of these forages (56–65%) is rapidly degradable in the rumen, and there is a rumen loss of nitrogen (N) in the form of ammonium (Min et al., 2000).

Some ingredients used in animal feed may contain composts with the potential to increase the contribution of metabolizable protein, improve animal performance, and reduce the environmental impact generated by N excretion (Alves, 2012). Among these composts, the tannins present antimicrobial properties that qualify them to control ruminal microbial activity in favorable directions such as decreasing ruminal protein digestion, increasing microbial protein synthesis, decreasing methanogenesis, and modifying two fatty acids (FAs) to biohydrogenation (Naumann et al., 2017). The tannins are framed in this context according to the concentration with which they appear in the forage or the diets (Makkar, 2003; Mupangwa et al., 2000).

In high dosages, tannins can be toxic, the components of the diet can become unavailable for intestinal absorption, the activities of ruminous bacteria can be reduced to critical levels, and the activities of intestinal enzymes can be compromised (Jones et al., 2000). Consequently, animal performance may be negatively affected. However, when tannins are used in low to moderate dosages, they can prevent bloating, increase the passage of proteins, some essential amino acids, and two unsaturated fatty acids (UFAs) to the intestine, increase the production/agir in the milk composition, and reduce the urinary excretion of N (Naumann et al., 2017; Vasta et al., 2009a).

The tannins of quebracho (*Schinopsis*) and chestnut (*Bertholletia excelsa*) can increase the content of rumenic acid in the milk of sheep supplemented with sunflower (Toral et al., 2013) or soybean oil (Buccioni et al., 2015). However, in vitro and in vivo studies that evaluated the effect of tannin on biohydrogenation in the rumen and no animal performance show conflicting results (Durmic et al., 2008; Vasta et al., 2009a, 2009b). This is probably due to differences in the source of tannins and the levels of supplementation, since the nutritional effects depend on the chemical composition, molecular structure, and dose (Toral et al., 2013).

The tannin interferes with the rumen biohydrogenation (Mcsweeney et al., 2001; Mueller-Harvey, 2006) and favorably modifies the dietary biohydrogenation of two polyunsaturated

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fatty acids (PUFA), increasing the accumulation of trans-11 18:1 acid due to the inhibition of the final step of biohydrogenation (Carreño et al., 2015), as well as the enrichment of conjugated linoleic acids (CLAs) in meat and non-milk (Carreño et al., 2015; Patra & Saxena, 2011). Therefore, the objective of this review was to discover the effects of two tannins on the composition, quality, and profile of two FAs in ruminant milk.

2 DEFINITION AND CHARACTERISTICS OF TANNIN

The word tannin — we see the term “tanning” in English, which means tanning — refers to sources of tannins used in leather tanning due to their ability to bind with skin proteins (Naumann et al., 2017). Scientifically, tannins are natural compounds called polyphenols, produced by various plants (Souza et al., 2019).

Tannins are composites from the secondary metabolism that occurs in tropical plants, chemically characterized as high-molecular-weight macromolecules (500–3,000 Da), capable of forming bonds with various types of proteins, polysaccharides, and amino acids (Aganga & Monase, 2001; Makkar, 2003). They differ from other polyphenols by their ability to precipitate proteins in aqueous solutions (Silanikove et al., 2001), metal ions, amino acids, and polysaccharides (Makkar, 2003).

Tannins are chemically heterogeneous, soluble in water, and synthesized in most plants, trees, shrubs, legumes, herbs, and cereal grains (Patra & Saxena, 2009). They are naturally occurring polyphenolic composites in plants that exert a great influence on the nutritional value of forages, capable of exerting inhibitory effects of enzymatic agents, and interfering with the organization of membrane systems of the cell wall (Carreño et al., 2015). Due to their presence, food can also be an alternative to control gastrointestinal parasites (Jaganath & Crozier, 2010).

Traditionally, tannins are divided into two groups based on structural types: hydrolyzable and condensed (CT) (Figure 1). Hydrolyzable tannins (HTs) are passive and will be degraded by chemical or enzymatic hydrolysis in various structural units that compose them. They are made up of a phenolic part (gallic acid and/or hexahydroxydiphenic acid) and a hexose unit (normally glucose) linked by an ester (McMahon et al., 2000). HT can be hydrolyzed by heating with frank acid; in contrast, CT can undergo oxidative degradation only by hot mineral acid (McMahon et al., 2000).

