

*Chapter***TANNINS IN RUMINANT NUTRITION: IMPACT ON ANIMAL PERFORMANCE AND QUALITY OF EDIBLE PRODUCTS**

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ABSTRACT

Tannins are widespread throughout the plant kingdom, occurring as hydrolysable and condensed tannins and at different levels in several animal feeding sources. Recent years have seen an increasing interest in the use of tannin-rich plants and plant extracts in ruminant diets for improving the quality of their edible products. Some results show that this strategy is effective in improving the fatty acid profile of meat and milk, increasing the level of health-beneficial fatty acids as well as enhancing the oxidative stability of the products. However, the use of tannin-rich feed in animal diets requires great care, due to its possible detrimental effects on animal performance and induction of metabolic disorders. Although promising, the results of studies on the effects of tannins on animal performance and quality of their products are still controversial, probably depending on type and chemical structure of tannins, amount ingested, composition of diet, and species of animal. In this chapter, the current knowledge regarding the effect of dietary tannins on animal performance and the quality of their products (meat and milk),

particularly on the fatty acid profile, oxidative stability, and organoleptic properties, is reviewed. The tannin chemistry diversity and its occurrence in ruminant diets, as well as its beneficial and adverse effects on ruminants, will be briefly reviewed, and aspects related to oral cavity physiology, saliva production/composition, and postingestive effects will also be discussed.

1. INTRODUCTION

Sheep, cattle and goat are domestic ruminants of significant economic interest in the Mediterranean region, where the extensive livestock production systems are often based on the consumption of tannin-rich feed, such as shrub and tree foliage, or even agro-industrial by-products, which provide energy and nutrients during periods of pasture scarcity. In addition to their potential as nutritive ingredients, these plants have attracted increasing interest due to their tannin content, which may have an important effect on animal performance and also on the quality of their products.

Tannins are considered to have both adverse and beneficial effects, depending on chemical structure and concentration in diets, composition of basal diet, and other factors intrinsically related to the animals, such as animal species and physiological stage (Makkar et al., 2007; Waghorn, 2008; Piluzza et al., 2014). Adverse effects include reduction of feed intake, digestibility of fibre and nitrogen, and animal performance (Min et al., 2003; Makkar et al., 2007; Waghorn, 2008). Conversely, tannins may prevent bloat, enhance protein utilization during digestion, act to control internal parasites, and induce improvements in growth performance, wool growth, and milk production (Min et al., 2003; Waghorn, 2008; Piluzza et al., 2014). Moreover, tannins are also known to have antioxidant activity, and some studies show that dietary tannins may improve the animal antioxidant status (Gladine et al., 2007; López-Andrés et al., 2013).

Recent years have seen an increasing interest in plants and plant extracts rich in tannins, for use in nutritional strategies for improving some aspects of the quality of products from ruminants, particularly fatty acids (FA) composition and oxidative stability. Some studies have shown that utilization of plants or plant extracts rich in tannins in ruminant diets conduces to an increase in the levels of health-beneficial FA in meat and milk and improves the oxidative stability of meat. However, the possible adverse effects of tannins on animal performance and organoleptic properties of products constitute a major restriction on the practical application of this nutritional strategy.

With a focus on ruminant species, the main objective of this chapter is to review the current knowledge regarding the effect of dietary tannins on animal performance and on the quality of their products (meat and milk), particularly on the FA profile, oxidative stability, and organoleptic properties. Moreover, the diversity of tannin chemistry and its occurrence in ruminant diets, as well as its beneficial and adverse effects on these animal species, will be briefly reviewed. Aspects related to oral cavity physiology and saliva production/composition will be discussed, due to the known differences among ruminant species and their possible relation to different acceptance/avoidance and tolerance to these compounds. A number of aspects related to postingestive effects will also be discussed.

2. TANNINS IN FEED RESOURCES

Tannins are phenolic secondary compounds of plants that are found in approximately 80% of woody perennial dicotyledons and 15% of annual and herbaceous perennial dicotyledon species, and are present in feeds, foods, and drinks (Mueller-Harvey, 1999). They are present in almost every part of a plant – seeds, fruit, leaves, wood, bark and root, where their principal function is to provide protection against microbial pathogens, insect, pests, and herbivores (Dixon et al., 2005). The level of tannins found in most plant tissues, such as fruit and leaves, is normally in the range of 2-5% of the fresh weight, but in pathological conditions a rapid accumulation of tannins may occur (Haslam, 2007; War et al., 2012). The induction of tannin in plant tissues is also stimulated by abiotic stresses such as UV-light (Mellway and Constabel, 2009), hydric stress, temperature, ozone, and nutrient availability (Treutter, 2006).

Tannins are a heterogeneous group of polymeric phenolic compounds that possess 12-16 phenolic groups and 5-7 aromatic rings per 1000 units of relative molecular mass (Lamy et al., 2011b). They are usually classified into two groups based on their chemical structures: hydrolysable (HT) and condensed tannins (CT). Hydrolysable tannins are constituted from a carbohydrate core (often glucose) esterified with gallic acid (gallotannins) or ellagic acid (ellagitannins). Condensed tannins are oligomers or polymers of flavanol units (flavan-3-ols) commonly linked by carbon-carbon bonds in the 4/6 or 4/8 position (Hagerman and Butler, 1991; Le Bourvellec and Renarda, 2012). These compounds are also known as proanthocyanidins because in acid conditions they can be oxidatively degraded, yielding anthocyanidin pigments. Under mild or anaerobic conditions the polymer is stable (Hagerman et al., 1992). The structure of the CT varies depending on the nature of the constitutive units (number and positions of the hydroxyl groups, stereochemistry), the type and position of linkage between successive monomeric units, and the degree of polymerization (Dixon et al., 2005). Different monomeric units of flavanol, such as (+) catechin, (-) epicatechin, (+) gallo catechin and (-) epigallocatechin, lead to different classes of polymers, such as procyanidin (polymers of catechin and epicatechin) and prodelfinidins (polymers of gallo catechin and epigallocatechin). In temperate forages the number of flavanol units may range in chain length from dimmers to over 20 flavanol units, and each polymer can be composed of several flavan-3-ol structures (Waghorn, 2008).

Hydrolysable tannins are more soluble in water, and have a lower molecular weight and higher susceptibility to enzymatic and non-enzymatic hydrolysis than CT. Hydrolysable tannins are found only in dicotyledonous plants, while CT are found both in angiosperms and gymnosperms (Scalbert et al., 1989; Silanikove et al., 2001). However, according Waghorn (2008), both HT and CT can be found in the same plant.

Condensed tannins are the most common type of tannin in forage legumes, shrubs and the leaves of trees (Min et al., 2003). Many of these plants are widely available and suitable for ruminant nutrition (Vasta et al., 2008). Their utilization in ruminant nutrition has therefore increasingly seen them being exploited as alternative feeding resources with which to replace part of the cereal concentrate in animal diets, particularly in small ruminants, not only to reduce the production costs associated with livestock feeding but also as part of nutritional strategies to improve the quality of their edible products (Vasta and Luciano, 2011).

Some of these feed resources assume high importance in the Mediterranean region, where the seasonal feed deficits, particularly during the drought period, may require expensive feed supplementation. Table 1 presents the tannin levels of some feed resources widely available in Mediterranean.

Table 1. Tannin levels of feed resources widely available in the Mediterranean region

Species	Plant part	Tannin levels (g/kg DM)	Reference
<i>Arbutus unedo</i>	Foliage	232 ¹	Ammar et al. (2005)
<i>Ceratonia siliqua (carob)</i>	Leaves	50 ¹	Silanikove et al. (1996)
<i>Cistus ladanifer</i> L.	Leaves and soft stems	32.1-161 ²	Guerreiro et al. (2015)
<i>Cistus monspeliensis</i> L.	Foliage	16.1 ⁵	Unpublished data from our laboratories
<i>Cistus salvifolius</i> L.	Leaves	28-74 ³	Bruno-Soares et al. (2011)
	Flower buds	9-25 ³	
	Fruits	12 ³	
	Leaves and soft stems	133 ⁴	Unpublished data from our laboratories
<i>Hedysarum coronarium</i> L. (sulla)	Fresh plant	17.8 ⁵	Priolo et al. (2005)
<i>Olea Europaea</i> L. var. <i>europaea</i>	Foliage	0.280 ⁵	Unpublished data from our laboratories
<i>Olea Europaea</i> L. var. <i>sylvestris</i>	Foliage	0.631 ⁵	Unpublished data from our laboratories
<i>Pistacia lentiscus</i> L.	Foliage	383 ¹	Ammar et al. (2005)
	Foliage	205 ¹	Silanikove et al. (1996)
	Foliage	129 ⁶	Unpublished data from our laboratories
<i>Quercus ilex</i>	Foliage	106 ⁷	Unpublished data from our laboratories
	Fruit	47 ⁷	
<i>Quercus calliprinos</i>	Foliage	95 ¹	Silanikove et al. (1996)
<i>Quercus coccifera</i> L.	Foliage	9.25 ⁵	Unpublished data from our laboratories

