Ability of tannins to modulate ruminal lipid metabolism and milk and meat fatty acid profiles

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ABSTRACT

Tannins are a large, diverse and complex group of phenolic compounds that may be detrimental, innocuous or beneficial to animal nutrition and health depending on a number of factors (e.g., type, amount ingested, consumer animal species or the basal diet). The ability of tannins to modulate ruminal biohydrogenation and, consequently, the fatty acid (FA) composition of milk and meat is a relatively recent finding that has attracted interest among ruminant nutritionists. In this review, we take a close look at the effects of tannins on the broadest possible range of FA, including less abundant compounds that have previously received little attention. Tannins are able to improve the concentrations of potentially beneficial FA, such as 18:3n-3, 18:2n-6, *trans*-11 18:1 and conjugated linoleic and linolenic acids, both in milk and meat, which may contribute to meeting consumer demand for health-promoting foods. These positive responses to tannins can be explained by their modulatory action on specific biohydrogenation steps and pathways. Thus, higher contents of dietary polyunsaturated FA in ruminant-derived products, as well as increased or decreased accumulation of other biohydrogenation intermediates (e.g., *cis* and *trans* 18:3, 18:2 and 18:1 isomers) and changes in lipids of bacterial origin (odd- and branched-chain FA and dimethylacetals), are somewhat common findings when the effects of tannins are examined. In contrast, *de novo* synthesized FA are less frequently affected by these plant secondary metabolites, which also applies to other FA (e.g., *trans*-10 18:1) that might be detrimental to animal performance (i.e., milk fat synthesis) and perhaps to human health. However, further studies are required to unravel the reasons for certain erratic responses to tannins. This paper reviews the roles of tannin chemical and structural diversity, dosage, interaction with other dietary ingredients, differences among ruminant species and variations over time in the reaction of rumen microbiota in the influences of these phenolic compounds on milk and meat FA profiles.

Key words: biohydrogenation, conjugated linoleic acid, phenolic compound, polyunsaturated fatty acid, ruminant, *trans* fatty acid

Abbreviations: BH, biohydrogenation; CLA, conjugated linoleic acid; CLnA, conjugated linolenic acid; CT, condensed tannin; DM, dry matter; DMA, dimethylacetal; F:C, forage to concentrate ratio; FA, fatty acid; HT, hydrolyzable tannin; PUFA, polyunsaturated fatty acid; SCD, stearoyl-CoA desaturase.

1. Introduction

At the beginning of this century, Mueller-Harvey (2006) wrote a review aiming at unravelling the conundrum of tannins in animal nutrition and health. She started this widely cited article highlighting the odd persistency of generalizations that tannins were harmful or toxic to animals. It is surprising that this erroneous perception persists nowadays, and it is long past time that we move beyond this idea.

Tannins can be detrimental, innocuous or beneficial depending on several factors, such as their type and chemical structure, the amount ingested, the consumer animal species, and the basal diet (Makkar, 2003; Patra and Saxena, 2011). All these factors together are probably responsible for the apparently inconsistent findings in the literature. First, tannins are a large and complex group of phenolic compounds with a great chemical and structural diversity. However, according to Mueller-Harvey (2006), this diversity has unfortunately been ignored or not sufficiently appreciated in many feeding trials, which has led to considerable confusion. Despite the widely used classification into hydrolyzable (HT) and condensed (CT) tannins, results obtained with a particular type of tannins cannot be extrapolated to other tannin classes (Mueller-Harvey, 2006; Patra and Saxena, 2011). In fact, tannins of different origins may differently affect nutrient availability and utilization, even when ingested at the same concentration (Schofield et al., 2001; Bueno et al., 2008). In this regard, estimations of the biological effects or reactivity of tannins would be more useful when associated with a certain type and extent of response (Makkar et al., 1995; Rodríguez et al., 2014).

Another key issue is the dietary concentration of tannins and consequently the amount that is actually consumed. The lack of standardization in the analysis of this large group of phenolic compounds and the use of different standards to express their content (e.g., tannic acid, quebracho, catechin, internal standards extracted from the plants themselves, etc.), means that direct comparisons between experiments can seldom be made (Schofield et al., 2001; Makkar,

2003; Álvarez del Pino et al., 2005), not to mention those cases where no standards are reported.

Variations due to ruminant species, which are known to differ in their capacity to tolerate or degrade plant secondary metabolites (Pell et al. 2000; Frutos et al., 2004), and on the basal diet (Vasta et al., 2009b) are two further factors accounting for the apparent controversy in the results reported in the literature.

Thus, with all due caution, it might be said that high concentrations of tannins would reduce voluntary feed intake and nutrient digestibility (Makkar, 2003; Frutos et al., 2004), while low to moderate concentrations may exert beneficial effects on ruminant nutrition and health. These benefits would include protection of dietary protein against ruminal degradation (Frutos et al., 2004; Patra and Saxena, 2011), prevention from bloat (e.g., Wang et al., 2012; Luscher et al., 2014), anthelmintic activity (e.g., Hoste et al., 2015), reduction of methane emissions (e.g., Beauchemin et al., 2008; Bodas et al., 2012; Hristov et al., 2013), mitigation of oxidative stress (Ciampi et al., in press), or improvement of animal performance and product quality (e.g., Wang et al., 1996; Vasta and Luciano, 2011). Another relatively recent finding on the benefits of tannins is their ability to modulate ruminal biohydrogenation (BH) and, consequently, the fatty acid (FA) compositions of milk and meat (Priolo et al., 2005; Buccioni et al., 2012; Carreño et al., 2015), which has attracted immediate interest from ruminant nutritionists and will represent the focus of this review.

The first studies reporting a positive effect of tannin consumption on milk and meat FA profile date from the early 2000s (Roy et al., 2002; Priolo et al., 2005; Turner et al., 2005). The mechanism of action was presumed to derive from changes in ruminal lipid metabolism, which was then confirmed with the use of purified tannin extracts in batch cultures of ruminal microorganisms (Kronberg et al., 2007; Vasta et al., 2009a). A number of trials on this topic, both in vitro and in vivo, have been published since then, but the results are still controversial (e.g., Jeronimo et al., 2010; Carreño et al., 2015; Buccioni et al., 2017a).

In this review, we will first provide background information on bioactive FA in ruminant-derived products and their metabolic origin. Then, we will examine the existing literature on the modulatory effects of tannins on ruminal lipid metabolism and their consequences on milk and meat FA profiles. It is worth noting that the earliest studies and reviews on this topic mostly focused on variations in major FA (e.g., α -linolenic, rumenic, vaccenic and stearic acids; Vasta and Luciano, 2011; Morales and Ungerfeld, 2015; Toral et al., 2018), whereas changes in less abundant FA received little attention. In this article, we will try to review the effects of tannins on the broadest possible range of FA.