The tannin content of the plants can vary according to the climatic and geographical conditions. Tannins present a varied chemical composition, which is often less known (Carreño et al., 2015). The CT and HT are classified according to their chemical structure (Naumann et al., 2017; Souza et al., 2019).

The CT is the most common type of tannin. It is found in forage legumes, trees (leaves, fruits, seeds, galhos, husks, and wood), vagens, and roots (Poquet et al., 2010), and in shrubs in tropical areas (Hagerman & Butler, 1981). The CTs are polymers and have received attention due to their ability to protect ingested protein from ruminal degradation (by pass) when in low concentration and because they present important

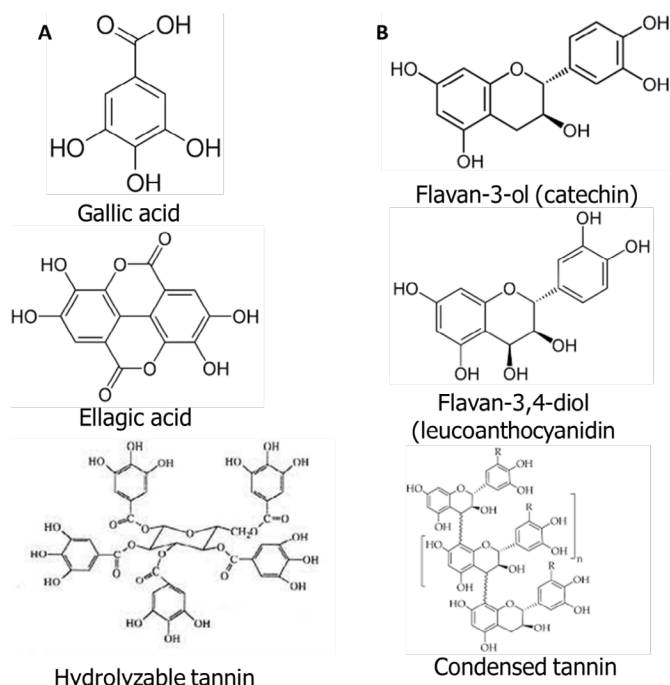


Figure 1. Structural components of (A) and hydrolyzable (B) condensed tannins.

antinutritional action when in high concentration (above 5% of dry matter (DM) in diet). CTs are seen as potential sources for new natural drugs, antibiotics, insecticides, and herbicides (Jaganath & Crozier, 2010). In animal feeding, CTs are quickly released during chewing or by manual cutting of the forages. In this first moment, the tannins confer a sensation of astringency, immediately impairing the palatability of the forage, or generally leading the animal to reduce the voluntary consumption of DM (Kondo et al., 2016).

The HTs are made up of phenolic acid polyesters that have a carbohydrate (D-glucose) in the central core (Mueller-Harvey, 2006). These tannins are found in low concentrations in plants and can undergo hydrolysis. They are hydrolyzed by free acids or free bases to produce carbohydrates and phenolic compounds, such as gallic acid or ellagic acid (Bele et al., 2010). The HT occurs more in the vagens of the two fruits and galhos of the plants (Min et al., 2003). Ruminous microorganisms are capable of degrading HT, which becomes apt for blood absorption (Bele et al., 2010; Mueller-Harvey, 2006) and, contrary to CT, its degradation products are absorbed in the small intestine of two animals, potentially causing intoxication due to the absorption of degraded HT products and a greater load of phenotypes in the bloodstream, or that is beyond the capacity of the liver to detoxify them (Makkar, 2003; Min et al., 2003).

The CTs have a more profound effect on digestibility than the HT, insofar as the second can cause various toxic manifestations due to hydrolysis in the rumen. Microbial metabolism and gastric digestion convert HT into absorbable toxic metabolites of low molecular weight. It was observed that HT is degraded to gallic acid, pyrogallol, phloroglucinol, and, finally, to acetate and butyrate, through the sequential actions of different enzymes.