Table 1. (Continued)

Species	Plant part	Tannin levels (g/kg DM)	Reference
<i>Quercus robur</i> L.	Foliage	6.46 ⁵	Unpublished data from our laboratories
<i>Quercus suber</i>	Foliage	151 ¹	Ammar et al. (2005)
<i>Rhamnus alaternus</i> L.	Foliage	2.70 ⁵	Unpublished data from our laboratories
<i>Smilax aspra</i>	Foliage	8.15 ⁵	Unpublished data from our laboratories

¹Quantified by butanol-HCl assay using purified quebracho condensed tannins as standard; ²Quantified by butanol-HCl assay using purified *Cistus ladanifer* L. condensed tannins as standard; ³Quantified by vanillin assay using catechin as standard; ⁴Quantified by butanol-HCl assay using purified *Cistus salvifolius* L. condensed tannins as standard; ⁵Quantified by butanol-HCl assay and expressed as leucocyanidin equivalent; ⁶Quantified by butanol-HCl assay using purified *Pistacia lentiscus* L. condensed tannins as standard; ⁷Quantified by butanol-HCl assay using purified *Quercus ilex* condensed tannins as standard.

3. ADVERSE AND BENEFICIAL EFFECTS OF TANNINS

For a long time, tannins were considered as antinutritive and/or toxic compounds when present in feeds due to their decreasing the intake, digestion and absorption of proteins, polysaccharides and minerals and impacting negatively on animal performance, and because they can cause ulcers, irritation and desquamation of the intestinal mucosa, lesions in the liver and kidneys, and, in extreme conditions, even death (Kumar and Singh, 1984; Kumar and Vaithyanathan, 1990; Reed, 1995; Mueller-Harvey, 2006). The severe damage caused by tannins in ruminants (renal and hepatic lesions and death) are normally associated with the ingestion of HT, which are enzymatically depolymerised in the rumen by cleaving the ester linkages between glucose and phenolic sub-units, yielding gallic acid, which is metabolized to pyrogallol and resorcinol, which are absorbed and responsible for cellular damage (Spier et al., 1987; Murdiati et al., 1992). In cattle, sheep, and llamas, several cases of acute intoxication related to intake of large amounts of HT from leaves of oak (*Quercus spp.*) and yellow-wood (*Terminalia oblongata*) were diagnosed (Garg et al., 1992; Chamorro et al., 2013) (Filippich et al., 1991). CT, by contrast, are not degraded, and absorbed into the bloodstream, and are therefore not likely to damage organs (McSweeney et al., 1988; Terrill et al., 1994; Makkar, 2003). According Makkar (2003) the organ damage caused by CT only occurs under situations of intestinal damage, where tannins can pass into the blood. The adverse effects associated with the presence of CT in animal diets are usually antinutritional due to their decreasing the intake and digestibility of proteins and carbohydrates, and inhibiting digestive enzymes and animal performances (Silanikove et al., 1996a; Barry and McNabb, 1999; Yisehak et al., 2014).

Tannins have the ability to complex with numerous types of molecules, including proteins, polysaccharides, and minerals (McSweeney et al., 2001; Min and Hart, 2003; Le Bourvellec and Renarda, 2012). The phenolic hydroxyl groups of tannins lead to the formation of complexes primarily with proteins, and to a lesser extent with polysaccharides, nucleic acids, and metal ions (Makkar, 2003). Tannins' ability to form complexes with proteins is the most important aspect of their nutritional and toxicological effects (Hagerman and Butler, 1981).

The complexes formed (tannins/proteins) can be reversible or irreversible depending on the type of linkage established, i.e. covalent non-covalent bonds. If the linkages are non-covalent (hydrogen, hydrophobic and van der Waals forces) the complexes are reversible. If the connections are covalent, the complexes formed are usually irreversible (Hagerman, 1989). The type of interactions between proteins and tannins is influenced by the relative concentration of both tannins and protein, by tannins' structure (molecular weight, conformation, flexibility of tannins, and water solubility) and protein structure (size, conformation [secondary or tertiary structure], and amino acid composition), and by the nature of the medium in which the interactions take place (solvent, temperature, pH, ionic strength) (Le Bourvellec and Renarda, 2012).

Tannins with high molecular weight, high flexibility and conformational mobility, and lower water affinity have greater ability to precipitate or interact with proteins (Hagerman, 1989). In CT, complex formation with protein is closely related to their molecular weight and to the prodelphinidin/procyanidin ratio in the molecule. Higher prodelphinidin/procyanidin ratio improves the capacity of CT to bind with protein (Aerts et al., 1999). The size and structure of the protein are other factors that determine protein binding capacity with tannins. Larger proteins with an open and flexible structure, high proline content and other hydrophobic amino acids have higher affinities for tannin than small proteins with compact globular structures (Hagerman and Butler, 1981; Asquith and Butler, 1986; Hagerman, 1989).

The solution conditions, such as pH, also play an important role in the formation of tannin/protein complexes. The optimum pH for the formation of tannin/protein complexes is near the isoelectric pH of protein, where the electrostatic repulsions that prevent aggregation of protein are minimized (Hagerman, 1989). This dependence of tannin-protein complexes on pH is of particular interest in animal nutrition because the nutritional behaviour of CT is defined along the digestive tract where pH changes occur.

So, tannins, by forming indigestible complexes, decrease the rumen turnover rate and digestibility of nutrients, which has a major impact on reducing feed intake (Makkar, 2003). Moreover, the low intake of feeds rich in tannins is attributed to a low palatability due to the sensation of astringency that tannins confer on feed by binding with salivary proteins, which fail to exercise their lubricant role in the mouth, giving an unpleasant feeling of dryness and harshness (Lesschaeve and Noble, 2005). While the interaction of salivary proteins with tannins can lead to the aversive sensation of astringency, such interaction can also result as a positive defence mechanism against the potential negative effects of tannin consumption (more details on this topic in section 4).

However, depending on factors such as tannin chemical structure and concentration in the diet, the composition of basal diet, and on other factors intrinsically related to the animals, such as species and physiological stage, the effect of dietary tannins in ruminants might be either detrimental or beneficial (Makkar et al., 2007; Waghorn, 2008; Piluzza et al., 2014). Condensed tannins have received special attention because, when present in diets at low-to-moderate concentrations, depending on the source, they may play an important role in increasing the nutritional value of feed, the quality of the products obtained, and the health and wellbeing of animals. One of the most important effects of CT ingestion by ruminants is associated with their ability to improve the digestive utilization of feed proteins. The potential of CT to increase the digestive utilization of dietary protein is associated with their ability to bind proteins under the rumen pH conditions (pH 5.5 to 7.0), preventing their excessive microbial degradation. The tannin-protein complexes are dissociated in the acidic pH of the abomasum (pH 2.5 to 3.5) and in alkaline conditions of the distal small intestine (pH <7.5), releasing protein for digestion and absorption (Jones and Mangan, 1977; Mueller-Harvey, 2006). *In vitro* and *in vivo* studies have confirmed the reduction of the effective degradability of protein induced by the presence of CT in the diet, mainly due to a marked reduction in initial solubilisation and a reduction of the fractional rate of degradation, increasing the flux of undegradable dietary protein into the post ruminal compartments without detrimentally affecting the post-ruminal digestion (Min et al., 2003; Theodoridou et al., 2010; Dentinho et al., 2014).

The reduction in rumen protein degradation leads to a lower level of N-NH₃ production in the rumen. As a result, the urinary N is reduced and the faecal N slightly increases, owing to the undegraded tannin-protein complexes formed along the digestive tract (Mueller-Harvey, 2006). At the environmental level this shift from urinary to faecal N is very important, because urinary N is predominantly urea, which is rapidly

converted to ammonia and nitrous oxide (N₂O), which has implications for environmental pollution, while the fecal N is retained in the soil and will benefit the content of organic matter (Hristov et al., 2013).

In addition, tannins are considered a promising group of compounds for decreasing enteric methane (CH₄) emissions from ruminants. Methane production in rumen represents not just an ecological problem, but also an economic one. As is well known, methane is a potent greenhouse gas, and methane produced by ruminants accounts for 28% of total anthropogenic methane (Beauchemin et al., 2008). Moreover, CH₄ produced during ruminal fermentation represents a loss of 5-8% of gross energy intake (Lopez and Newbol, 2007). The decrease of CH₄ production by tannins is achieved by their direct effect on growth and/or activity of methanogens and/or hydrogen-producing microbes or their indirect effect through reduction in fibre digestion (Tavendale et al., 2005; Goel and Makkar, 2012; Jayanegara et al., 2012). Results from a meta-analysis of *in vivo* experiments by Jayanegara et al. (2012) showed a relatively close relationship between dietary tannin concentration (HT and CT) and CH₄ production per unit of digestible organic matter. A meta-analysis conducted to compare the effects of C4 and C3 grasses, as well as warm and cold climate legumes, on CH₄ production of ruminants, concluded that CH₄ production was lower for animals fed high tannin legumes compared with animals fed low tannin legumes (Archimède et al., 2011).

