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

P. Frutos[a]*, G. Hervás[a], G. Luciano[b], M. Fondevila[c], A. Priolo[b] and P.G. Toral[a]

[a] *Instituto de Ganadería de Montaña (CSIC-University of León), Finca Marzanas s/n, 24346 Grulleros, León, Spain*

[b] *Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A), University of Catania, Via Valdisavoia 5, 95123, Catania, Italy*

[c] *Departamento Producción Animal y Ciencia de los Alimentos, Instituto Agroalimentario de Aragón (IA2), Universidad de Zaragoza-CITA, Miguel Servet 177, 50013, Zaragoza, Spain*

*Corresponding author. E-mail address: p.frutos@csic.es

ABSTRACT

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To be done

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

1. Introduction

In a review, Mueller-Harvey (2006) aimed to clarify the effects of tannins in animal nutrition and health. Such article highlighted the odd persistency of generalizations that tannins were harmful or toxic to animals. It is actually surprising that this erroneous perception still persists nowadays, so it is time that we get rid of 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 been ignored or not sufficiently appreciated in many feeding trials, which has led to considerable confusion. Despite the widely used classification into hydrolysable (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 origin may differently affect nutrient availability and utilisation, even when ingested at a same concentration (Schofield et al., 2001; Bueno et al., 2008). In this regard, it should be better referred to biological effect or reactivity of tannins in order to associate them to a certain type and extent of response (Makkar et al., 1995; Rodríguez et al., 2014, 2015).

Another key issue is the dietary concentration of tannins, and subsequently the amount that is actually consumed. The lack of standardisation on 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 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 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), or improvement of animal performance and product quality (e.g., Wang et al., 1996; Vasta and Luciano, 2011). Another relatively recent finding about tannin benefits is their ability to modulate ruminal biohydrogenation (BH) and, consequently, the fatty acid (FA) composition 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 the mechanism of the positive effect of tannin consumption on milk and meat FA profile (Roy et al., 2002; Priolo et al., 2005; Turner et al., 2005). presumed it derives from changes in ruminal lipid metabolism, which was 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 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 of 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 consequences on milk and meat FA profile. 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 range of FA as possible.

2. Bioactive fatty acids in meat and milk

Because of their potential long-term effect on human health, either beneficial or detrimental, a great deal of attention has been paid to animal fats over the past decades (Pariza et al., 2001; Aldai et al., 2013; Salter, 2013).

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 ruminants' fats, containing high proportions of saturated FA (Shingfield et al., 2008; Scollan et al., 2017) have been perceived as detrimental for health. Nevertheless, although 12:0, 14:0 and 16:0 would be cholesterol-raising, other saturated FA would be innocuous for cardiovascular diseases (e.g., 18:0) or may even be 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 consumer's health (Elwood et al., 2008) provide an example of the importance of considering that saturated FA from ruminants are not consumed as a single dietary entity but along with other compounds that might counteract possible negative effects (Lock and Bauman, 2004; Shingfield et al., 2008; Parodi, 2016).

Over the last two decades, especial attention has been given to research aimed to improve the concentration of bioactive FA potentially beneficial for health in ruminant-derived products (Lock and Bauman, 2004; Scollan et al., 2017). From all the factors that determine milk and meat FA profile, feeding strategies (e.g., changes in basal diet composition or supplementation with unsaturated lipids or other compounds) appear to have a greatest effect and offer the opportunity to modulate it in response to consumers' demand (Chilliard et al., 2007; Scollan et al., 2017).

In this section, only a few bioactive compounds will be mentioned as an example: conjugate linoleic acids (CLA), conjugate linolenic acids (CLnA), *trans* 18:1, n-3 polyunsaturated fatty acids (PUFA) and oxylipids. Milk and meat represent the major sources of CLA in human diet, in particular 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*-13 and *cis*-9 *trans*-11 *cis*-15 18:3; Hennessy et al., 2011; Saliba et al., 2014; Buccioni et al., 2015b). Yet, 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 some seed oils (e.g., from pomegranate, bitter gould and catalpa; Hennessy et al., 2011; Saliba et al., 2014).