2.1 Effect of two tannins on ruminants

Tannins are described as antinutritional factors because they can have a negative impact on animal production (Mueller-Harvey, 2006). According to Guimarães-Beelen et al. (2006), the soluble tannins present in the diet bind with cell wall components of two foods, resulting in indigestible complexes or inaccessibility to bacterial enzymes, reducing rumen degradability. The effects of these compounds include the inhibition of fermentation in the rumen, binding the proteins and fibers, making them resistant to digestion, or even binding with digestive enzymes, preventing their catalytic action. The growth of the microbial population can also be reduced when carbohydrates, proteins, and metal ions become inaccessible to ruminous microorganisms (Mueller-Harvey, 2006).

Tannins are found in various feeds and can produce numerous benefits to ruminants, such as preventing bloat (Makkar, 2003), favoring a better use of dietary protein, and increasing microbial protein synthesis, according to their concentration, molecular weight, and structure. Tannins affect protein digestibility as they form complexes with dietary proteins, improving the duodenal flow of proteins, some essential amino acids, and UFAs, modifying the profile of two FAs in meat and milk, and rumen biohydrogenation (Patra & Saxena, 2011). They also improve rates of growth of live weight, production of higher milk, an increase in fertility, and a reduction of parasite load, improving animal health (Mueller-Harvey, 2006).

The nutritional effects of two tannins can vary according to the type of tannin used and the level of inclusion in the diet (Muniz et al., 2022a; 2022b; Santos, 2022), their chemical structure and molecular weight, the amount ingested, and the species or animal category involved in the energy and protein balance of the diet (Muniz et al., 2022a). Muniz et al. (2022a) observed that cows receiving diets with 5.2% tannin did not modify DM intake, milk production, fatness, protein, grain, total solids, acidity, or pH. We also observed that the FAs of the milk of the cows will not change with the inclusion of tannin in the diet.

Min et al. (2005) reported that high concentrations of free CT can react with other sources of protein after chewing, as well as the enzymes secreted by ruminal bacteria, and thus inhibit the fermentation of carbohydrates in the rumen. The main forces acting on these interactions have hydrophobic effects, and these are increased by hydrogen bridges from CT, the proteins in particular, or the carbonyl group of tertiary peptides (Haslam, 1989).

Silanikove et al. (2001) revealed a hypothesis that the tannin-protein complexes were formed at the prevailing pH in the rumen ($pH = 6-7$) and that post-ruminal changes of the pH in the abomasum ($pH < 3.5$) and in the small intestine ($pH > 7$) release proteins from these complexes, making them available for gastric or pancreatic digestion. This form, or tannin-protein complex, can be formed in the rumen environment ($pH \cong 7$) and dissociated in the abomasum ($pH \cong 3.5$) or duodenum ($pH \cong 7-8$), allowing the protection of feed protein from rumen digestion and increasing the intestinal flow of protein. Tannins can impede intestinal digestion when tannin-protein complexes are formed at the beginning of the small intestine

($pH \cong 5.5$) (McNabb et al., 1993). They can also reduce protein digestion when tannin-protein complexes are formed with digestive enzymes (Silanikove et al., 2001). However, most of the studies do not take into account the factors such as the presence of bile ducts that can prevent tannins from binding to digestive enzymes.

The tannin from Trevina (*Lotus pedunculatus*) caused a greater reduction in the degradation of the forage protein than the tannin from the perennial Cornichão (*Lotus corniculatus*) (Min et al., 2005). Apparently, this difference is related to the higher molecular weight of the tannin from *Lotus pedunculatus* (12,300 Da) than the tannin from *Lotus corniculatus* (< 5,300 Da). Other studies confirmed the effect of tannin present in *Lotus corniculatus* in reducing the population of non-rumen proteolytic bacteria and observed an increase in milk production in cows (Hart et al., 2008).

Some authors advised that the inclusion of tea at 50 g of CT/kg of DM is beneficial for animal performance (Hervás et al., 2003; Min et al., 2003). Most of these recommendations originate from tests with the *Lotus* species, and many times they cannot be applied to other species. Some examples are *Hedysarum coronarium* and *O. viciifolia*, which have a beneficial effect in sheep with 72 g of CT/kg of DM and 80 g of CT/kg of DM, respectively (Ulyatt et al., 1976). On the contrary, the inclusion of only 25 g of CT/kg of DM of alfalfa pulp (*Ceratonia siliqua*) reduced the growth rates in lambs (Priolo et al., 2000). According to these differences, it becomes important to know the nutritional effects of two different types of tannins available for use in the ruminant diet, as well as the adequate dosage to achieve the intended objective.