Bloat is a digestive disorder that occurs in ruminants grazing on highly digestible forage legumes (e.g., alfalfa or clove). Digestion of legumes high in soluble protein can cause the formation of stable foam that limits the release of fermentation gas by eructation. The gas builds up, and the rumen becomes distended or stretched. It has been demonstrated that legumes containing CT, such as *Lotus corniculatus*, *Coronilla varia*, *Onobrychis viciifolia* or *Astragalus cicer* L., either as sole feeds or in mixtures with bloat-forming forages, prevents bloat in ruminants (Mueller-Harvey, 2006; Rochfort et al., 2008; Wang et al., 2012).

The importance of tannins in animal diets is also associated with their effects in respect of reducing intestinal parasites. Control of ruminant gastrointestinal parasites has usually been achieved by the use of anthelmintic drugs. However, this procedure is becoming more difficult due to increased parasite resistance to common anthelmintics (Pomroy et al., 2002; Min and Hart, 2003) and due to the adverse impact that chemical treatments in animal production have on the environment, animal health, and food safety. Alternative parasitic control strategies have been studied, and the use of tannin-rich feeds has been one of the proposed alternatives (Niezen et al., 1995; Barry et al., 2001; Min and Hart, 2003). Feeding studies have shown a direct effect of tannins on nematodes by their inhibition of eggs and infective larvae and reduction of larvae mobility (Butter et al., 2000; Molan et al., 2000), and an indirect effect by increasing protein availability, which strengthens the immune system, thus increasing the resistance to infections (Min and Hart, 2003).

More recently, research into the impact of dietary tannins in ruminants has been focused on lipid metabolism, and a beneficial effect of the inclusion of tannin-rich plants and plant extracts in diets on FA profile of ruminant edible products has been reported (more details in section 5.1.). Phenolic compounds such as tannins are known to have antioxidant properties, and positive impacts on antioxidant status of animals due to the presence of tannins in the diet were observed in *in vivo* studies conducted with sheep and cattle (Gladine et al., 2007b; Luciano et al., 2011; López-Andrés et al., 2013; Dey and De, 2014). Moreover, some feeding studies have also shown that dietary tannins may improve the oxidative stability of meat during storage (more details in section 5.2).

3.1. Effect of Tannins on Ruminants' Performance

Most of the effects of CT on animal performance are interlinked with feeding value. Feeding value is described as nutritive value (NV) \times intake (Waghorn and Clark, 2004). Although not directly an indication of attainable animal performance, the concept reflects the quality of forages (Martens and Barnes, 1988), comprehending the potential nutritive value (type and amount of digestible nutrients and the efficiency of their use), extent of occurrence of anti-nutritive factors, and potential voluntary dry matter intake (related to chemical and physical characteristics of feedstuffs and influenced by combination with other feeds as well as by animal-specific factors).

In this context, and as a rule, high concentrations of CT will reduce the total amount ingested due to the decrease of overall palatability and of dry matter potentially available for digestion. However, a combination of field trials, digestive physiology and chemistry research over the last 20 years has demonstrated clear benefits for lamb growth, wool production, fertility and tolerance of intestinal parasites (for a comprehensive review see Waghorn, 2008; Patra and Saxena, 2011; Pilluza et al., 2014). The apparent incongruity could be explained by the circumstances of the trials that support those findings.

Despite the wide diversity of experimental designs and results, a number of factors that can affect the impact of dietary tannins on animal performance have been identified. The physiological status of the animal seems to be one of the factors that determine the response of ruminants to diets containing CT, since physiological condition affects the animal's nutrient needs (Waghorn, 2008). Numerous trials have been conducted with lambs (e.g., Niezen et al., 1998; Ramírez-Restrepo et al., 2005) or with lactating ewes and cows (Wang et al., 1996; Woodward et al., 2004), animals with higher protein requirements, capable of responding to an increase of dietary protein and thus improving performance in response to a surplus of amino acids (Barry and Manley, 1984). Under such circumstances, the productive response to forages containing CT mainly have a positive benefit, with increases potentially ranging from 8-38% for daily gain, or 10-21% for milk production (Waghorn, 2008) compared to controls. In respect of milk production, greater availability of amino acids, particularly the essential amino acids methionine, lysine, and branched-chain amino acid, may promote milk protein and also lactose synthesis (via neoglucogenesis), contributing to increases in overall production (Wang et al., 1996).

On the other hand, the amount consumed, and also the overall diet quality, will also affect animal performance. An intake of below 50 g CT/kg DM may contribute to greater availability of amino acids absorption (Min et al., 2003). With regard to diet quality, intake of CT from browse, in combination with a medium-poor quality diet, is detrimental to performance (Waghorn, 2008). In animals fed fibrous diets, energy is likely to be the first limiting factor for performance. This type of diet conduces to low yield of volatile fatty acids, and addition of CT to the diet may contribute to depressing fibre digestibility due to inhibition of fibre-degrading bacteria, and therefore any increase in amino acid absorption will contribute to the energy balance, and not to protein synthesis (Waghorn, 2008). However, even among high feeding value forages containing CT, a direct effect on animal performance is observed. For example, Waghorn (2008) states that in sainfoin (*Onobrychis*), sulla (*Hedysarum coronarium*) and lotus major (*Lotus pedunculatus*) the CT do not appear to have a beneficial effect on productivity, while the CT in birdsfoot trefoil (*Lotus corniculatus*) have a favourable impact on ruminant production.

In conclusion, the use of low-to-moderate CT in diets allows for increases in the efficiency of protein digestion and may improve animal health and production under grazing, depending upon the concentration and chemical structure of these compounds (reviewed by Min et al., 2003; Frutos et al., 2004; Ramírez-

Restrepo and Barry, 2005). Additionally, and from an ecological point of view, this may enable the development of more sustainable grazing systems (Min et al., 2003; Ramírez-Restrepo and Barry, 2005).

4. TANNINS AND FEEDING BEHAVIOUR IN RUMINANTS

Grazing animals eat a wide variety of plants, but often prefer some and avoid others. Animals select the plants they eat based on the flavour, defined as the perceptual combination of three anatomically distinct chemical senses: taste, smell, and chemosensory irritation (Beauchamp and Mennella, 2009). Food flavour is determined by its physical and chemical characteristics, and during ingestion, flavour cues are integrated with the postingestive effects of nutrients and toxins, influencing its palatability (Provenza, 1995; Mayland and Shewmaker 1999; Atwood et al., 2001). Several studies indicate an important role of saliva in the perception of taste and texture sensations (Engelen et al., 2007), whereby the saliva composition can modulate food perception and, simultaneously, be modulated by the type of diet (Dawes, 1970; Mese and Matsuo, 2007). On the other hand, salivary proteins have been identified as one of the animal's defence mechanisms against tannins (a detailed list of studies is reviewed in Shimada, 2006).

4.1. Saliva Composition and Tannin Intake

As stated above, the low intake of feeds rich in tannins is attributed to a low palatability because of the sensation of astringency that tannins confer on feed by binding with salivary proteins (Lesschaeve and Noble, 2005). Two mechanisms have been proposed to explain the astringency: 1) The interaction of tannins with glycoproteins causes a rupture of the lubricating salivary pellicle, which covers all of the oral surfaces in the oral cavity, causing friction; 2) such rupture of the salivary pellicle leads to exposure of the oral mucosa, allowing tannin-protein aggregates to interact directly with the oral tissues, possibly via receptors (Gibbins and Carpenter, 2013). It is also possible that the free tannins themselves interact directly with the mucosa/receptors after disruption of the salivary pellicle by tannin-protein aggregates (Gibbins and Carpenter, 2013). This salivary pellicle contains many proteins, especially mucins, salivary proteins with high molecular weight, highly glycosylated (Tabak et al., 1982; Morzel et al., 2004), and some authors suggest that its composition can be altered by dietary polyphenols (Davies et al., 2014).

Tannin-binding salivary proteins (TBSPs) are proteins which present a particularly high affinity for tannins, allowing the complexes formed to be stable at the different pH conditions of the gastrointestinal tract. Such stability allows the passage of tannins through the digestive system, in a bound form, preventing them from being degraded and absorbed (HT) or interacting with other food or endogenous molecules. The salivary proteins usually reported as TBSPs are proteins belonging to two different families: proline-rich proteins (PRPs) and histatins. Whereas PRPs have been identified in different animal species [e.g., Patamia et al. (2005) and Fanali et al. (2008)], salivary histatins have only been reported in humans (de Sousa-Pereira et al., 2013). Shimada (2006) studied the presence of TBSP in several species of animal, concluding that their levels can be related to the amount of tannins constitutive of each species' regular diet. For example, in rodent species (e.g., rats and mice), PRPs are not constitutively present in saliva, but feeding tannin-rich diets for 3-4 days is sufficient to induce a dramatic increase in the secretion of these salivary constituents (Carlson, 1988).