2. Bioactive fatty acids in meat and milk

Dietary guidelines aimed at reducing the risk of coronary heart disease generally recommend a low consumption of saturated fats (Salter, 2013; Parodi, 2016), so it is not surprising that ruminant fats containing high proportions of saturated FA (Shingfield et al., 2008; Scollan et al., 2017) have been perceived as detrimental to human health. Nevertheless, although 12:0, 14:0 and 16:0 can be cholesterol raising, other saturated FA may be innocuous for cardiovascular diseases (e.g., 18:0) or even healthy for consumers (e.g., 4:0 and odd- and branched-chain FA; Shingfield et al., 2008; Parodi, 2016). The demonstrated beneficial effects of dairy products on consumers' health (Elwood et al., 2008) provide an example of the importance of recognizing that saturated FA from ruminants are not consumed as a single dietary entity but along with other compounds that might counteract their possible negative effects (Lock and Bauman, 2004; Shingfield et al., 2008; Parodi, 2016). In addition, research on ruminant nutrition has demonstrated the effectiveness of some feeding strategies to improve the concentrations of bioactive FA potentially beneficial for health in milk and meat (Lock and Bauman, 2004; Scollan et al., 2017).

In this section, only a few bioactive compounds will be mentioned by way of example:

conjugated linoleic acids (CLA), conjugated linolenic acids (CLnA), *trans* 18:1, n-3 polyunsaturated fatty acids (PUFA) and oxygenated FA. Milk and meat represent the major sources of CLA in the human diet, particularly the *cis*-9 *trans*-11 isomer (rumenic acid; Palmquist et al., 2005). Numerous biological properties have been attributed to this and other less prevalent CLA isomers, such as inhibition of the growth of cancer cell lines; antiatherogenic, antidiabetogenic and immunomodulatory activity; and stimulation or inhibition of lipogenesis in body tissues (Pariza et al., 2001; Shingfield et al., 2008; Pachikian et al., 2018).

Ruminants' fats may also contain CLnA isomers (e.g., *cis*-9 *trans*-11 *cis*-15 and *cis*-9 *trans*-13 *cis*-15 18:3; Hennessy et al., 2011; Saliba et al., 2014; Buccioni et al., 2015b). Nonetheless, despite the potential positive role that CLnA might play in the prevention of disease, their amount in conventional ruminant products is much lower than in certain seed oils (e.g., from pomegranate, bitter melon and cashew; Hennessy et al., 2011; Saliba et al., 2014).

Ruminant-derived products are major sources of *trans* FA. However, observations thus far suggest that the advice on limiting *trans* FA consumption would often be restricted to those from industrial foods, which have negative health effects, whereas the implications of ruminant *trans* FA intake is under debate (Kühlsen et al., 2005; Shingfield et al., 2008; Aldai et al., 2013). This is probably because *trans*-11 18:1 (vaccenic acid), the major *trans* FA in ruminant-derived products, is suggested to be health promoting (Roy et al., 2007; Tyburczy et al., 2009; Aldai et al., 2013) and is converted to *cis*-9 *trans*-11 CLA in body tissues (Palmquist et al., 2005).

Opportunities to improve the content of n-3 PUFA in meat and milk have been explored, but with inconsistent effectiveness (e.g., Chilliard et al., 2007; Dewhurst and Moloney, 2013). Evidence indicates that these PUFA are essential for neuronal development and functions and may have beneficial cardiovascular and anti-inflammatory properties (Calder, 2012; Salter, 2013). In recent years, there has been much debate about the benefits of decreasing the n-6:n-3

PUFA ratio in the diet, but conflicting results suggest that this advice might be misguided and strategies should focus on increasing total PUFA consumption, both n-3 and n-6 (Salter, 2013)

Oxygenated FA (i.e., FA with hydroxy, epoxy, methoxy and oxo substituents) have received increasing interest in recent years because of their role in the regulation of mammary inflammatory responses in dairy ruminants (Sordillo, 2018). However, we are not aware of targeted research examining the biological effects of certain oxygenated FA that are found in milk (e.g., 10-O-18:0 and 10-OH-18:0) and whose concentrations are known to be affected by diet composition, with significant increases under some milk fat-depressing conditions (Kairenius et al., 2018; Toral et al., 2018). Therefore, their actual implications for animal performance and consumer health are unknown. Although oxygenated FA are produced in the rumen during microbial FA metabolism (Alves et al., 2013) and are likely to be deposited in the intramuscular fat, fragmentary information is available on the occurrence, concentration and dietary-related changes of these compounds in meat. This paucity of information is mainly due to the fact that these FA are not reported in most studies, with only one exception (i.e., oxo-18:0; Parente et al., 2020). In other instances, dietary hydroxy-FA (e.g., ricinoleic acid; 12-OH, *cis*-9 18:1) were detected in meat (Maia et al., 2012).

3. Metabolic origin of fatty acids in ruminant-derived products

From a general standpoint, FA found in ruminant milk and meat have two main origins: direct uptake of preformed FA transported in plasma and *de novo* synthesis in body tissues (Vernon, 1980; Palmquist, 2006). Under conventional feeding conditions, 40 to 60% of FA would derive from each source (Palmquist, 2006). In addition, both types of FA can be subjected to the action of desaturases and elongases (Jakobsson et al., 2006; Bernard et al., 2013).

3.1. Fatty acids of dietary and ruminal origin (preformed FA uptake)

Preformed FA derive from the absorption of lipids in the digestive tract, so they have a dietary or ruminal origin (Moore and Christie, 1981; Jenkins et al., 2008). Body fat mobilization may also contribute but to a lower extent (Palmquist, 2006).

The major sources of dietary lipids consumed by ruminants are forages, cereal grains and oilseeds (Buccioni et al., 2012; Dewhurst and Moloney, 2013). Once in the rumen, fat is first subjected to hydrolysis by lipases, releasing free unsaturated FA (Buccioni et al., 2012). Then, rumen microorganisms biohydrogenate them to saturated FA to reduce their toxicity (Figure 1; Harfoot and Hazlewood, 1997; Jenkins et al., 2008). Nevertheless, the BH process is typically incomplete, and a vast array of intermediate metabolites leave the rumen and, after intestinal absorption, are available for incorporation into milk and meat (Palmquist, 2006). It is worth mentioning that despite the frequent low concentrations of certain BH intermediates (e.g., *trans*-10 *cis*-12 CLA or *cis*-9 *trans*-11 *cis*-15 CLnA), their biological consequences on the host ruminant or the consumer may be of relevance (Shingfield et al., 2008; Hennessy et al., 2011).