Ruminant-derived products are also major sources of dietary *trans* FA. However, observation thus far tend to suggest that the advice on limiting *trans* FA consumption would often be restricted to those from industrial foods, which have proven to be negative for health, whereas the implications of ruminant *trans* FA intake is still under debate (Kühlsen et al., 2005; Shingfield et al., 2008; Aldai et al., 2013). This is probably due to the fact that *trans*-11 18:1 (vaccenic acid), the major *trans* 18:1 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 in a number of works, but with unequal 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 potential benefits of decreasing the n-6:n-3 PUFA ratio of the diet, but conflicting results in the literature suggest that this advice might be misguided and strategies should focus on increasing total PUFA consumption, both n-3 and n-6 (Salter, 2013)

Oxylipids have raised increasing interest in recent years because of their role in the regulation of mammary inflammatory responses in dairy ruminants (see the recent review by Sordillo, 2018). The concentration of some milk oxylipids (e.g., 10-O-18:0 and 10-OH-18:0) is known to be affected by dietary changes, with significant increases under some milk fat depressing conditions (Kairenius et al., 2018; Toral et al., 2018). However, their actual implications for animal performance and consumer's health is yet unknown. Is there any information in meat?

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 lipids, and de novo synthesis in the mammary gland and adipose 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, in some tissues, of elongases (Jakobsson et al., 2006; Bernard et al., 2013).

3.1. Fatty acid 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). Mobilization of body fat reserves 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). Extensive metabolism in the rumen of these feeds results in remarkable differences between the FA profile of lipids entering (mostly unsaturated; 18:2n-6 and 18:3n-3) and leaving (mostly saturated; 18:0) this fermentation chamber (Harfoot and Hazlewood, 1997; Jenkins et al., 2008).

Once in the rumen, dietary 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 with various degrees of unsaturation and positional isomerisation 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 concentration of some BH intermediates (e.g., *trans*-10 *cis*-12 CLA or *cis*-9 *trans*-11 *cis*-13 CLnA), their biological consequences on the host ruminant or the consumer may be of particular 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 represent 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 now been described (Jenkins et al., 2008; Kairenius et al., 2018).

Ruminal bacteria are also able to synthesize a particular type of saturated FA: the odd- and branched-chain FA (Vlaeminck et al., 2006). Since each bacterial population has specific FA synthetases that determine the end products of their activity, differences in the concentration of each odd- and branched-chain FA leaving the rumen and appearing 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 FA 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). Some milk odd-chain FA may have a mammary origin through the occasional use of propionate as precursor (Vlaeminck et al., 2006, 2015).

In adipose tissue….

3.3. Desaturation and elongation of fatty acids

As mentioned before, preformed and de novo synthesized FA can be substrates for desaturase and elongase enzymes to produce new FA in mammary or adipose tissues (Jakobsson et al., 2006). The most active, and studied, FA desaturase is the stearoyl-CoA desaturase (SCD), also known as Δ^9-desaturase because it introduces a *cis* double bond at the Δ^9 position (Palmquist et al., 2005; Bernard et al., 2013). Although many medium- and long-chain FA serve as substrates for SCD, only 2 will be cited here: first, the 18:0 (stearic acid), which is the main substrate for SCD and is desaturated to the health promoting *cis*-9 18:1 (oleic acid; Bernard et al., 2013); and second, 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).

Concerning elongases, their role in milk fat synthesis, if any, is assumed to be negligible (Moore and Christie, 1981; Palmquist, 2006)….. MEAT?

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 would most likely be mediated by tannin-induced changes in the rumen microbial community (Mannelli et al., 2019; Vasta et al., 2019). However, the specific bacteria actually 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, 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 by-products) are used as non-conventional ingredients in ruminant diets. Nevertheless, based on increasing world population, land degradation and food-fuel-feed competition, the use of alternative feed resources should be of relevance at a global level (Makkar and Ankers, 2014).

The following subsections aims to provide 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., Loor 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 aforesaid geographical origin of the studies. In the absence of direct interspecies comparisons, caution should be taken before extrapolating the knowledge gained from a ruminant species to others.

4.1. Effects on PUFA of dietary origin

The more knowledge on the benefits for human health of PUFA consumption (Calder, 2012; Salter, 2013), the more interest of ruminant nutritionists in developing strategies to protect dietary unsaturated FA from ruminal BH and so 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. Please refer to Figure 2, which 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, chebulic 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).