In ruminants, it has been suggested that TBSPs are present in concentrate selectors or browsers (which thrive on tannin-rich diets), such as deer, absent in grazers, such as sheep and cattle, and present in intermediate amounts in mixed feeders (Austin et al., 1989; Shimada, 2006). Nevertheless, there is no consensus as to the presence or absence of salivary proteins with affinity for tannins in ruminant species. The question remains particularly controversial for domestic species such as sheep and goats. Changes in salivary protein profiles after the ingestion of a CT (from quebracho extract) enriched diet were observed in both sheep and goats, although the salivary proteins whose levels changed differed between the species (Lamy et al., 2011a). The presence of a relatively high percentage of the amino acids proline (6.5%), glutamine (16.5%) and glycine (6.1%) in goat parotid saliva, together with the observation that these animals' salivary secretion was altered in the presence of a tannin-rich diet compared to one with lower levels of tannins, suggested the presence of TBSPs in goat saliva (Silanikove et al., 1996b). In addition, Alonso-Díaz et al. (2010) suggested the presence of TBSPs in goats. These authors observed that these animals were able to ingest considerable levels of plant secondary metabolites without suffering negative effects, and attributed this capacity to saliva. Moreover, they observed that goat saliva has the capacity to precipitate tannins extracted from tropical plants (Alonso-Díaz et al., 2012). Changes in saliva secretion (increase in the production of parotid saliva in response to ingestion of quebracho tannin) were suggested as increasing the ability of Alpine goats to deal with tannin consumption (Salem et al., 2013). Distel and Provenza (1991) concluded that goats do not secrete TBSPs, since they did not find salivary PRPs in this species. Corroborating this conclusion, other studies, using proteomic approaches, found none of these salivary proteins in Mediterranean goat saliva (Lamy et al., 2008; Lamy et al., 2009). Hanovice-Ziony et al. (2010) did not find salivary proteins with affinity for tannic acid or quebracho tannins in Mediterranean goats, either constitutively or after three months' exposure to tannin-enriched diets.

There is a similar lack of consensus as to the presence/absence of tannin binding proteins in sheep saliva. As with goats, PRPs have not been observed in parotid (Lamy et al., 2008; Lamy et al., 2009) or mixed saliva (Austin et al., 1989). However, the ingestion of a CT (quebracho extract) enriched diet was observed to result in changes in salivary protein profiles (Lamy et al., 2011a). Nevertheless, the potential of such differently expressed salivary proteins in the binding of tannins was not explored in the study cited here. Salem et al. (2013), although not testing the tannin binding capacity of saliva, did not observe changes in parotid sheep saliva composition, in terms of electrolytes, after short- or medium- term quebracho or tannic acid consumption, and other authors have also suggested that sheep saliva may not be particularly important in neutralizing tannins (Ammar et al., 2013). However, other studies have observed that salivary proteins from sheep are capable of precipitating certain types of tannins (Vargas-Magaña et al., 2013).

The differing results obtained from the different studies of saliva-tannins interaction in ruminant species may be attributable to the diversity of different types of tannins, as has been previously mentioned. Although not in ruminants, it has been demonstrated that the salivary response to different types of tannins may differ according to tannin structure: salivary changes were different when mice consumed a diet enriched in CT or HT (da Costa et al., 2008; Lamy et al., 2010). Interestingly, Vargas-Magaña et al. (2013) also reported higher affinity of sheep saliva to one type of tannin and higher affinity of goat saliva to another type. Taken together, these results suggest the importance of considering the animal species and type of tannins when investigating the role of salivary proteins in tannin ingestion by ruminants. Moreover, knowledge about the interaction between saliva and tannins may be of particular relevance for the development of nutritional programs.

4.2. Tannins and Postingestive Effects

Ruminants have certain unique characteristics in terms of digestion and metabolism of nutrients. They consume an enormous amount of high fibre and low energy diets, which require ruminal fermentation. This results in some particularities in feed intake regulation. For example, unlike non-ruminants, blood glucose concentration in ruminants does not directly increase with feeding (Nagamine et al., 2003). Several factors have been found to affect ruminant feed intake. Studies based on each factor isolated have resulted in three main “feedback” theories: 1) “physical” theory, which suggested that intake is depressed when the rumen is filled (Allen, 1996); 2) “chemostatic” theory, for which the concentrations of nutrients and energy (including volatile fatty acids) are involved in controlling intake (Anil et al., 1993; Illius and Jessop, 1996); and 3) “oxygen efficiency” theory, which stated that ruminants eat the amount of forage that gives the optimum yield of net energy per unit of oxygen consumed (Ketelaars and Tolkamp, 1996). Additionally, ruminal and blood osmolality have also been observed to influence food intake (Carter and Grovum, 1990). However, these factors seem to act in concert, rather than in an isolated manner, and their integration has been studied (Forbes, 1996; Fisher, 2002).

Diet selection has been considered within a framework of feeding behaviour that views both selection and intake as outcomes of the animal’s internal state and knowledge of the feeding environment (Kyriazakis et al., 1999). Preference is demonstrated by the animal in relative consumption of one plant over another when given a free choice (Frost and Ruyle, 1993). Animals learn to avoid plants or plant parts through two interrelated systems: affective and cognitive. The affective system integrates the taste of feed with postingestive feedback. This system modulates the intake of feed items depending on whether the postingestive feedback is aversive or positive. The strength of aversion to toxic sources is known to depend on the strength of postingestive physiological effects (du Toit et al., 1991). Simultaneously, the cognitive system integrates the odour and sight of food with its taste. Animals use these senses to differentiate among feeds, and to select or avoid the ones for which postingestive feedback is either positive or aversive, respectively (Provenza et al., 1992). In a recent review, Ginane et al. (2015) provide a conceptual framework that emphasizes the critical involvement and interconnections of these two major regulatory systems of feeding behaviour: the reward and the homeostatic systems.

Gustation and olfaction are reported to be the principal senses in distinguishing food sensory properties, and are crucial for the detection and consumption of palatable foods, as well as for detection and rejection of bitter tasting substances (Mayland et al., 1997; Mayland and Shewmaker 1999; Ginane et al., 2011). Tannins are not only associated with a sense of astringency; Soares et al. (2013) show that several polyphenolic compounds classified as tannins activate various human bitter taste receptors. Outside the oral tissues, major receptors to bitter are also found in the gastrointestinal tract and other extra gustatory tissues (Wu et al., 2002; Rozengurt, 2006; Behrens and Meyerhof, 2011; Colombo et al., 2012; Cheled-Shoval et al., 2014), suggesting that a taste-sensing mechanism may also exist apart from the oral cavity, possibly participating in postingestive regulation.

A better understanding of the mechanisms associated with sensory cues and their effects on appetite and ingestive behaviour could be an interesting way to manipulate feedstuffs to alter feed preferences, including with the incorporation of tannins.

5. TANNINS AND THEIR EFFECTS ON CHARACTERISTICS OF RUMINANT EDIBLE PRODUCTS

5.1. Effect of Dietary Tannins on Meat and Milk Fatty Acid Profile

Ruminant fat is characterized by high levels of saturated fatty acids (SFA), low levels of polyunsaturated fatty acids (PUFA), and a variable content in *trans*-FA – properties that are regarded as being negative to human health. Such FA profile is mainly due to the intense metabolism that dietary lipids suffer in the rumen, where they are rapidly hydrolysed and the unsaturated FA released (mostly C18 PUFA) are biohydrogenated, with production of the high levels of SFA (mostly 18:0) as well as a variable amount of several isomeric C18 FA, including octatrienoic, octadecenoic and octamonoenoic acids, as result of the incomplete biohydrogenation of the unsaturated C18 FA (Harfoot and Hazelwood, 1997; Bessa et al., 2007). A considerable amount of research has therefore aimed at improving the FA composition of ruminant edible products, to reduce its SFA contents and increase its PUFA levels, particularly in *n*-3 PUFA and conjugated linoleic acid isomers (CLA).

The beneficial effects of both groups of FA have been the subject of numerous studies (see Palmquist, 2009; Dilzer and Park, 2012).