Thus, modulating the BH process with the aim of decreasing the disappearance of dietary PUFA and optimizing the outflow of bioactive intermediates represents an appealing opportunity for ruminant nutritionists (Chilliard et al., 2007; Scollan et al., 2017). Much progress has been made in characterizing the metabolism of FA in the rumen, which is no longer assumed to be a straightforward process with few enzymatic reactions and products (Harfoot and Hazlewood, 1997). Multiple BH pathways, together with alternative hydration and oxidation reactions, have been described (Jenkins et al., 2008; Kairenius et al., 2018).

Ruminal bacteria are also able to synthesize odd- and branched-chain FA (Vlaeminck et al., 2006). Since different bacterial populations have specific FA synthetases that determine the end products of their activity, odd- and branched-chain FA in meat and milk may reflect the abundance of particular microbial groups (Vlaeminck et al., 2006, 2015). Nonetheless, some

odd-chain FA may also have a mammary origin. Dimethylacetals (DMA) are another category of bacterial lipids that have been suggested as potential rumen microbial biomarkers and would derive from the vinyl ether chain of plasmalogens (Costa et al., 2017; Mannelli et al., 2019).

3.2. De novo fatty acid synthesis

Mammary and adipose tissues are the most active sites of de novo FA synthesis (Vernon, 1980; Palmquist, 2006). Rumen fermentation of carbohydrates provides the main substrates for this process: acetate and β -hydroxybutyrate for FA synthesis in mammary cells (Moore and Christie, 1981; Palmquist, 2006), and acetate and, to a lesser extent, lactate for their synthesis in adipocytes (Vernon, 1980). De novo synthesis in the mammary tissue yields the vast majority of even-chain saturated FA of 6 to 14 carbon atoms and approximately half of the 4:0 and 16:0 found in milk (Moore and Christie, 1981). Certain milk odd-chain FA may have a mammary origin through the occasional use of propionate as the precursor (Vlaeminck et al., 2006, 2015).

In non-lactating ruminants, most of the de novo FA are synthesized in adipose tissue (Vernon, 1980). This synthesis leads to the formation of FA with up to 16 carbons. Unlike the mammary tissue, where short- and medium-chain FA are incorporated rapidly into milk fat, the predominant end product of de novo synthesis in the adipose tissue is palmitic acid (16:0; Drackley et al., 2000). This FA can be used as substrate for further elongation and/or desaturation (Demeyer and Doreau 1999; Shingfield et al., 2013).

3.3. Desaturation and elongation of fatty acids

As noted above, preformed and de novo synthesized FA can be substrates for desaturase and elongase enzymes to produce new FA in body tissues (Jakobsson et al., 2006). The most active FA desaturase is the stearoyl-CoA desaturase (SCD), also known as Δ^9 -desaturase (Palmquist et al., 2005; Bernard et al., 2013). Although many medium- and long-chain FA serve

as SCD substrates, only 2 will be cited here: the 18:0 (stearic acid), which is desaturated to the health-promoting *cis*-9 18:1 (oleic acid; Bernard et al., 2013), and the *trans*-11 18:1 because its conversion to *cis*-9 *trans*-11 CLA represents the main origin of this bioactive CLA in ruminant-derived products (e.g., Palmquist et al., 2005; Shingfield et al., 2008; Hervás et al., 2020).

The role of elongases in milk fat synthesis, if any, seems negligible (Moore and Christie, 1981; Palmquist, 2006), while a relevant elongation activity occurs in the adipose tissue, where a significant portion of 16:0 is converted to 18:0 (Vernon, 1980; Drackley et al., 2000). Kadegowda et al. (2013) reported that the elongase processes can also involve palmitelaidic acid (*trans*-9 16:1), which could be first elongated to *trans*-11 18:1 and then desaturated to *cis*-9 *trans*-11 CLA. Further activity, such as the production of n-3 and n-6 very long-chain PUFA, has been detected in adipose tissue, although it mainly occurs in liver (Cherfaoui et al., 2012).

4. Tannins as feed additives to modulate the fatty acid composition of ruminant-derived products

Evidence supports that the effects of tannins on the FA profile of meat and milk are explained by modulation of ruminal lipid metabolism (Kronberg et al., 2007; Khiaosa-ard et al., 2009; Vasta et al., 2009b). There is also certain consensus that the mechanism is most likely mediated by tannin-induced changes in the rumen microbial community (Mannelli et al., 2019; Vasta et al., 2019). However, the specific bacteria involved in ruminal BH of dietary PUFA are largely unknown (Jenkins et al., 2008; Enjalbert et al., 2017; Carreño et al., 2019), and consequently, so is their response to tannins (Ishlak et al., 2015; Carreño et al., 2015; Mannelli et al., 2019). For that reason, this subject will only be mentioned in passing in this article and readers are referred to a recent review by Vasta et al. (2019) for further information on the impact of plant polyphenols on rumen microbiota.

Numerous studies have examined the effects of tannins on rumen FA metabolism with

variable success, due in part to the diversity of tannin types and concentrations that have been tested (Carreño et al., 2015; Abo-Donia et al., 2017; Alves et al., 2017). Much attention has been paid to this topic in Mediterranean and arid or semi-arid countries (e.g., Abarghuei et al., 2014; Correddu et al., 2015; Razzaghi et al., 2015), where local feed resources rich in polyphenols (e.g., bushes, spontaneous plants or agricultural byproducts) are used as non-conventional ingredients in ruminant diets. Nevertheless, based on the increasing world population, land degradation and food-fuel-feed competition, the use of alternative feed resources should be of interest at a global level (Makkar and Ankers, 2014).

The following subsections aim at providing a comprehensive review of the current knowledge on the effects of tannins on the FA composition of milk and meat. Fatty acids are grouped into 4 main categories based on their origin (i.e., dietary PUFA, ruminal intermediate metabolites, FA of bacterial origin and products of *de novo* synthesis). Particular attention will be paid to results of digesta FA profile because of both the consistency with milk and meat FA composition and the larger amount of information that is available, particularly *in vitro* (e.g., Jeronimo et al., 2010; Minieri et al., 2014; Natalello et al., 2020). In this respect, *in vitro* results often represent the first screening for selection of the most promising treatments to be applied *in vivo*. In fact, 60 out of the 72 experimental treatments that we compiled to prepare Figure 2 (please see the full list of references in Supplementary Material) showed a significant effect of CT or HT on digesta FA profile, supporting their potential application in ruminant feeding. Furthermore, as stated above, consistent changes in digesta and milk or meat FA composition have been reported in a number of studies (e.g., Looor et al., 2005; Vasta et al., 2009b; Jeronimo et al., 2010). In most cases, trials were conducted in small ruminants, especially in sheep (e.g., Correddu et al., 2015; Lobón et al., 2019; Maamouri et al., 2019), which may be related to the abovementioned geographical origin of the studies. In the absence of direct interspecies comparisons, caution should be taken before extrapolating the knowledge gained from one

ruminant species to others.