Nevertheless, studies on the effects of tannins on milk FA profile does no cover the wide diversity of FA, although 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 fresh or henified sainfoin, rich in CT (Addis et al., 2005; Bonanno et al., 2016; Pascual et al., 2019). Increments 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 and gallnut or mimosa tannin extracts 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). MEAT?

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, *cis*-9 *trans*-11 *trans*-13 CLnA; Natalello et al., 2020). On the contrary, chestnut, oak and lingonberry tannin extracts failed at reducing the BH extent of very long-chain n-3 PUFA from fish oil (Jellali, 2015; Szczechowiak et al., 2016), which might suggest that bacteria involved in metabolism of 20:5n-3 and 22:6n-3 are less sensitive to these plant polyphenols than those involved in the BH of C18 FA. Alternatively, this ineffectiveness may be explained by the low inclusion rate of the tannin extracts (0.45 to 2% of the diet), which is consistent with their general lack of effects on other ruminal FA (Jellali, 2015; Szczechowiak et al., 2016).

Low levels of tannin supplementation may also explain unaffected dietary PUFA proportions in digesta, milk and meat lipids in several studies examining the effects of CT from sainfoin, birdsfoot trefoil and quebracho, and HT from chestnut (Toral et al., 2011; Girard et al., 2016; Lobón et al., 2019; MEAT?). 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 (Alipanahi et al., 2019). In this regard, erratic effects of tannin consumption (see Figures 2, 3 and 4) may be related not only to factors anticipated 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), so the lack of direct comparative studies recommends caution in making speculations.

Finally, mechanisms explaining the above-mentioned effects are yet uncertain. Some secondary compounds, 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 which is 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 about the ability of tannins to modify the concentration of vaccenic acid, CLA, *trans*-10 18:1, less-well-known BH intermediates (namely, some *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 multiplied 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 lower (<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. MEAT? Increments in the accumulation of this desirable CLA isomer in digesta are also common in response to the dietary use of quebracho, chestnut, oak, chestnut, chebulic myrobalan or pomegranate tannin extracts (Rana et al., 2012; Carreño et al., 2015; Natalello et al., 2020).

Nevertheless, 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 similar or even greater than that described above for dietary PUFA (see Figures 2, 3 and 4). For instance, both lack of variation and decreases in their ruminal or milk concentrations have been described for CT and HT (e.g., sainfoin, quebracho and chestnut) that were 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 inconstant effects of tannins might be the dosage. Significant dose-dependent responses were reported by Carreño et al. (2015), who suggested that low and moderate diet supplementation with a CT extract from grape (from 2 to 4%, on DM basis) decreased the first steps of 18:2n-6 BH, while higher doses (from 6 to 8% of 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).

Interspecies differences are likely another key reason explaining the controversy about 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 (Toral 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 perhaps be expected that differences between 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 tannins 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 some *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). This contrasts 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 (about 2% of total FA) seems much lower than that observed in response to marine lipid supplements (up to approx. 12% of total FA; Loor et al., 2005; Shingfield et al., 2006; Boeckaert et al., 2008).

MEAT?

Less-well-known BH pathways can also be affected by tannins. For example, variations in some isomers belonging to the *trans*-13 pathway (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 were used. Nevertheless, information on these minor intermediate metabolites is too scarce to stablish generalizations on their reaction to tannins.

Characterization of the effects of tannins on the isomerization and hydrogenation steps (Figure 1) that explain variations of other 18:2 intermediate metabolites is complicated because of scarce data in the literature, and 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, 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; 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 (references?). Little information is available about changes in minor 18:3 intermediate metabolites, although increases in ruminal *cis*-9 *trans*-11 *cis*-15 and *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 biohydrogenation of certain *cis* 18:1 isomers with tannins have 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?. In the case of the oleic acid, given its dual origin (i.e., from diet or from ruminal BH of some PUFA) it is 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 so 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 concentration 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, some 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 this, 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 mentioned 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 concentration 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), the lower concentration of *iso* FA and the increment in *anteiso* 15:0 may suggest, respectively, reduced and enhanced abundance of cellulolytic and 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). On the contrary, 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 (≤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 instead of 8% of DM. Altogether, and once again, these findings prevent from establishing 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. Yet, 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 de novo synthesized fatty acids

Effects of tannins on *de novo* synthesized FA are less frequent than those described on FA deriving from diets or ruminal metabolism, and are usually of much lower extent (e.g., Aprianita et al., 2014; Buccioni et al., 2015a; MEAT?).