Conjugated linoleic acid isomers are produced during ruminal biohydrogenation, and some can also be produced endogenously in tissues from the *trans* monoenes, which are also produced during ruminal biohydrogenation (Shingfield and Wallace, 2014), making the edible products of ruminants naturally rich in CLA. Rumenic acid (18:2 *cis*-9, *trans*-11, RA) is the major CLA isomer found in ruminant fat (Parodi, 2003), and is formed by ruminal biohydrogenation of linoleic acid (18:2 *n*-6, LA) (Harfoot and Hazelwood, 1997) and mainly by endogenous conversion of vaccenic acid (18:1 *trans*-11, VA) by the action of stearoyl-CoA desaturase (SCD) in tissues (Griinari et al., 2000). The VA is an intermediate product of the ruminal biohydrogenation of both LA and α -linoleic acid (18:3 *n*-3, LNA) (Harfoot and Hazelwood, 1997). So, the enrichment of RA in ruminant fats can be achieved by increasing the rumen outflow of RA and VA and by increase of the endogenous conversation of VA to RA by the SCD (Bessa et al., 2015).

The possibility of improving the nutritional value of ruminant fat via manipulation of the ruminal biohydrogenation through modulation of dietary factors has been recognised. Several dietary factors capable of modulating the ruminal biohydrogenation have been identified, such as the amount and type of lipid supplement or basal diet, but in recent years much of the search for ruminal biohydrogenation modulators has been dedicated to secondary compounds of plants, such as tannins. The results of *in vitro* and *in vivo* studies indicate that both tannin types – CT and HT – are able to influence the ruminal biohydrogenation. However, the results of studies of tannin effects on ruminal biohydrogenation are controversial. Some *in vitro* studies have reported the accumulation of the VA and decrease of the stearic acid (18:0, SA) in ruminal fluid and in rumen solid associated bacteria after incubations with tannin sources, either CT or HT, suggesting the inhibition of the last step of the ruminal biohydrogenation (Durmic et al., 2008; Khiaosa-Ard et al., 2009; Vasta et al., 2009a; Buccioni et al., 2011). In accordance with *in vitro* results, *in vivo* studies conducted with lambs also observed the increase in VA production and decrease of the SA in digesta (ruminal fluid and abomasal digesta) from lambs fed diets supplemented with quebracho (*Schinopsis lorentzii*) tannin extract (Vasta et al., 2009b; Vasta et al., 2010b) and *Cistus ladanifer* (rockrose, a shrub rich in CT, distributed widely over the marginal fields of Mediterranean countries) (Jerónimo et al., 2010). The

same trend was also observed in ruminal fluid from goat kids supplemented with aqueous extract of a tanniferous plant – *Terminalia chebula* (Rana et al., 2012). However, other *in vitro* results on the influence of sources of CT and HT on ruminal biohydrogenation suggest the induction of the general inhibition of the biohydrogenation rather than the specific negative effect on the last step, observing the reduction of the LA and LNA biohydrogenation but not the effect on the last step of biohydrogenation (Khiaosa-Ard et al., 2009; Minieri et al., 2014; Carreño et al., 2015).

In addition to these findings on the impact of dietary tannins on ruminal biohydrogenation, the effect on FA composition of meat and milk of inclusion of several plant extracts and plants rich in tannins in ruminant diets has been extensively scrutinized (Tables 2 and 3 for meat and milk, respectively).

5.1.1. Meat

In the first studies on the effect of dietary tannins on the FA profile of meat, the tannin-containing diets were compared to control diets without tannins and to diets with the same levels of tannins and supplemented with polyethylene glycol (PEG), a binding agent that deactivates the effects of tannins. Priolo et al. (2005) reported that lambs fed fresh sulla (*Hedysarum coronarium*, 17.8 g of CT per kg DM) with or without PEG had higher levels of RA, LNA and MUFA, and lower levels of LA and SFA in intramuscular fat, than those fed a concentrate diet; only a slight reduction in the LNA was observed in meat from lambs fed sulla supplemented with PEG when compared with meat from lambs that received sulla without PEG. So, the differences in FA profile found in the intramuscular fat of lambs fed concentrate or sulla seem to derive from the well-known effect of concentrate and green herbage/grass on the FA profile of ruminant edible fat (Vasta et al., 2008) and not from the dietary tannins. Conversely, Vasta et al. (2007) showed the reduction of RA and VA in intramuscular fat by inclusion of carob pulp (*Cerotonia siliqua*) in lamb diets (45% carob pulp, which results in diets with 27 g of CT per kg DM) compared to control diet. However, the PEG supplementation of the lamb diets containing carob pulp increased the levels of RA and VA in meat, suggesting the effect of the carob CT on ruminal biohydrogenation. Change in the FA profile of intramuscular fat was also observed in lambs fed diets with increasing levels of carob (24 and 35% of feed; 3.4 and 4.5 g total tannins/kg DM, respectively) (Gravador et al., 2015). Inclusion of carob-pulp in lamb diets resulted in lower amounts of SA and total SFA, lower n-6/n-3 ratio, and higher levels of total PUFA, RA, LA and LNA in meat compared to control treatment (Gravador et al., 2015), thus leading to the production of meat with a FA profile that is considered more beneficial to health.

Table 2. Effect of tanniferous diets on fatty acid profile of meat from ruminants

Tannin source	Species	Tannin Levels (g/kg DM)	Duration of treatment	Effects	Reference
Sulla (<i>Hedysarum coronarium</i>)	Ovine	17.8 ²	63 days	sulla (with or without PEG) ↑ RA, LNA and MUFA and ↓ LA and SFA PEG ↓ LNA compared to sulla	Priolo et al. (2005)
Carob pulp	Ovine	27.0 ²	45 days	↓ RA, VA and SA PEG ↑ RA and VA compared to carob	Vasta et al. (2007)
Carob pulp	Ovine	3.4-4.5 ³	56 days	Carob ↓ SA, total SFA and <i>n-6/n-3</i> ratio and ↑ total PUFA, RA, LA and LNA	Gravador et al. (2015)
Quebracho tannin extract (<i>Schinopsis lorentzii</i>)	Ovine	40.4-40.6 ³	60 days	↑ VA and RA ↓ SA in concentrate diet ↓ PUFA and LA in herbage diet ↓ SFA in concentrate and herbage diets	Vasta et al. (2009b)
Quebracho tannin extract (<i>Aspidosperma quebracho</i>) ¹	Ovine	-	12 days	Minor effect on fatty acid profile	Borgna et al. (2014)
Grape seed extract	Ovine	13.3-14.4 ⁴	42 days	Minor effect on fatty acid profile	Jerónimo et al. (2012); Jerónimo et al. (2010)
Rockrose (<i>Cistus ladanifer</i>)	Ovine	20.7-21.0 ⁵	42 days	↑ VA and RA in lambs feed diets supplemented with vegetable oils	Jerónimo et al. (2012); Jerónimo et al. (2010)
Rockrose (<i>Cistus ladanifer</i>)	Ovine	2.7-14.1 ⁵	42 days	20% CL ↑ total <i>trans</i> -MUFA Without effect on VA	Francisco et al. (2015)
Redberry juniper (<i>Juniperus pinchotii</i>)	Ovine	-	86 days	↑ RA and ↓ SA	Whitney et al. (2011)

Table 2. (Continued)

Tannin source	Species	Tannin Levels (g/kg DM)	Duration of treatment	Effects	Reference
Redberry juniper (<i>Juniperus pinchotii</i>)	Ovine	-	96 days	↑ 18:1 <i>trans</i> -10 and total 18:1 <i>trans</i>	Whitney and Smith (2015)
Sainfoin (<i>Onobrychis viciifolia</i>)	Ovine	104 ⁶	103-145 days	↓ SFA and ↑ PUFA n-3 LC-PUFA and PUFA/SFA	Girard et al. (2015)
Birdsfoot trefoil (<i>Lotus corniculatus</i> L.)	Ovine	21 ⁷	103-145 days	↓ SFA and ↑ PUFA, LNA, LA, n-3 LC-PUFA and PUFA/SFA	Girard et al. (2015)
Birdsfoot trefoil (<i>Lotus corniculatus</i> L.)	Caprine	-	180-187 days	↓ LA and 18:1 <i>trans</i> -10	Turner et al. (2015)
<i>Terminalia chebula</i>	Caprine	-	90 days	↑ RA and MUFA and ↓ SA and SFA	Rana et al. (2012)
<i>Acacia Karroo</i>	Bovine	74 ⁸	60 days	↑ VA, LNA and n-3 LC-PUFA	Mapiye et al. (2011)
High-tannin sorghum	Bovine	-	102-123 days	Without effect in meat fatty acid profile	Larraín et al. (2008)

LA – linoleic acid, 18:2 *n*-6; LNA – linoleic acid, 18:3 *n*-3; MUFA – sum of monounsaturated fatty acids; *n*-3 LC-PUFA – sum of *n*-3 long chain polyunsaturated fatty acids; PEG – polyethylene glycol; PUFA – sum of polyunsaturated fatty acids; RA – rumenic acid, 18:2 *cis*-9, *trans*-11; SA – stearic acid, 18:0; SFA – sum of saturated fatty acids; VA – vaccenic acid, 18:1 *trans*-11; ¹diet with tannins supplied during 12 days followed by the consumption of alfalfa pellets during two weeks; ²Quantified by butanol-HCl assay and expressed as leucocyanidin; ³Quantified by the method of Makkar et al. (1993) and expressed as tannic acid equivalents; ⁴Quantified by butanol-HCl assay using purified grape seed condensed tannins as standard; ⁵Quantified by butanol-HCl assay using purified *Cistus ladanifer* L. condensed tannins as standard; ⁶Quantified by butanol-HCl assay using purified *Onobrychis viciifolia* condensed tannins as standard; ⁷Quantified by butanol-HCl assay using purified *Lotus corniculatus* L. condensed tannins as standard; ⁸Quantified by butanol-HCl assay using *Desmodium intortum* condensed tannins as standard.