4.1. Effects on PUFA of dietary origin

The more knowledge acquired on the benefits for human health of PUFA consumption (Calder, 2012; Salter, 2013), the greater the interest among ruminant nutritionists in developing strategies to protect dietary unsaturated FA from ruminal BH and thereby increase their concentration in meat and milk (Dewhurst and Moloney, 2013; Scollan et al., 2017). However, rumen protection technologies are not always readily available and often have a relatively low effectiveness (Lacasse et al., 2002; Dewhurst and Moloney, 2013). This fact has prompted the search for feasible alternatives, such as the use of tannins (Kronberg et al., 2007; Minieri et al., 2014; Alves et al., 2017). Reduction in the BH extent of major dietary PUFA (i.e., 18:3n-3 and 18:2n-6) in the presence of tannins, either CT or HT, is rather common in the literature. Figure 2 reports the proportion of experimental treatments causing increases, no significant changes or decreases in specific FA concentrations in digesta. Similar results for milk and meat responses are shown in Figures 3 and 4, respectively.

Increases in 18:2n-6 and 18:3n-3 concentrations in the digesta have been reported in response to very diverse tannin types (e.g., from quebracho, grape seed, chestnut, *Cistus ladanifer*, sainfoin, chebolic myrobalan, carob, oak or pomegranate) in trials conducted both in vitro (Vasta et al., 2009a; Rana et al., 2012; Carreño et al., 2015; Natalello et al., 2020) and in vivo (Campidonico et al., 2016; Alves et al., 2017; Buccioni et al., 2017a).

Studies on the effects of tannins on milk FA profile cover a lower diversity of tannin types than those reported above for digesta. Nevertheless, some works have confirmed the positive effects of CT from quebracho and HT from chestnut and oak on milk 18:3n-3 concentration (Buccioni et al., 2015b; Henke et al., 2017; Focant et al., 2019). This beneficial effect seems even higher (up to 2-to-3.5-fold increases) with the consumption of sainfoin hay and fresh sulla,

rich in CT (Addis et al., 2005; Bonanno et al., 2016; Pascual et al., 2019). Increases in milk 18:2n-6 concentration with either tannin extracts or tannin-rich forages are usually less marked (Henke et al., 2017; Bonanno et al., 2016; Focant et al., 2019), which is consistent with this PUFA being rarely affected by feeding strategies (Mele et al., 2006; Glasser et al., 2008; Bernard et al., 2009). Birdsfoot trefoil pasture (rich in CT) and gallnut or mimosa tannin extracts (mostly hydrolyzable and condensed tannins, respectively) have also been proved to beneficially modify the concentration of these dietary PUFA in milk (Turner et al., 2005; Abo-Donia et al., 2017; de Lucena et al., 2018).

Regarding the effects of dietary tannins on meat FA composition, most studies have tested mainly CT. Notwithstanding, a rather wide selection of tannin sources has been used, including tannin extracts, as well as different tanniniferous plants and alternative feed resources. As shown in Figure 4, while in most cases the content of 18:2n-6 and 18:3n-3 in meat was not affected by feeding tannins, a number of studies reported increases in these FA. For example, quebracho tannin extracts were able to enrich lamb meat with 18:2n-6 (Kamel et al., 2018) or 18:2n-6 and 18:3n-3 (Vasta et al., 2009b). Desirable increases of these PUFA in meat were also found when animals were fed with tannin-containing forages (Priolo et al., 2005; Girard et al., 2016b) or alternative feeds (Mapiye et al., 2011; Gravador et al., 2015).

Besides 18:3n-3 and 18:2n-6, tannins can also decrease the BH extent of less-common PUFA in vegetable feeds, such as CLnA present in pomegranate seed oil (e.g., punicic acid, *cis*-9 *trans*-11 *cis*-13 CLnA, and α -eleostearic acid, and *cis*-9 *trans*-11 *trans*-13 CLnA; Natalello et al., 2020). On the contrary, lingonberry tannin extract (*Vaccinium vitis-idaea*; rich in CT) failed to reduce the BH extent of very long-chain n-3 PUFA from fish oil (Szczechowiak et al., 2016), although this may be explained by the low inclusion rate of the extract (0.45% of the diet), which is consistent with the general lack of effects on other ruminal FA.

Low levels of tannin supplementation may also account for unaffected dietary PUFA

proportions in digesta, milk and meat lipids in several studies examining the effects of CT from sainfoin, rockrose, birdsfoot trefoil and quebracho, and HT from chestnut (Toral et al., 2011; Campidonico et al., 2016; Girard et al., 2016a; Lobón et al., 2019; Guerreiro et al., 2020). Surprisingly, Buccioni et al. (2011) observed decreases in 18:2n-6 and 18:3n-3 concentration in rumen bacteria in response to quebracho and chestnut extracts, although no similar findings have been described in total digesta content, milk or meat, except for the use of oak acorns (rich in HT; Alipanahi et al., 2019) and lotus pasture (rich in CT; Turner et al., 2015). In this regard, the inconsistent effects of tannin consumption (see Figures 2, 3 and 4) may be related not only to factors described in the Introduction, such as the type and dose of tannins or differences among ruminant species in their capacity to deal with these phenolic compounds but also to differences in experimental conditions, sample type or interactions with other dietary ingredients (see Section 5). In any event, the lack of direct comparative studies recommends caution in making speculations.

Finally, the mechanisms explaining the abovementioned effects remain uncertain. Some plant endogenous factors, such as polyphenol oxidase from red clover, decrease the overall BH extent of plant PUFA by protecting them from the action of lipases (Buccioni et al., 2012; Lee, 2014). Yet, this does not appear to be the mechanism of action of tannins. The ruminal response to tannins is assumed to be mediated by changes in rumen microbiota (Mannelli et al., 2019; Vasta et al., 2019), but further research is required to elucidate the main responsibility of microorganisms affected by these polyphenols (e.g., lipolysis, isomerization of free PUFA, etc.).

4.2. Effects on intermediates of ruminal fatty acid metabolism

This section focuses on the literature describing the ability of tannins to modify the concentration of vaccenic acid, CLA, *trans*-10 18:1, less-well-known BH intermediates

(namely, certain *cis* and *trans* 18:1, 18:2, and 18:3 isomers), stearic acid, and oxo-FA.