2.2 Effect of two tannins on rumen biohydrogenation and synthesis of CLA

Due to the concern of two consumers with the quality of two products of animal origin and the growing demand for healthier products, researchers and nutritionists assist in the production of products with this function. For this reason, in recent years, some studies have focused on the composition of two FAs present in animal products and their effects on human health, especially PUFAs (AGP) and CLA (Lock & Bauman, 2004).

Lipids from the diet are rapidly metabolized by ruminous microorganisms through two processes: lipolysis and biohydrogenation. Lipolysis is responsible for the hydrolysis of two esterified dietary lipids and for the action of extracellular microbial lipases, which release free FAs and allow glycerol to be used for the production of short-chain FAs. Still, the bacteria are not capable of using these FAs as an energy source because they will be fairly reduced compounds, but they can incorporate some into their cytoplasmic membrane (Kozloski et al., 2012).

The majority of the two FAs resulting from the lipolysis process go through a process known as ruminal biohydrogenation, altering these free FAs by the addition of hydrogen instead of the double bonds, transforming the unsaturated into saturated bonds (Kozloski et al., 2012). This process is important because the UFAs have a more toxic effect on the rumen microbiota.

To circumvent this effect, the microorganisms transform the UFAs into saturated FAs by a process called biohydrogenation (Russell, 2002).

Biohydrogenation is carried out by ruminous bacteria, including *Butyrivibrio fibrisolvens*, the best-known species (Rana et al., 2012). The bacteria that carry out biohydrogenation are divided into two groups: A and B. In rumen, the bacteria of group A are more abundant than the bacteria of group B. Group A transforms linoleic acid (C18:2) or vacênic acid (C18:1 trans-11), and group B reduces vacênic acid to stearic acid (Demeyer & Doreau, 1999). Therefore, the complete pathway favors the formation of stearic acid (C18:0). Such a mechanism would be a defense against IFAs because they will be toxic to bacteria (Maia et al., 2010). The initial stage of the action of these microorganisms on the AGP (C18:2 cis-9, trans-12) begins with the isomerization of cis-9, trans-12 for cis-9, trans-11 and subsequent reduction for C18:1 trans-11 (vacênic acid). From this stage, the group B bacteria *Butyrivibrio proteoclasticus* (previously classified as *Clostridium proteoclasticum*) carry out the hydrogenation action of C18:1 trans-11 non-stearic acid (C18:0) (Demeyer & Doreau, 1999).

Thus, when an increase in the accumulation of this isomer (trans-11) occurs, this hydrogenation stage becomes inefficient, favoring a greater escape of trans-11. CLA is formed as an intermediate during the biohydrogenation of linoleic acid (Cys 18:2) in the rumen by *Butyrivibrio fibrisolvens* with the help of an enzyme linoleic acid isomerase, or through vacênic acid (C18:1 trans-11), resulting from biohydrogenation incomplete that serves as a substrate for the endogenous synthesis of CLA (cis-9, trans-11) in the mammary gland due to the action of the enzyme Δ^9 -desaturase or trans-11 (Rana et al., 2012; Vasta et al., 2009a).

Food products obtained from ruminants are an important source of CLA, two of them being the most important functional food components (Rana et al., 2012). The increase in CLA concentrations in foods derived from ruminants has been the subject of studies due to its beneficial effects on human health such as anticancer properties, reduction of two risks of cardiovascular diseases, reduction of growth of body fat and altered partition of nutrients, better modulation of the immune system (Patra & Saxena, 2011; Vasta et al., 2009b), anti-atherogenic and anti-diabetic properties, reduction and development of arteriosclerosis, and improvement of bone mineralization (Rana et al., 2012).