Table 3. Effect of tanniferous diets on fatty acid profile of milk from ruminants

Tannin source	Animals	Tannin Levels in diet (g/kg DM)	Duration of treatment	Effects	Reference
Sulla (<i>Hedysarum coronarium</i>)	Ewes	-	3 weeks	↑ short-chain FA, LNA and atherogenicity index and ↓ 18:1 <i>cis</i> -9 = RA to annual ryegrass and < than burr medic and daisy forb.	Addis et al. (2005)
Sulla (<i>Hedysarum coronarium</i>)	Ewes	-	-	↓ RA, 18:1 <i>cis</i> -9 and total MUFA ↑ LA, LNA and total PUFA	Cabiddu et al. (2005); Piredda et al. (2002)
Sulla (<i>Hedysarum coronarium</i>)	Ewes	26.6 ¹	56 days	↑ RA and VA and ↓ LA and LNA by supplementation with PEG	Cabiddu et al. (2009)
Quebracho (<i>Schinopsis lorentzii</i>) and chestnut (<i>Castanea sativa</i>) tannin extracts	Ewes	10 ²	4 weeks	Minor effect on milk fatty acid profile	Toral et al. (2011)
Quebracho tannins extract (<i>Schinopsis lorentzii</i>)	Ewes	18 ²	4 weeks	Minor effect on milk fatty acid profile	Toral et al. (2013)
Quebracho tannins extract (<i>Schinopsis lorentzii</i>)	Ewes	24 ²	4 weeks	↑ LA, RA and VA ↓ total SFA and SA	Buccioni et al. (2015)
Chestnut tannins extract (<i>Castanea sativa</i>)	Ewes	40 ²	4 weeks	↑ LA, VA and SA ↓ total SFA and RA	Buccioni et al. (2015)

Table 3. (Continued)

Tannin source	Animals	Tannin Levels in diet (g/kg DM)	Duration of treatment	Effects	Reference
Quebracho tannins extract (<i>Schinopsis balansae</i>)	Cows	150 g/dia 0.45% DMI	28-d period (4 x 4 Latin square)	Without effect in milk fatty acid profile	Benchaar and Chouinard (2009)
Quebracho tannins extract (<i>Schinopsis</i> spp.)	Cows	22.5 ²	21-d period (4 x 4 Latin square)	↑ LNA and total 18:1 <i>trans</i>	Dschaak et al. (2011)
Birdsfoot trefoil (<i>Lotus corniculatus</i>)	Cows	-	14 days	↑ RA, LA and LNA and ↓ VA and SA	Turner et al. (2005)

FA – fatty acids; LA – linoleic acid, 18:2 *n*-6; LNA – linoleic acid, 18:3 *n*-3; MUFA – sum of monounsaturated fatty acids; *n*-3 LC-PUFA – sum of *n*-3 long chain polyunsaturated fatty acids; PEG – polyethylene glycol; PUFA – sum of polyunsaturated fatty acids; RA – rumenic acid, 18:2 *cis*-9, *trans*-11; SA – stearic acid, 18:0; SFA – sum of saturated fatty acids; VA – vaccenic acid, 18:1 *trans*-11; ¹Quantified by butanol-HCl assay and expressed as leucocyanidin; ²Value calculated using the commercial indications about amount of condensed or hydrolysable tannins in extracts.

Vasta et al. (2009b) demonstrated that inclusion of quebracho (*Schinopsis lorentzii*) tannin extract either to barley-based concentrate (40.4 g total tannins/kg DM) or to fresh vetch herbage-based (40.6 g total tannins/kg DM) diet affects the FA profile of intramuscular fat from lambs, but that the effect was dependent on the basal diet. In concentrate diets, the addition of quebracho tannins to diets led to an increase of the VA, RA, and total 18:1 *trans* amounts in intramuscular fat, while SA decreased. Lambs that received the herbage diet supplemented with quebracho tannins showed higher levels of total PUFA and LA in meat than those fed herbage without tannin addition. In both diets, tannin supplementation reduced the SFA content in meat (Vasta et al., 2009b). Such results indicate that tannins may affect differentially the FA profile of ruminant edible products depending on the basal diet. Studies using *Cistus ladanifer* also identified a distinct effect on the FA profile of meat when the composition of the basal diet varied. Inclusion of leaves and soft stems of *Cistus ladanifer* in lamb diet (250 g/kg DM) composed of dehydrated lucerne supplemented with 6% of vegetable oil blend (diets with 21 g CT/kg DM) resulted in an increase of the VA, RA and depression of SA in intramuscular fat (Jerónimo et al., 2010). However, in lambs that received diets without oil supplementation, the *Cistus ladanifer* had no major effects on meat FA profile (Jerónimo et al., 2010). Recently, Francisco et al. (2015) evaluated the effect of the inclusion of increasing levels of *Cistus ladanifer* (5, 10 and 20%, resulting in diets with 2.7, 6.9 and 15.6 g/CT kg DM, respectively) in a basal diet of forage:concentrate (1:1), with or without supplementation with a vegetable oil blend (4 and 8% of vegetable oil blend in diet) on lamb meat FA profile. Increasing levels of *Cistus ladanifer* oil in diet promoted the increase of the total MUFA *trans*, but not of the VA, in contrast to results obtained when *Cistus ladanifer* was added to dehydrated lucerne-based diet (Jerónimo et al., 2010).

The effect of a number of other plant extracts and plants rich in CT on FA profile of ruminant meat, such as *Juniperus pinchotii* (redberry juniper), *Acacia Karroo* and *Terminalia chebula*, was also evaluated. The replacement of cottonseed hulls with dry redberry juniper leaves (0, 50 and 100% of replacement) in the lamb diets increased the concentration of RA and decreased the SA levels in meat (Whitney et al., 2011). On the other hand, the replacement of oat hay with ground redberry juniper in dried distilled grains-based diets (0, 33, 66 and 100% of replacement) increased linearly the concentrations of 18:1 *trans*-10 and total 18:1 *trans* isomers. The LA, total MUFA and total PUFA showed a quadratic response, with higher levels of total MUFA in treatments with 33 and 66% of replacement and higher levels of LA and total PUFA in diets without redberry juniper and with 100% of replacement (Whitney and

Smith, 2015). Mapiye et al. (2011) showed that beef from steers fed a natural pasture supplemented with *Acacia karroo* leaf-meal (1500 g/day, 74 g CT/kg DM) had higher concentration of the VA and LNA, and of the two *n*-3 long chain PUFA (20:3*n*-3 and 22:5*n*-3), than beef from steers fed a control diet. The supplementation of the diet of goat kids with aqueous extract of *Terminalia chebula* (0.59 and 1.79% of DMI) also induced changes in the FA profile of meat, but the effect depended on the supplementation levels (Rana et al., 2012). Dietary supplementation with the highest levels of *Terminalia chebula* resulted in meat with lower levels of SA and SFA than in the control. Inclusion of both levels of *Terminalia chebula* in diet increased the total MUFA, total CLA and RA in meat, with highest levels of total CLA and RA in meat from kids supplemented with highest levels of *Terminalia chebula*.

Lambs fed silages of plants rich in CT – birdsfoot trefoil (*Lotus corniculatus* L.) and sainfoin (*Onobrychis viciifolia*) (21 and 104 g CT/kg DM, respectively) also showed meat FA composition distinct from lambs fed alfalfa or red clover silages, with lower levels of SFA and higher content of PUFA and higher PUFA/SFA ratio observed in lambs that received CT-rich silages than in those fed with other silages (Girard et al., 2015). The lower SFA and higher PUFA contents in meat from lambs fed CT-rich silages was mainly due to decrease of the 16:0 and SA, and increase of the LA, LNA and *n*-3 long chain PUFA, respectively. However, such FA changes were more pronounced with sainfoin, which contains 5 times more CT than birdsfoot trefoil. Conversely, meat from goat kids finished on birdsfoot trefoil pastures had lower levels of LA and 18:1 *trans*-10 than in those fed red clover; equal levels of SFA, PUFA, *n*-6 PUFA and *n*-3 PUFA were observed in meat from lambs fed birdsfoot trefoil and red clover (Turner et al., 2015).