The inhibitory action of tannins on the last step of ruminal BH (Figure 1) and, in particular, on the saturation of vaccenic acid, is probably the best characterized effect attributed to these compounds (Khiaosa-ard et al., 2009; Vasta et al., 2009a; Rana et al., 2012). The concentration of *trans*-11 18:1 can be increased twofold in digesta with the use of quebracho, grape and chestnut tannin extracts or *Cistus ladanifer* (Jeronimo et al., 2010; Vasta et al., 2010; Buccioni et al., 2017a; Costa et al., 2017). Changes in this 18:1 isomer in milk usually seem less dramatic (<100% increase; Addis et al., 2005; Bonanno et al., 2016; de Lucena et al., 2018), which may be partly due to its conversion to *cis*-9 *trans*-11 CLA in the mammary gland (Palmquist et al., 2005; Hervás et al., 2020). In fact, extracts from quebracho (Buccioni et al., 2017b) and mimosa (de Lucena et al., 2018), as well as birdsfoot trefoil (Turner et al., 2005), sulla (Bonanno et al., 2016), *Flemingia macrophylla* (das Neves Oiticica et al., 2019) and oak acorns (Alipahani et al., 2019) are examples of feeds rich in either CT or HT that potentially enhance the content of *cis*-9 *trans*-11 CLA in milk. Increments in the accumulation of this desirable CLA isomer in digesta are also quite common in response to the dietary use of extracts rich in CT from quebracho, or HT from chestnut, oak, chebulic myrobalan or pomegranate (Rana et al., 2012; Carreño et al., 2015; Natalello et al., 2020). Nevertheless, in meat (Figure 4), the effectiveness of tannins in increasing the concentrations of *trans*-11 18:1 and *cis*-9 *trans*-11 CLA is still controversial, with most studies reporting no effect (Brognia et al., 2014; Campidonico et al., 2016; Biondi et al., 2019; Larrain et al., 2008). In some instances, vaccenic acid was increased by rockrose or quebracho extracts (CT), with no effect on *cis*-9 *trans*-11 CLA (Vasta et al., 2009b; Guerreiro et al., 2020), while other studies reported that *Terminalia chebula* and carob tannins (mostly hydrolysable) increased *cis*-9 *trans*-11 CLA with no effect on vaccenic acid (Rana et al., 2012; Gravador et al., 2015). However, it should be highlighted that, differently from milk, most of the meat studies evaluated the effects of tannins using basal

diets with a low lipid content, thus having a limited view of the possible modulatory effects of tannins on BH. Diets supplemented with lipid sources (BH substrates) could facilitate the study of BH process (Jeronimo et al., 2012; Francisco et al., 2016). Furthermore, many of these intramuscular FA profiles were not comprehensive enough to study even the most relevant FA. Indeed, it is of note that *trans*-11 18:1 is not reported in several studies (Lobón et al., 2017; Kamel et al., 2018; Seoni et al., 2018; Gesteira et al., 2019). Overlooked coelutions between *trans*-11 18:1 and other 18:1 isomers (in particular *trans*-10 18:1) may also exist, which obliges to be cautious in the interpretation of poorly detailed FA profiles. Finally, while most of the studies reported the FA profile of the total intramuscular lipids, only in few instances the effects of tannins were described for individual lipid fractions composing that intramuscular fat (i.e., phospholipids and triacylglycerols; Jerónimo et al., 2010; Francisco et al., 2016).

The variability in the effects of tannins on the relative proportion of *trans*-11 18:1 and *cis*-9 *trans*-11 CLA in digesta, milk or meat seems to be similar or even greater than that described above for dietary PUFA (see Figures 2, 3 and 4). For instance, both no effects and decreases in their ruminal or milk concentrations have been described for CT and HT (e.g., sainfoin, quebracho and chestnut) that were previously observed to induce positive changes under different experimental conditions (Buccioni et al., 2015b; Campidonico et al., 2016; Toral et al., 2016; Henke et al., 2017). In most cases, the inhibition of the initial BH of dietary PUFA, which would cause a general slowdown of the process, could account for reductions in these intermediate metabolites (Minieri et al., 2014; Campidonico et al., 2016; Henke et al., 2017).

A key reason behind the conflicting effects of tannins might be the dosage. Significant dose-dependent responses were reported by Carreño et al. (2015), who suggested that low and moderate dietary supplementation with a CT extract from grape (2 and 4% on a DM basis) decreased the first steps of 18:2n-6 BH, while higher doses (6 and 8% DM) tended to improve *trans*-11 18:1 accumulation. It is worth mentioning that high levels of tannin inclusion may be

intrinsically interesting in scientific research but will likely be impractical under farm conditions because of their cost and risk of toxicity (Makkar, 2003; Mueller-Harvey, 2006).

The role of different lipid pools in meat might also contribute to explain some inconsistent responses to tannins reported in the literature. For example, Francisco et al. (2016) reported a positive dose-dependent relationship between the level of inclusion of *Cistus ladanifer* (a CT-rich shrub) in the diet and the concentration of total C18 BH intermediates in the neutral lipid fraction of muscle in lambs, whereas no effect was found in polar lipids. On the other hand, Jeronimo et al. (2010) observed that CT from grape seed and *Cistus ladanifer* increased total C18 BH intermediates in muscle polar but not neutral lipids.

Interspecies differences are likely another key reason explaining the controversy regarding the ability of tannins to interfere with BH and modulate the FA profile of milk and meat. In a work conducted with marine lipids rich in 20:5n-3 and 22:6n-3 (Torales et al., 2017), which are known to inhibit the saturation of *trans*-11 18:1, significant differences between cows and ewes were observed in the in vitro accumulation of this 18:1 isomer. On this basis, it may be expected that differences among ruminant species also exist in the effects of tannins on the last BH step, but this speculation must be taken with due caution.

Rumen BH is a very complex process with a multitude of steps and pathways, and although many authors have focused their attention on *trans*-11 18:1, studies in which detailed FA profiles are reported suggest that CT and HT also cause alterations in other BH products (Minieri et al., 2014; Campidonico et al., 2016; Alves et al., 2017). In this regard, the *trans*-10 pathway has attracted the interest of the scientific community due to the potential effects of certain *trans*-10-containing FA not only on human health but also on animal performance (Chilliard et al., 2007; Shingfield et al., 2008; Aldai et al., 2013). As shown in Figure 2, tannin-induced changes in *trans*-10 18:1 often differ from those obtained for *trans*-11 18:1. Beneficial reductions in *trans*-10 18:1 have been described in the digesta with diets containing quebracho,