When 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 increasing PUFA concentration 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 are reported in some assays with tannins (Addis et al., 2005; Henke et al., 2013; Lobón et al., 2019). This might also explain the infrequent effects of these phenolic compounds decreasing milk fat content and yield, which could be associated to increments in total milk yield and a subsequent dilution effect (Addis et al., 2005; Bonanno et al., 2016).

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 addition of a quebracho tannin 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 profile of milk or suckling lambs' muscle.

Concerning the presence or not of plant oils in the basal diet, CT in *Cistus ladanifer* were only effective to increase *trans*-11 18:1 and *cis*-9 *trans*-11 CLA muscle lipid concentrations when the basal diet contained sunflower and linseed oils (6% in total), but no effects were observed without oil supplementation (Jeronimo et al. 2010). This interaction was then confirmed on digesta FA composition (Alves et al., 2017). On the contrary, 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 from establishing 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, neither Jellali (2015) nor 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, effects of sainfoin (rich in CT) and red clover (rich in polyphenol oxidase) on ruminal concentrations of 18:3n-3 and some 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 different ruminal mechanisms underlying the response to each fodder legume (Buccioni et al., 2012; Lee, 2014; Vasta et al., 2019).

Pomegranate by-products 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 by-product (a mixture of peels, pulp and seeds) was known 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). Yet, it remained unclear whether this enrichment was due to the action of the tannins present in peels and pulp (mostly hydrolysable), the bioactive CLnA in seeds (mostly *cis*-9 *trans*-11 *cis*-13 CLnA), or perhaps their interaction. In a recent *in vitro* study, Natalello et al. (2020) showed that pomegranate tannins and CLnA played different roles in modulating ruminal BH: they would favour 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 derive from biohydrogenation 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 explain some discrepancies in the literature. Changes in digesta odd- and branched-chain FA concentration in the aforesaid study by Natalello et al. (2020) were clearer at 24 than 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, some reductions in minor 18:1 isomers were only significant in the short-term (12 h; Natalello et al. 2020). In general, 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 were speculative and prevents from establishing generalizations.

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 × time interactions (Buccioni et al., 2015a, 2017b; Lobón et al., 2018), feeding fresh sulla 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 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 onwards (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 mean that long-term tannin supplementation would necessarily prompt a shift in BH pathways from *trans*-11 to *trans*-10 18:1. In fact, dietary addition of quebracho tannins for 70 days enhanced ruminal *trans*-11 18:1 proportion and reduced that of *trans*-10 18:1 in lambs (Vasta et al., 2010). Similarly, 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).

7. Conclusions and future perspectives

Tannins may modulate ruminal lipid metabolism and, in consequence, modify the FA profile of milk and meat to meet the consumer’s demand for health-promoting foods. Tannins can improve the concentration of some 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) than might be detrimental for human health or animal performance (i.e., milk fat synthesis). 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 reason for some erratic and dose-dependent responses to different tannins. Concretely, interactions between the specific tannins and the particular feeding conditions must be tested before implementing any strategy in practical farming. Evaluation of agricultural by-products 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 reduce 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 hydrolysable or condensed tannins (HT and CT, respectively) that cause increases, no significant changes or decreases in the concentration of selected fatty acids in digesta. Data derived from 23 individual studies, reported in Supplementary Material, corresponding to 72 dietary treatments.

Figure 3

Proportion of experimental treatments containing hydrolysable or condensed tannins (HT and CT, respectively) that cause increases, no significant changes or decreases in the concentration of selected fatty acids in milk. Data derived from 25 individual studies, reported in Supplementary Material, corresponding to 46 dietary treatments.