CLA isomers are present mainly in the fat of ruminant animals, dairy products, and partially hydrogenated vegetable oils (Rana et al., 2012). The theory of CLA in meat and milk is strongly linked to ruminal biohydrogenation of cis-9, cis-12, C18:2 (linoleic acid) and cis-9, cis-12, cis-15 C18:3 (linolenic acid, LNA). The taxa and extent of this pathway are strongly dependent on the diet of the animal (Kozloski et al., 2012). The concentrations of CLA in foods derived from ruminants can be increased by feeding and management practices that facilitate the increase in the rumen production of CLA and vacênic acid (trans-11 C18:1) for absorption and incorporation in two animals (Patra & Saxena, 2011; Rana et al., 2012).

The increase in vacênic acid in the rumen was observed in sheep receiving a concentrate with 6.4% tannin (*Schinopsis* spp., 9.57% of DM) (Vasta et al., 2010). In the same work, the rumen population of *B. proteoclasticus* was lower (30.6%; $P < 0.1$) and that of *B. fibrisolvens* and protozoa foram was higher (107 and 56.1%, respectively) in lambs supplemented with tannin. In controlled diet, sheep supplemented with tannin (*Schinopsis* spp., 4% of DM of concentrate) were observed a reduction in the concentration of stearic acid (-49%) and an increase in the concentration of vacênic acid (+97%), not fluid rumen, or that it reduced the concentrations of SFA in blood and increased the concentration of PUFA and ruminic acid (100%) in milk (Vasta et al., 2009b). These in vivo results suggest that tannin may be useful in the accumulation of vacênic acid that does not enter the rumen through the change of the rumen population and favors the endogenous synthesis of ruminic acid.

Buccioni et al. (2015) using a mixture of tannins (*Bertholletia excelsa* and *Schinopsis* spp.) at concentrations of 53 g/kg DM observed a slight increase in the concentration of C18:2, C18:1, and CLA cis-9, trans-11 and a reduction of stearic acid and AGS, and Dschaak et al. (2011) observed higher total concentrations of trans C18:1 and C18:3 with tannin supplementation (3% condensed tannin from *Schinopsis* spp.).

Toral et al. (2011), while studying eggs supplemented with sunflower oil and a commercial extract containing quebracho CT (*Schinopsis brasiliensis*) and castanheiros (*Castanea sativa*) hydrolyzate (1% of the diet), concluded that no important alterations were verified in the profile of two FAs for AGP, mainly from CLA (cis-9, trans-11) of milk, by adding tannin extract with sunflower oil in the diet of lactating sheep when compared to a control diet that contains sunflower oil. We also observed that the composition of two milky FAs has an increase in the AGI with 18 carbons, mainly of cis and trans 18:1, and a concomitant reduction in most of the two saturated FAs of short and medium chains (6:0 to 12:0 and 16:0; $P < 0.05$). The authors suggested that possibly the dose used and the type of tannins (1:1 quebracho CT and HT extract) or both may have been responsible for not modifying the FA profile due to increased rumen acid.

Khiaosa-ard et al. (2009) observed an inhibition in the final stage of biohydrogenation and also an accumulation of vacênic acid with the supplementation of *Acacia mearnsii* at 7.89% of DM. When *Acacia iteaphylla* extracts (CT) were incubated in vitro, there was also an increase in vacênic acid and a reduction in stearic acid (Durmic et al., 2008). The reduction in C18:3 biohydrogenation was observed with tannin from *Schinopsis* spp. (Kronberg et al., 2007). Buccioni et al. (2011), in an in vitro study using two sources of tannin (Portuguese chestnut (*Castanea sativa*) HT or *Schinopsis balansae* CT) in doses of 49 and 82 g/kg of DM, verified that the two main AGI theories were from liquid bacteria ruminal foram shaved by the presence of tannins in the diets, mainly the content of C18:1 trans-11, or which was significantly higher, especially with hydrolyzed tannin at 49 g/kg of DM.

Vasta et al. (2009a) studied the in vitro effect of two tannins from *Ceratonia siliqua*, *Acacia cyanophylla*, and *Schinopsis lorentzii* in three concentrations (0.0, 0.6, and 1.0 mg/mL cow rumen fluid) in ruminal biohydrogenation and verified the

highest concentration of C18:1 (23%), but the total concentration of CLA isomers was not affected. In this way, the in vitro studies demonstrated that feeding ruminants with tannin can favorably alter the final stage of rumen biohydrogenation of linoleic acid, favoring the accumulation of vacênico acid and a reduction of stearic acid in the rumen by the inhibitory effect of tannin on the bacteria of group B, which are responsible for performing the final stage of biohydrogenation, during ruminal fermentation (Khiaosa-Ard et al., 2009; Vasta et al., 2009a; 2009b). The contents of FAs, such as vacênico acid and rumenic acid, which are reported as promoters of human health, can be increased in dairy products or meat (Toral et al., 2011).