acacia or chestnut tannins (Khiaosa-ard et al., 2009; Carreño et al., 2015; Costa et al., 2017) and in milk after fresh sulla consumption (Cabiddu et al., 2009). These findings contrast with expectations based on the effect of other modulators on the last BH step (e.g., marine lipids; Boeckaert et al., 2008; Toral et al., 2017; Kairenius et al., 2018). On the other hand, increases in milk *trans*-10 18:1 have also been described (Toral et al., 2013; das Neves Oiticica et al., 2019; Pascual et al., 2019), but the maximum concentration reached (approximately 2% of total FA) seems much lower than that observed in response to marine lipid supplements (up to approx. 12% of total FA; Looor et al., 2005; Shingfield et al., 2006; Boeckaert et al., 2008). As shown in Figure 4, the available information on meat seems to suggest that both CT and HT often have no appreciable effects on the concentration of *trans*-10 18:1 (Biondi et al., 2019; Campidonico et al., 2016; Guerreiro et al., 2020; Larrain et al., 2008; Turner et al., 2015), although tannin-induced increases in this 18:1 isomer have been described under certain feeding conditions (e.g., when the basal diet was supplemented with plant oils; Jeronimo et al., 2010; Francisco et al., 2016). Again, as noted for the *trans*-11 18:1, it should be stressed that the *trans*-10 18:1 isomer is not reported in several studies.

Less well-known BH pathways can also be affected by tannins. For example, variations in certain *trans*-13-containing FA (e.g., *trans*-13 18:1, *cis*-9 *trans*-13 18:2, *cis*-11 *trans*-13 CLA and *trans*-11 *trans*-13 CLA) were found when quebracho (Vasta et al., 2009a,b; Toral et al., 2013), sainfoin (Campidonico et al., 2016) or sulla (Cabiddu et al., 2009) tannins (mostly CT) were used. Nevertheless, information on these minor intermediate metabolites is too scarce to establish generalizations on their reactions to tannins.

Characterization of the effects of tannins on the isomerization and hydrogenation steps (Figure 1) that explain variations in other 18:2 intermediate metabolites is complicated because of scarce data in the literature as well as their transient accumulation in digesta and lower concentrations in milk and meat (Toral et al., 2013; Alves et al., 2017; Alipanahi et al., 2019).

However, effects of feeding quebracho, gallnut, sainfoin and *Cistus ladanifer* tannins on *cis*-9 *trans*-12, *trans*-9 *cis*-12, *trans*-11 *cis*-15, *trans*-10 *cis*-15, *trans*-11 *trans*-15 18:2 or *trans*-10 *cis*-12 and *trans,trans* CLA have been reported in digesta (Vasta et al., 2009b; Campidonico et al., 2016; Abo-Donia et al., 2017; Alves et al., 2017; Buccioni et al., 2017a), milk (Toral et al., 2013; Bonanno et al., 2016; Henke et al., 2017) and meat (Vasta et al., 2009b; Jerónimo et al., 2010; Francisco et al., 2015). In like manner, little information is available about changes in minor 18:3 intermediate metabolites. Yet, increases in ruminal *cis*-9 *trans*-11 *cis*-15 18:3 concentration have been described after the addition of a quebracho tannin extract to a diet containing linseed oil (Minieri et al., 2014).

Promotion or inhibition of the BH of certain *cis* 18:1 isomers with tannins has also been described (e.g., *cis*-9, -11 and -13 18:1; Khiaosa-ard et al., 2009; Buccioni et al., 2017a) and subsequently confirmed in milk (e.g., Bonanno et al., 2016; das Neves Oiticica et al., 2019; Pascual et al., 2019) and meat (Vasta et al., 2009b; Gravador et al., 2015; Gesteira et al., 2019; Guerreiro et al., 2020). In the case of the oleic acid, SCD activity would act as a confounding factor when examining the effects of tannins on meat and milk (Rana et al., 2012; Bernard et al., 2013). In digesta, the dual origin of this *cis* 18:1 isomer (i.e., from diet or from ruminal BH of certain PUFA) also makes it difficult to discern whether changes are due to the action of tannins on the first or last steps of BH. In any event, as shown in Figure 2, its variations in digesta seem to follow a similar pattern to that observed for *trans* 18:1 and dietary PUFA.

Most changes described thus far, and specifically those involving inhibition of BH (at the initial, intermediate or last steps), would explain the regular effects of both CT and HT by decreasing the accumulation of 18:0 (Figure 2; Vasta et al., 2009a; Rana et al., 2012; Ishlak et al., 2015). These reductions are relatively small, especially when compared with those observed with the use of other rumen BH modulators, such as marine lipids (Toral et al., 2017; Kairenius et al., 2018). In general, variations in 18:0 concentrations in milk and meat (Figures 3 and 4)

are also limited, with minor consequences on the concentration of *cis*-9 18:1, the product of 18:0 desaturation in body tissues (Turner et al., 2005; Buccioni et al., 2017b).

Concerning oxo-acids, certain nutritional strategies aimed at modulating the FA profile of milk and meat, such as diet supplementation with fish oil and marine microalgae, may cause large increases in 10-oxo-18:0 (Frutos et al., 2017; Kairenius et al., 2018). In line with these results, CT from *Cistus ladanifer* have been found to increase 10-oxo-18:0 and decrease 16-oxo-18:0 in digesta (Alves et al., 2017), whereas HT from pomegranate peels and pulp decreased not only 10- and 16- but also 13-oxo-18:0 (Natalello et al., 2020). Nevertheless, the magnitude of these changes was smaller than that obtained with marine lipid supplementation.

Finally, as described in Section 4.1 for dietary FA, in-depth information on the mechanisms explaining the effects of tannins on intermediates of ruminal FA metabolism is also missing.

4.3. Effects on fatty acids of bacterial origin

Figure 5 reports the effects of HT and CT on the concentration of odd- and branched-chain FA in digesta. Despite the great variability in the response reported in the literature, a high proportion of decreases in 17:0 and *iso* 17:0 concentrations is observed with both tannin types, and in 15:0, *iso* 15:0 and *iso* 16:0 with CT. Increases in *anteiso* 15:0, *iso* 14:0 and *anteiso* 17:0 are also reported, particularly with HT. According to Vlaeminck et al. (2006), a lower concentration of *iso* FA may suggest a reduced abundance of cellulolytic bacteria, while an increase in *anteiso* 15:0 would point to enhanced pectin- or sugar-fermenting bacteria. These mechanisms would explain the observed lower fiber degradability with high levels of tannins (Patra and Saxena, 2011; Vasta et al., 2019). In contrast, changes in availability of precursors (i.e., propionate and minor volatile FA) have been suggested to play a minor role in odd- and branched-chain FA content (Vlaeminck et al., 2006), even though tannins are known to modify

the volatile FA profile in the rumen (Patra and Saxena, 2011).