On the other hand, some of the dietary sources of CT tested only induced a minor effect on meat FA profile. The effect of grape seed extract on lamb intramuscular FA profile was also investigated by Jerónimo et al. (2010), who reported that inclusion of 2.5% of grape seed extract (diets with 13.3-14.4 g CT/kg DM) in dehydrated lucerne-based diet supplemented or not with vegetable oil had no major effect on the meat FA profile. Using *Aspidosperma quebracho* tannin extract, Brogna et al. (2014) observed in lambs that intake of dried beet pulp supplemented with 80 g/kg DM of tannin extract over the course of 12 days, followed by the consumption of alfalfa pellets for two weeks, has a negligible effect on the meat FA profile, resulting only in the increase of the concentration of C14:1 *cis*-9 comparatively to control treatment.

Finishing steers with high-tannin sorghum also did not induce changes in the FA composition of beef (Larraín et al., 2007).

5.1.2. Milk

Sulla has been one of the most explored tannin sources in terms of its impact on the FA profile of meat and milk from ruminants. Addis et al. (2005) observed that, in the winter and spring periods, milk from sulla-fed ewes showed higher levels of short-chain FA and LNA and higher atherogenicity index, but lower content of 18:1 *cis*-9 when compared to milk from ewes fed other Mediterranean forages – annual ryegrass (*Lolium rigidum* Gaudin), burr medic (*Medicago polymorpha* L.) and daisy forb (*Chrysanthemum coronarium* L.). Regarding CLA concentrations of milk, in both experimental periods, sulla resulted in similar milk concentration in RA to annual ryegrass, but lower than burr medic and daisy forb. Cheeses (1- and 60-d-old) manufactured with milk from sulla-fed ewes showed similar FA profile to milk (Addis et al., 2005). In another study, where the effect of increasing grazing time on sulla in two phenological stages (growth vs reproductive) was evaluated, in both stages were observed a decrease of 18:1 *cis*-9 and total MUFA and increase of LA, LNA, and total PUFA in sheep milk when the grazing time on sulla increased from 0 h (24h on ryegrass pasture) to 24h (0h on ryegrass pasture) (Piredda et al., 2002; Cabiddu et al., 2005). In contrast to the results obtained by Addis et al. (2005), the CLA content of milk decreased with increasing sheep grazing time on sulla, but such reduction depended on the phenological stage of the plant, with a slight decrease observed in the growth stage (-9.1%), while in the reproductive stage the CLA content in milk from sulla-fed ewes decreased by 41% compared to milk from ewes fed on ryegrass pasture (Piredda et al., 2002; Cabiddu et al., 2005). The FA profile of milk from ewes grazing on sulla (26.6 g CT/kg DM, an average of three sampling diets) supplemented or not with PEG was evaluated by Cabiddu et al. (2009), who reported the increase of RA and VA and reduction of LA and LNA in milk as a result of sulla supplementation with PEG. On the other hand, PEG supplementation of dairy cows fed birdsfoot trefoil (*Lotus corniculatus*) induced increases of RA, LA and LNA and reduction of VA and SA in milk (Turner et al., 2005).

Benchaar and Chouinard (2009) observed no effect of diet supplementation with quebracho (*Schinopsis balansae*) tannins extract (150 g/day, 0.45% dry matter intake) on the milk FA composition of dairy cows. Dschaak et al. (2011) also reported that supplementation both of low-forage and high-forage diets with quebracho (*Shinopsis* spp.) tannins extract (30 g of extract/kg DM, resulting in diets with 22.5 g CT/kg DM) had limited effect on milk FA profile of dairy cows, observing only that the diets with quebracho led to increase of the total 18:1 *trans* and LNA in milk comparatively to diets without tannin

addition. Moreover, it was reported that addition of the mixture of quebracho (*Schinopsis lorentzii*) and chestnut tannin extracts (10 g tannins/kg DM) or quebracho tannins extract (20 g of extract/kg DM, resulting in diets with 18 g CT/kg DM) to ewes' diets supplemented with sunflower oil had a minor effect on FA composition of milk (Toral et al., 2011; Toral et al., 2013). Conversely, Buccioni et al. (2015) reported a significant change in the FA profile of ewes' milk by inclusion of quebracho (*Schinopsis lorentzii*) or chestnut (*Castanea sativa*) tannins extracts (52.8 g extracts/kg DM, resulting in diets with 24 g CT/kg DM and 40 g HT/kg DM, respectively) in diets supplemented with soybean oil. Both tannins sources resulted in an increase of the LA and VA and reduction of the total SFA, but the quebracho was able to induce more pronounced changes in this FA than chestnut extracts. On the other hand, the milk content in RA and SA was affected differentially by the two tannins sources: quebracho led to RA increase (+24.2%) and SA reduction (-11.5%), while chestnut resulted in lower levels of RA (-1.9%) and higher levels of SA (+8.7%) than a control diet without the incorporation of tannins. The authors suggest that the distinct effects of the two different tannins sources on milk FA probably stem from the different ability of CT and HT to interfere with the microbial metabolism of the rumen (Buccioni et al., 2015). Moreover, the result they obtained also suggested that these tannin sources affect differentially the endogenous syntheses of FA, with a positive effect of quebracho tannins on the activity of SCD resulting in higher levels of oleic acid (18:1 *cis*-9, OA) in milk from quebracho treatment than from control, probably due to the increased endogenous conversion of SA to OA, while milk from cows fed chestnut showed lower OA content and higher levels of SA than milk from cows fed a control diet (Buccioni et al., 2015). Such suggestion is consistent with the increase of the SCD protein expression and activity in lamb muscle induced by quebracho and *Terminalia chebula* extracts, respectively (Vasta et al., 2009c; Rana et al., 2012). The effect of tannins on FA composition of meat and milk of ruminants therefore seems to result from its effect not only on ruminal biohydrogenation, but also on endogenous syntheses of FA, and further studies are needed in order to attain a deeper understanding of these mechanisms.

The studies of tannins' effect on the FA profile of ruminant edible products show a wide diversity in tannins dietary sources and levels of inclusion, supplementation time, diet composition and animal physiological stages, resulting in controversial results. Tannins are heterogeneous compounds, variable in structure and size, and it is expected that their metabolism and activity are dependent on tannin type, which helps to explain the controversial results as regards their ability to modulate the FA profile of ruminant edible

product. Moreover, the results suggest that the effect of tannins on the FA profile in milk and meat is dependent on the composition of the diet and the dose of tannins included therein. Higher levels of tannins in the diet seem to be more effective in modulating the FA composition of ruminant fat; however, the utilization of such high levels may have detrimental effects on animal performance.

5.2. Effect of Dietary Tannins on Meat Oxidative Stability

Oxidative reactions have a negative effect on the quality and acceptability of meat and meat products, because they lead to the development of off-flavours and colour deterioration as a result of the lipid and myoglobin oxidation respectively (Wood and Enser, 1997). Moreover, the nutritive value and sensory properties of meat may be impaired by oxidation of proteins (Lund et al., 2011). Synthetic antioxidants are widely used in animal nutrition, but in recent years, in response to consumer concerns about food safety and toxicity associated with synthetic antioxidants, an interest in natural antioxidants has developed. The results of inclusion of CT-rich plants and plant extracts in animal diets show that this is an effective nutritional strategy to improve the oxidative stability of meat.

Inclusion of quebracho (*Schinopsis lorentzii*) tannins (40.4 g/kg DM) in lamb diet delays the myoglobin oxidation and improves the colour stability of meat stored in high-oxygen modified atmosphere and in aerobic conditions (Luciano et al., 2009; Luciano et al., 2011). Despite the beneficial effect on meat colour, the dietary supplementation with quebracho tannin extract did not result in improvement of the lipid stability in lamb meat, evaluated by measuring the 2-thiobarbituric acid reactive substances (TBARS) (Luciano et al., 2009). However, higher overall antioxidant status was observed in *longissimus dorsi* muscle from lambs fed the tannin-supplemented diet than in those from lambs that received a control diet, which may have contributed to improving the meat colour stability (Luciano et al., 2011). In accordance with earlier results obtained by Luciano et al. (2009), other authors (Brognia et al., 2014) likewise did not observe any influence of the incorporation of quebracho extract (*Aspidosperma quebracho*) in lamb diets on meat lipid oxidation. The supplementation of goat diets with increasing levels of pine bark (0, 15 and 30%) similarly had no effect on the lipid stability of the meat (Leick et al., 2012).