In studies with higher doses of TC in the diet of dairy sheep supplemented with a mixture of tannin extract from *Bertholletia excelsa* and *Shinopsis* spp. (10 g/kg CMS) (Toral et al., 2011), or only *Shinopsis* spp. (20 g/kg CMS) (Toral et al., 2013), the authors did not observe alterations in the concentrations of FAs in milk without a sense of a potentially healthier profile. Root supplementation of ruminants with oils rich in linoleic acid, such as sunflower oil, is known to increase the CLA content of milk, presumably through increased vacênico acid formation in the rumen (Toral et al., 2011). Another way to accumulate this acid is to inhibit the final stage of biohydrogenation in the rumen using marine lipids, oils from fish and algae (Toral et al., 2010), or, according to other studies, through tannins (Khiaosa-Ard et al., 2009; Patra & Saxena, 2011; Vasta et al., 2009b).

In cows supplemented with 150 g/day of condensing tannin (*Schinopsis balansae*-70% tannins; 0.45% of CMS), the milk FA profile was not affected; therefore, the results revealed a potential effect of tannin in altering biohydrogenation rumen with the dosage used (Benchaar & Chouinard, 2009). Patra and Saxena (2011) reported that the capacity of vegetable extracts, such as tannins, will modify the composition of FAs in food products derived from ruminants (milk or meat) that have received attention in recent years. However, information about the effects of the two tannins in the rumen biohydrogenation process and CLA theory in meat and milk is somewhat limited. CTs can inhibit the growth of many bacteria, including ruminal bacteria associated with rumen biohydrogenation. This activity can be beneficial because the inhibition of the final stage of the rumen biohydrogenation process accumulates intermediates between the quasis or CLA and its precursor for endogenous synthesis (C18:1 trans-11) (Khiaosa-Ard et al., 2009; Patra & Saxena, 2010; Vasta et al., 2009a, 2009b).

Kolling (2016) evaluated the inclusion of oregano extract (*Origanum vulgare*) and green tea (*Camellia sinensis* L.) and their non-concentrated association fed to 32 cows of the Dutch and mixed breed (Holandês and Gir) on production and milk composition. The animals supplemented with the association of oregano extract and green tea produced greater amounts of FAs in their majority of short and saturated chain, while the control group produced milk with a greater proportion of C21:0.

Dias (2016) evaluated the effect of the supplementation of the tanniferous extract of black acácia (*Acácia mearnsii*) on the profile of FAs in the milk of sheep and cows in pasture. The author observed that supplementation with tannin extract

in a proportion of 1.0–1.6% of DM consumption increased the concentrations of beneficial FAs for human health (rumenic acid, 18:1 cis-9) and observed a theory higher CLA. The sheep of the leiteira breed should be milked without harming the animal performance when the tanniferous extract is mixed with the concentrate in the proportion of 30 g/kg. No effects were observed in the cows on the lipid profile of milk; apparently, the cows are less susceptible to tannin than the sheep, or the feeding method can influence the nutritional effect of tannin.

Dairy sheep fed with lentilha palm (*Lens culinaris* Medik) rich in CT and with olive leaves rich in HT show that the inclusion of 0.84% and 1.12%, respectively, in DM increased total CLA in milk fat. The olive leaves increased vacênico acid and decreased stearic acid (Abbeddou et al., 2011). Lucena et al. (2015) evaluated the profile of FAs of eight milk multiparous goats of the Saanen breed fed with silagens from Pornunça with increasing levels of commercial tannin. We observed an increasing linear behavior with the inclusion of tannin in the silage of two respective acids: stearic acid (C18:0), oleic acid (C18:1 c9), vacênico acid (C18:1 trans), and rumenic acid (C18:2 c9t11). These acids are important for the neutral effect on cholesterol metabolism due to hypocholesterolemic action, with the advantage of not reducing HDL cholesterol, playing a role in protection against coronary heart disease and biohydrogenation. The authors also observed that the alpha-linolenic acid (C18:3 n-3) presented a linearly increasing behavior, with an increase of 41.62% when compared to the treatment without the addition of tannin with the addition of 4.8% of tannin. These lipids are essential FAs belonging to the omega 3 series, respectively, and promoters of lowering blood cholesterol, reducing the risks of cardiovascular diseases (Santos et al., 2013).