As expected, consequences of tannin consumption on odd- and branched-chain FA are dose dependent. High levels of acacia, quebracho and carob tannin extracts (16% of the substrate) were found by Vasta et al. (2009a) to induce a consistent decrease in the in vitro ruminal concentrations of these bacterial FA. Alves et al. (2017) reported similar results, together with decreased volatile FA concentration, when examining the in vivo effects of *Cistus ladanifer* supplementation (50 vs. 200 g/kg diet DM). Nevertheless, substantial variations in odd- and branched-chain FA in digesta and milk have also been described with low tannin amounts ($\leq 3\%$ in diet; Jeronimo et al., 2010; Buccioni et al., 2017a; Henke et al., 2017), suggesting relevant changes in specific rumen bacterial populations. In line with this, Carreño et al. (2015) reported more pronounced changes in digesta odd- and branched-chain FA with the use of HT extracts from chestnut and oak at 2 than at 8% of DM. Taken together, and once again, these findings prevent generalizations about the effects of tannins on ruminal microbiota and would support significant interactions between bacterial groups (Vasta et al., 2019).

Concerning dimethylacetals (DMA), little information is available on the effect of tannins. The results from recent in vitro trials have shown greater concentrations in total and individual DMA (e.g., DMA 13:0, 14:0, *anteiso* 15:0 and *cis*-9 18:1) in incubations with chestnut HT extracts compared with controls (i.e., no tannin addition) or CT from mimosa or grape seed (Costa et al., 2017, 2018; Mannelli et al., 2019). In any event, it is yet unclear whether these changes in DMA reflect variations in the abundance of specific microbial species or adaptations of bacterial membrane composition in response to environmental alterations (Costa et al., 2017; Mannelli et al., 2019).

4.4. Effects on fatty acids synthesized *de novo* in body tissues

The effects of tannins on *de novo* synthesized FA are less frequent than those described

on FA derived from diets or ruminal metabolism and are usually of much lower extent (e.g., Aprianita et al., 2014; Buccioni et al., 2015a; Valizadeh Yonjalli et al., in press). Nevertheless, van Leeuwen et al. (2017) reported that CT from rockrose were able to mitigate the inhibition of de novo FA synthesis in meat due to dietary FA supplementation.

When the diet is supplemented with PUFA, there is a regular inhibition of mammary de novo synthesis, which is either counteracted by increased preformed FA uptake or induces milk fat depression (Bauman and Griinari, 2001; Frutos et al., 2017). However, we have found no evident relationship between the effect of tannins in increasing PUFA concentrations in digesta or milk and inhibition of mammary de novo FA synthesis (Cabiddu et al., 2009; Buccioni et al., 2015a; Pascual et al., 2019). Indeed, concomitant increases in 18:3n-3, 18:2n-6 and short- and medium-chain saturated FA concentrations in milk have been reported in assays with tannins (Addis et al., 2005; Henke et al., 2013; Lobón et al., 2019). These findings might also explain the infrequent effects of these phenolic compounds in decreasing milk fat content and yield, which could be associated with increments in total milk yield and a subsequent dilution effect (Addis et al., 2005; Bonanno et al., 2016).

Regarding the intramuscular fat, it is important to stress that tannins could have an indirect effect on de novo FA synthesis. Indeed, in cases where tannins exhibit anti-nutritional effects, with consequent reduction in ingestion and growth, the intramuscular fat deposition can be impaired (Girard et al., 2016a). Therefore, the synthesis of de novo fatty acids may also be depressed, as this lipogenic process is favored by highly energetic diets (Scollan et al., 2006). This reduction mainly concerns triglycerides (neutral lipid), as phospholipids (polar lipid) are fairly constant in the muscle (Wood et al., 2008). As a consequence, considering that phospholipids have a higher PUFA content than triglycerides (Wood et al., 2008), the PUFA proportion in the total intramuscular fat may be increased, which might lead to an incorrect interpretation of tannin effects on the modulation of BH.

5. Interaction with other dietary components

Features of basal diet composition affecting rumen microbial diversity may interfere with tannin actions on BH. Particular focus is placed in this section on interactions with diet concentrate level, lipid-rich supplements, and other bioactive compounds.

Vasta et al. (2009b) reported that the addition of a quebracho CT extract to a forage (100% fresh vetch) or a concentrate (30:70 alfalfa hay:concentrate) diet enhanced 18:3n-3 in the muscle of lambs, supporting an inhibition of initial BH regardless of the diet. Nonetheless, increases in *trans*-11 18:1 and *cis*-9 *trans*-11 CLA were only observed when tannins were included in the concentrate diet, suggesting that the forage diet prevented the perturbation of the last BH step. This interaction was attributed to the presence of concentrate rather than to the forage type, which agrees with results by Lobón et al. (2019) in ewes fed diets with a constant 80:20 forage to concentrate ratio: no interactions between the effects of quebracho tannin supplementation and forage type (pasture or hay) were found in the FA profiles of milk or suckling lambs' muscle.

Concerning the presence or absence of plant oils in the basal diet, CT in *Cistus ladanifer* only increased *trans*-11 18:1 and *cis*-9 *trans*-11 CLA muscle neutral lipid concentrations when the basal diet contained sunflower and linseed oils (6% in total), but no variation was observed without oil supplementation (Jeronimo et al. 2010). These effects on *trans*-11 18:1 and *cis*-9 *trans*-11 CLA were not replicated on meat and digesta in a subsequent trial showing, in addition, that the highest *trans*-10 18:1 concentration in muscle neutral lipids was reached when the basal diet contained plant oils (Francisco et al., 2016; Alves et al., 2017). In contrast, quebracho tannin extract was able to increase the content of 18:2n-6 in lamb meat whether or not the basal diet was supplemented with PUFA (Kamel et al., 2018). Similarly, Pascual et al. (2019) reported rather consistent effects of CT-rich sainfoin hay on milk FA profile regardless of the lipid composition of the basal diet. Given the complexity of the rumen microbiota, numerous

interactions may occur between dietary components and tannin sources, preventing generalizations on the final effects on BH (Minieri et al., 2014; Carreño et al., 2015; Vasta et al., 2019).

Regarding other bioactive compounds, the combined use of different modulators of rumen BH might result in additive, synergistic or even antagonistic effects, depending on their different mechanisms of action. For example, Szczechowiak et al. (2016) found any interaction between tannin extracts (from oak, grape or lingonberry) and fish oil on the inhibition of the last BH step. However, the effects of sainfoin (rich in CT) and red clover (rich in polyphenol oxidase) on ruminal concentrations of 18:3n-3 and certain 18:1 isomers were greater when used in combination (Campidonico et al., 2016). In this latter work, the fact that sainfoin and red clover did not affect rumen odd- and branched-chain FA in the same way when they were used separately would point to the involvement of different ruminal mechanisms underlying the response to each fodder legume (Buccioni et al., 2012; Lee, 2014; Vasta et al., 2019).