Conversely, using a methodology that entailed the induction of oxidation followed by the measurement of oxidized lipids to evaluate the meat lipid

stability, Jerónimo et al. (2012) showed that incorporation of grape seed extract or *Cistus ladanifer* in lamb diets enhances meat resistance against lipid oxidation, including in PUFA-enriched meat, which is thus more susceptible to lipid oxidation (Wood and Enser, 1997). The improvement of the lipid stability of lamb meat by dietary *Cistus ladanifer* was confirmed by Francisco et al. (2015), measuring the lipid oxidation by TBARS without oxidative induction in meat. In this study, in the oil supplemented and unsupplemented diets, increasing levels of *Cistus ladanifer* (50, 100 and 200 g/kg DM) were incorporated, resulting in significant reduction of the lipid oxidation between meats from lambs that received 50 and 200 g/kg DM of *Cistus ladanifer*. Moreover, the highest levels of *Cistus ladanifer* in diets (100 and 200 g/kg DM) reduced the meat colour changes during 7 days of refrigerated storage (Francisco et al., 2015). However, Jerónimo et al. (2012) reported that inclusion of 250 g/kg DM of *Cistus ladanifer* in lamb diets had no effect on colour variation of meat after 7 days of storage. Recently, Gravador et al. (2015) found that the inclusion of 24 and 35% of carob pulp in diets (3.4 and 4.5 g total tannins/kg DM) for lambs had no effect on lipid and protein oxidation and the colour stability of meat. However, the carob pulp inclusion in diets resulted in an increase of the PUFA concentration in meat, which increases the susceptibility of the meat to oxidation (Wood and Enser, 1997). It is therefore suggested that the oxidative stability verified in PUFA-enriched meat may result from the antioxidant activity of bioactive compounds present in the carob pulp (Gravador et al., 2015).

However, the mechanisms by which dietary sources of CT are able to improve the meat's oxidative stability remain unclear. The direct antioxidant activity of a dietary compound would imply its absorption along the gastrointestinal tract and deposition in the tissues (Vasta and Luciano, 2011). However, the polymeric nature and high molecular weight of CTs should in principle limit their absorption – and it is unlikely that oligomers larger than trimers could be absorbed in the small intestine (Manach et al., 2004). Some of the dietary tannins sources that induced improvements in the oxidative stability of meat, such as *Cistus ladanifer* and carob pulp, contain other bioactive compounds besides CT (Barrajón-Catalán et al., 2010; Karim and Azlan, 2012; Barros et al., 2013) which may also contribute to this effect. However, grape seed and quebracho extracts are composed almost exclusively of CT. Degradation of CT into compounds with low degree of polymerization or monomers would allow their absorption; however, the occurrence of CT degradation in the gastrointestinal tract of ruminants and its absorption still remain unclear. Gladine et al. (2007b) reported the presence of epicatechins and

unknown phenolic compounds in the plasma of sheep that received grape extract (seed and peel) directly in the rumen. Considering the minor proportion of monomeric compounds in grape extract, the results suggested that a high level of epicatechins in the plasma resulted from the biodegradation of polymeric CT by rumen microorganisms. In fact, *in vivo* studies using ¹⁴C-labelled CT showed a substantial disappearance of CT from ruminant gastrointestinal tract (Terrill et al., 1994; Perez-Maldonado and Norton, 1996), although little ¹⁴C-CT was absorbed from the small intestine (Terrill et al., 1994). However, Makkar et al. (1995) demonstrated that rumen microorganisms do not degrade CT. Conversely, results from studies in rats and humans showed that their intestinal microflora is able to induce structural modifications in CT (Déprez et al., 2000; Abia and Fry, 2001).

On the other hand, recent results suggest that the antioxidant effect induced in sheep by dietary CT is not related to their absorption (López-Andrés et al., 2013). Local activity in the gastrointestinal tract has been proposed as an indirect antioxidant mechanism for the phenolic compounds which are poorly absorbed in the digestive tract, potentially resulting in improvements of the animal's antioxidant status (Halliwell et al., 2005). Moreover, it is hypothesized that the improvement of the meat's oxidative stability induced by dietary CT may result from its interaction with other components of the antioxidant system (Jerónimo et al., 2012). Inclusion of rosemary and grape extract or pure compounds such as catechins and epicatechins in rat diets increased the vitamin E content in plasma and liver (Frank, 2005; Gladine et al., 2007a). Moreover, previous studies showed that the inclusion of plant extracts – *Gymnema montanum*, grape and marigold – in diets increases the activity of antioxidant enzymes in rat tissues (Ananthan et al., 2004; Gladine et al., 2007a). The increase of the gene expression of antioxidant enzymes is observed in sheep that received grape skin extract (Sgorlon et al., 2006).

5.3. Effect of Dietary Tannins on Meat Organoleptic Properties

Some authors have reported that utilization of tanniferous plant species, such as carob, *Acacia cyanophylla* or sulla, in lamb diets produces lighter meat [higher *L** (meat lightness)] compared to diets without tannin sources or with the same tanniferous diet but supplemented with PEG (Priolo et al., 1998; Priolo et al., 2000; Priolo et al., 2002a; Priolo et al., 2005).

Dietary supplementation with PEG seems to limit the impact of tannins on meat colour (Priolo et al., 2000; Priolo et al., 2002a; Priolo et al., 2002b; Priolo

et al., 2005). Conversely, at day 0 of storage the meat colour from lambs that received diets supplemented with *Cistus ladanifer* (Jerónimo et al., 2012; Francisco et al., 2015) or quebracho extract (Luciano et al., 2009) did not differ from the meat from lambs fed diets without tannin sources. Moreover, in goats the meat colour was also unaffected by inclusion of pine bark in diets (Leick et al., 2012; Min et al., 2012).

The inclusion of 200 g of carob pulp/kg (diets 12.4 g CT/kg DM of diet) in lamb diets does not seem to affect the sensorial properties of the meat, since the trained panellists were unable to distinguish between the meat of lambs fed diets with carob pulp from that of lambs fed control diets (Priolo et al., 1998). However, the inclusion of higher levels of carob pulp in diets (560 g/kg, 25 g CT/kg DM of diet) affected the flavour and overall acceptability of lamb meat, producing less acceptable meat, judged by the panellists to be “bland” or with “foreign flavours”, in comparison to meat from lambs fed control diet or carob diet supplemented with PEG (Priolo et al., 2000). Reduction of the sheep meat odour was induced in lamb meat by inclusion of 10% of quebracho (*Schinopsis lorentzii*) extract either in herbage or in concentrate-based diets (40 g CT/kg DM of diet) (Priolo et al., 2009). Moreover, the supply of 33 g/d of grape seed extract as a liquid supplement to lambs fed white clover or perennial ryegrass reduced the intensity of overall and sweet odour in the intramuscular fat (Schreurs et al., 2007). In both forage diets, the sheepy, camphor, faecal and barnyard flavours were also less intense in meat samples from lambs supplemented with grape seed extract (Schreurs et al., 2007). The replacement of cottonseed hulls with dry redberry juniper leaves (0, 50 and 100% of replacement) in lamb diets induced a linear increase of the off-flavour in meat (Whitney et al., 2011). Conversely, in dried distillers grain with solubles-based diets the replacement of oat hay by ground redberry juniper (0, 33, 66 and 100% of replacement) did not affect off-flavour in the lamb chop, but increased the juiciness, tenderness and flavour intensity (Whitney and Smith, 2015). Leick et al. (2012) reported that inclusion of 10 and 30% of pine bark in goat diets increased the texture, flavour and overall acceptability of loin chop.

Jerónimo et al. (2012) also reported that a consumer panel did not detect any effect of the grape seed extract and *Cistus ladanifer* (250 g/kg DM) inclusion in diets on the sensory properties of lamb meat, although both CT sources have been found to affect the volatile profile of meat (Vasta et al., 2010a). Conversely, recent results regarding the effect of inclusion of increasing levels of *Cistus ladanifer* in diets on flavour and off-flavour intensity in lamb meat were inconclusive, but suggest that *Cistus ladanifer* inclusion in diets may be associated with an increase in both parameters (Francisco et al., 2015).

CONCLUSION

Although the results of studies into the effect of including tannins in ruminant diets on animal performance and FA profile, oxidative stability and organoleptic properties of meat and milk are still not consistent, many have shown that this nutritional strategy is a promising tool for improving the FA profile of ruminant fat and oxidative stability of their products. The effect of dietary tannin sources on the FA profile of ruminant products has been evaluated both in meat and in milk, however, regarding its impact on oxidative stability and organoleptic properties, to the best of our knowledge, existing studies have focused mainly on the meat. Therefore, studies in milk and dairy products are needed.

From the studies reviewed here it is possible to observe a wide variety of tannins sources and inclusion levels, both in the context of supplementation and diet composition, which contribute to the substantial diversity in the results found. However, it seems clear that the tannin effects are dependent on several factors, such as tannin source and levels, and composition of the diet. So, to establish nutritional strategies based on the use of tannins to improve product quality without compromising animal health and performance, further research needs to be conducted to assess for each tannin source the optimal conditions of application, such as supplementation level and composition of basal diet. Moreover, the underlying mechanisms of tannins' ability to manipulate the FA profile and improve the oxidative stability of products should also be target of further studies.

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