Some in vitro studies suggests that supplementation with these phenolic compounds can modify the biohydrogenation of two PUFAs present in the animal diet and promote the accumulation of vacênico acid through the inhibition of the final stage of biohydrogenation (Buccioni et al., 2011; Khiaosa-Ard et al., 2009; Vasta et al., 2009a). Other studies reported a general inhibition in biohydrogenation rather than a specific inhibition in the conversion of vacênico acid to stearic acid (Kronberg et al., 2007; Minieri et al., 2014). This beneficial effect has rarely been observed in vivo (Khiaosa-Ard et al., 2009; Vasta et al., 2009b), and other studies suggest positive effects (Vasta et al., 2009b; 2010) or are not significant (Benchaar & Chouinard, 2009; Toral et al., 2011). Due to the large variation in the structural characteristics and relativity of different tannins (Álvarez Del Pino et al., 2005; Mueller-Harvey, 2006), all these inconsistent results are possibly related to the type and/or concentration of the two tannins used. Data from studies carried out with tannins evaluating their effect on the composition of two FAs from ruminant milk are presented in Table 1.

According to Grainger et al. (2009), the use of the tanniferous extract from *Acacia mearnsii* in the diet of dairy cows supplemented with 0.9 and 1.5% of DM intake showed a low content of crude protein from the diet (15%) fed to the cows at the beginning of lactation, resulting in the reduction of milk production and its components (protein and fat) (Grainger et al., 2009). The results of these studies show that supplementation

with this tannin extract in 1.5% DM intake can reduce the rumen degradability of proteins, causing negative effects on production, chemical composition, milk FA profile, and fecal N concentration in ruminants.

The supply of a moderate source of proanthocyanidins in the ovine diet and supplementing the feed with *Lotus corniculatus* (cornichão) increase the production of milk, protein, and lactose. It is suggested that the increase in milk protein yield may be related to the fact that there is no rumen reticulum (which has a pH of 6.0–7.0), the phenolic compounds interact with proteins, thus inhibiting the utilization of protein in the rumen by local microorganisms (it is estimated that rumen reticulum microflora degrades 75% of ingested protein), and that more often than the complex phenolic-protein composites pass to the abomasum (pH 2.5–3, 5), the complexes break down the protein released, degraded, and used by ruminant hairs (O'Connell & Fox, 2001).

Toral et al. (2011) observed sheep in half lactation to assess the effects of adding a mixture (1:1) of two commercial oenological extracts of quebracho, condensed and chestnut tannins, and HT for a diet containing sunflower oil (SO) on animal performance, production, and composition of milk and ruminal fermentation. All the sheep receive supplementation with 20 g of SO/kg (DM) plus 0 (control) or 10 g of tannins/kg DM, or consumption of DM, milk, or its components, and the yield for shaved hair treated with tannin.

Souza et al. (2016) evaluated the milk production of eight cows from the cross between Dutch and Zebu fed with diets containing sun-dried whole banana peel with or without additives. The banana peel is a potential option for animal feed and stands out for presenting 10–21% pectin (Mohapatra et al., 2010) and a high content of soluble carbohydrates, which can reach 32.4% of DM. The cultivar gives the ethereal extract theory that can vary from 2 to 10.9% (Emaga et al., 2011). However, the banana peel has an astringent flavor due to its high amounts of tannins, which can be reduced or consumed when added at high levels in the diet (Martinez & Moyano, 2003). The authors concluded that the inclusion of 20% banana peel with or without additive

in the diet, in substitution for sorghum silage, does not harm the contribution of nutrients for average production (16.88 kg of milk corrected for 3.5% fat). Meanwhile, it reduces the digestibility of the DM and two nutrients, limiting the weight gain of two animals.