Pomegranate byproducts represent a particular case of feed containing different bioactive compounds, which makes it more complicated to elucidate the origin of changes. Consumption of whole pomegranate byproduct (a mixture of peels, pulp and seeds) was shown to enhance meat and milk contents of *trans*-11 18:1, *cis*-9 *trans*-11 CLA and CLnA in goats (Modaresi et al., 2011; Emami et al., 2015), sheep (Natalello et al., 2019; Valenti et al., 2019) and cows (Kotsampasi et al., 2017). Nonetheless, it remained unclear whether this enrichment was due to the action of the tannins present in peels and pulp (mostly hydrolyzable), the bioactive CLnA in seeds (mostly *cis*-9 *trans*-11 *cis*-13 CLnA), or perhaps their interaction. We showed in a recent in vitro study (Natalello et al., 2020) that pomegranate tannins and CLnA played different roles in modulating ruminal BH: the former would favor the accumulation of dietary PUFA (18:2n-6 or 18:3n-3) and *cis*-9 *trans*-11 CLA, whereas increases in *trans*-11 18:1 would mainly arise from BH of CLnA isomers.

6. Time-dependent effects of tannins

Variation over time in the response of rumen microbiota to tannins may be another factor contributing to the discrepancies in the literature. Starting with in vitro assays, changes in digesta odd- and branched-chain FA concentration in the study by Natalello et al. (2020) were clearer at 24 than at 12 h of incubation, in agreement with consequences of gallnut extract (rich in HT) on *trans*-11 18:1 accumulation (Abo-Donia et al., 2017). On the other hand, reductions in certain minor 18:1 isomers were only significant in the short term (12 h; Natalello et al. 2020). In general, the results are highly inconsistent and even apparently contradictory, with the greatest effects of tannins being observed at shorter or longer in vitro incubation times depending on a multitude of variables (type, dose and reactivity of tannins, basal diet, interactions, etc.), which makes extrapolations speculative and prevents the establishment of generalizations. In any case, we consider it is worth mentioning these short-term variations because in vitro trials often constitute the first step to select treatments to be tested in vivo.

In vivo experiments including repeated measurements over time seem particularly relevant to characterize the long-term persistency of tannin-induced changes in FA metabolism, but few reports are available in this regard (e.g., Cabiddu et al., 2009; Toral et al., 2013; Buccioni et al., 2017b). Although some studies using tannin extracts have found no significant treatment $\times$ time interactions (Buccioni et al., 2015a, 2017b; Lobón et al., 2018), feeding fresh sulla (rich in CT) induced a gradual decline in *trans*-11 18:1 and *cis*-9 *trans*-11 CLA over time in ovine milk, with opposite changes in *trans*-9 *trans*-11 and *trans*-11 *trans*-13 CLA (Cabiddu et al., 2009). In lactating ewes, we also observed that the effects of a quebracho tannin extract on milk *trans*-11 18:1 and *cis*-9 *trans*-11 CLA concentrations were only transient, during the first week, and they were subsequently displaced by a gradual increase in *trans*-10 18:1 from day 12 onward (Toral et al., 2013). Thus, we speculated that some positive effects of tannins on

BH might be compromised by time because of the potential adaptation of the ruminal microbiota. Nevertheless, changes in *trans*-10 18:1, which agree with slow responses reported when other rumen BH modulators were used (e.g., fish oil; Shingfield et al., 2006; Boeckaert et al., 2008), do not indicate that long-term tannin supplementation would necessarily prompt a shift in BH pathways from *trans*-11 to *trans*-10 18:1. In fact, decreased milk *trans*-10 18:1 concentration was found in milk from ewes fed fresh sulla for a 5-week period (Cabiddu et al., 2009). Similarly, dietary addition of 10% DM of quebracho CT for 70 days enhanced the ruminal *trans*-11 18:1 proportion and reduced that of *trans*-10 18:1 in lambs (Vasta et al., 2010), although a lower level of inclusion (4.7% DM) of the same tannins increased the *trans*-10:*trans*-11 FA ratio in the muscle of lambs after a similar period of supplementation (60 days; Vasta et al., 2009b). Again, differences in experimental conditions, tannin doses and basal diets, and perhaps in individual rumen microbial composition, may contribute to explain seeming contradictions in the literature.

7. Conclusions and future perspectives

Tannins may modulate ruminal lipid metabolism and, in consequence, modify the FA profile of milk and meat to meet consumer demand for foods perceived as health promoting. Tannins are able to improve the concentration of certain potentially beneficial FA, such as 18:3n-3, 18:2n-6, CLA, CLnA or vaccenic acid. However, unlike other feeding strategies aimed at modifying the FA profile of ruminant-derived products, tannins seem to induce smaller increases in other FA (e.g., *trans*-10 18:1) that might be detrimental for animal performance (i.e., milk fat synthesis) and perhaps human health. The tentative classification into HT and CT is not helpful for predicting the effect of tannins on FA metabolism.

Further studies are required to unravel the reasons for the erratic and dose-dependent responses to different tannins. Interactions between the specific tannins and the particular

feeding conditions must be tested before implementing any strategy in practical farming. Finally, evaluation of agricultural byproducts rich in tannins represents a topic with increasing interest in this research field because their use may decrease feeding costs while improving the nutritional value of ruminant-derived products and reducing the food-fuel-feed competition at a global level.

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Figure 1

Pathways of dietary unsaturated fatty acid (FA) metabolism in the rumen.

Figure 2

Proportion of experimental treatments containing hydrolyzable or condensed tannins (HT and CT, respectively) that cause increases, no significant changes or decreases in the concentrations of selected fatty acids in digesta. Data are derived from 23 individual studies, reported in Supplementary Material, corresponding to 72 dietary treatments.

Figure 3

Proportion of experimental treatments containing hydrolyzable or condensed tannins (HT and CT, respectively) that cause increases, no significant changes or decreases in the concentrations of selected fatty acids in milk. Data are derived from 26 individual studies, reported in Supplementary Material, corresponding to 46 dietary treatments.

Figure 4

Proportion of experimental treatments containing hydrolyzable or condensed tannins (HT and CT, respectively) that cause increases, no significant changes or decreases in the concentrations of selected fatty acids in meat. Data are derived from 22 individual studies, reported in Supplementary Material, corresponding to 40 dietary treatments.

Figure 5

Proportion of experimental treatments containing hydrolyzable or condensed tannins (HT and CT, respectively) that cause increases, no significant changes or decreases in the concentrations of odd- and branched-chain fatty acids in digesta. Data are derived from 13 individual studies, reported in Supplementary Material, corresponding to 52 dietary treatments.

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

SUPPLEMENTARY MATERIAL

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