Silva (2013) evaluated the effect of sorghum silage with high and low tannins supplemented with and without carob bean in feeding four Dutch-Zebu crossbred cows in lactation (Table 2), concluding that sorghum silage with high tannin and concentrate containing carob can be used in the diet of lactating cows without negative influences on milk production and composition. Dias (2016) evaluated the effect of the supplementation of the tanniferous extract of black acácia (*Acácia mearnsii*) on the milk production of sheep and cows in the pasture.

Supplementation with tanniferous extract in a proportion of 1.0–1.6% of DM consumption did not harm animal performance; when the tanniferous extract was mixed with or concentrated in a proportion of 30 g/kg of DM and offered to the cows, there were no observed effects on either production or chemical composition of milk.

Flores et al. (2013), when evaluating the milk production of Dutch breed cows supplemented with three different doses of oregano extract (200, 400, and 600 mg/day), did not observe a significant difference between the groups in relation to the

Table 2. Production and composition of the milk of lactating cows as a function of the experimental diet.

Variables	Sorghum silage with low tannin		High tannin sorghum silage	
	C/ FVA	S/ FVA	S/ FVA	C/ FVA
Milk production (kg/day)	9.97	8.43	9.03	9.74
Fat	3.43	3.97	3.44	3.48
Protein	3.06	4.05	3.49	3.21
Lactose	3.57	4.52	4.45	4.41
Total solids	10.68	13.49	12.30	12.04
Fattened dry extract	7.25	9.28	8.77	8.56

Source: adapted from Silva (2013).

Table 1. Effects of dietary tannins on the composition of two fatty acids of ruminant milk.

Species	Tannin source	Dosage and fraction of tannin	Duration of experiment	Effects	References
Bovine	<i>Schinopsis lorentzii</i>	150 g/day in DM 70%, CT	28 days/periods (4×4 DQL)	I have no effect on or PMFA	Benchaar and Chouinard (2009)
Ovine	<i>Lens culinaris</i> ; <i>Olea europaea</i>	0.84% in DM 74%, CT 1.12% in DM 94%	50 50	↑ LA, CLA total In milk fat ↓ LA, LNA, c9-18:1 and 18:0. ↑ RA and VA	Abbeddou et al. (2011)
Bovine	<i>S. balansae</i>	3% in DM, CT	21 days/periods (4×4 DQL)	↑ LNA total	Dschaak et al. (2011)
Ovine	<i>S. quebracho colorado</i> ; <i>Castanea sativa</i>	1% in DM 50% HT	28	I have no effect on or PMFA	Toral et al. (2011)
Ovine	<i>S. quebracho colorado</i>	2% in DM, CT	28	I have no effect on or PMFA	Toral et al. (2013)
Bovine	Extract <i>acácia Mearnsii</i>	400 g/day CT	21 days/periods (4×4 DQL)	I have no effect on or PMFA	Aprianita et al. (2014)

CT: condensed tannins; HT: hydrolyzed tannins; DM: dry matter; LA: linoleic acid; LNA: linolenic acid; RA: rumenic acid (C18:2 cis-9, trans-11); VA: vacenic acid (C18:1 trans-11); PUFA: polyunsaturated fatty acids; PMFA: profile of two milky fatty acids.

control. Similarly, Benchaar et al. (2006) supplemented cows with 2 g/day of oregano extract and found no differences in milk production corrected to 4% fat compared to cows from the control group.

3 CONCLUSION

With the beginning of the use of phenolic composts in animal feed, numerous studies are being conducted to assess their real beneficial capacity for ruminants. Tannin supplementation has favorable potential for the rumen environment and its activities on microorganisms, although there is variability in the results attributed to the differences in the source of tannins and our levels of supplementation, since the nutritional effects depend on the chemical composition, molecular structure, and dosage. However, research shows that tannins can act positively on animal performance, production, composition, and profile of two milk FAs.

The beneficial and antinutritional effects of two tannins are linked to their ability to form complexes with other organic molecules, or the power of adaptation and use of these properties by ruminant hairs so that tannins become an important tool to make production systems more sustainable and efficient. It is becoming necessary to conduct a more complete characterization of the effects of the tannins on the most important ruminous microbial species involved in the biohydrogenation process and how they can be affected by diet, which is important for the advancement of knowledge in the area.

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