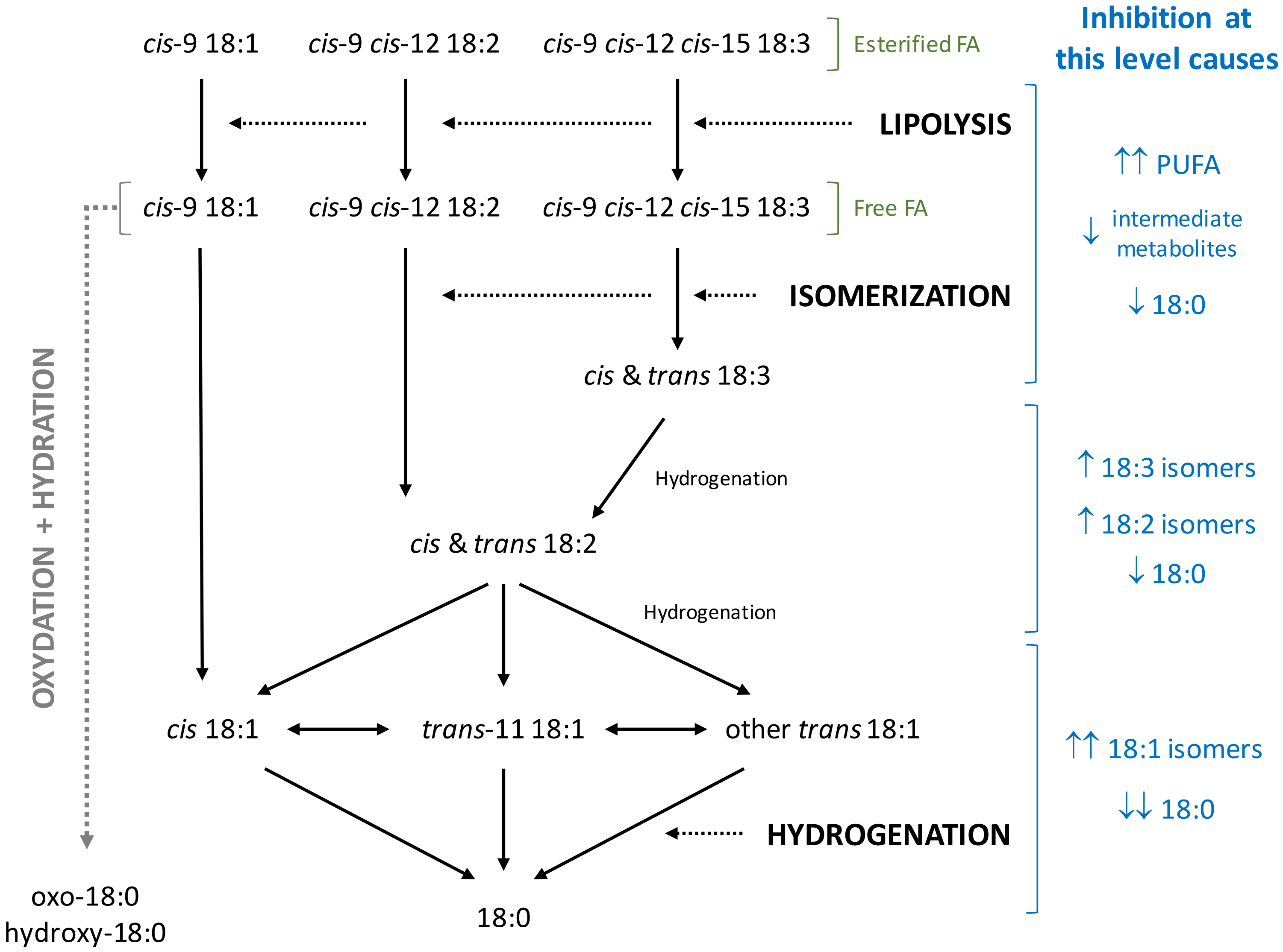
Figure 4

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

Figure 5

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

FIGURE 1



cis-9 18:1
cis-9 cis-12 18:2
cis-9 cis-12 cis-15 18:3
Esterified FA
Inhibition at this level causes
LIPOLYSIS
↑↑ PUFA
cis-9 18:1
cis-9 cis-12 18:2
cis-9 cis-12 cis-15 18:3
Free FA
↓ intermediate metabolites
↓ 18:0
ISOMERIZATION
cis & trans 18:3
OXYDATION + HYDRATION
Hydrogenation
↑ 18:3 isomers
↑ 18:2 isomers
↓ 18:0
cis & trans 18:2
Hydrogenation
cis 18:1
trans-11 18:1
other trans 18:1
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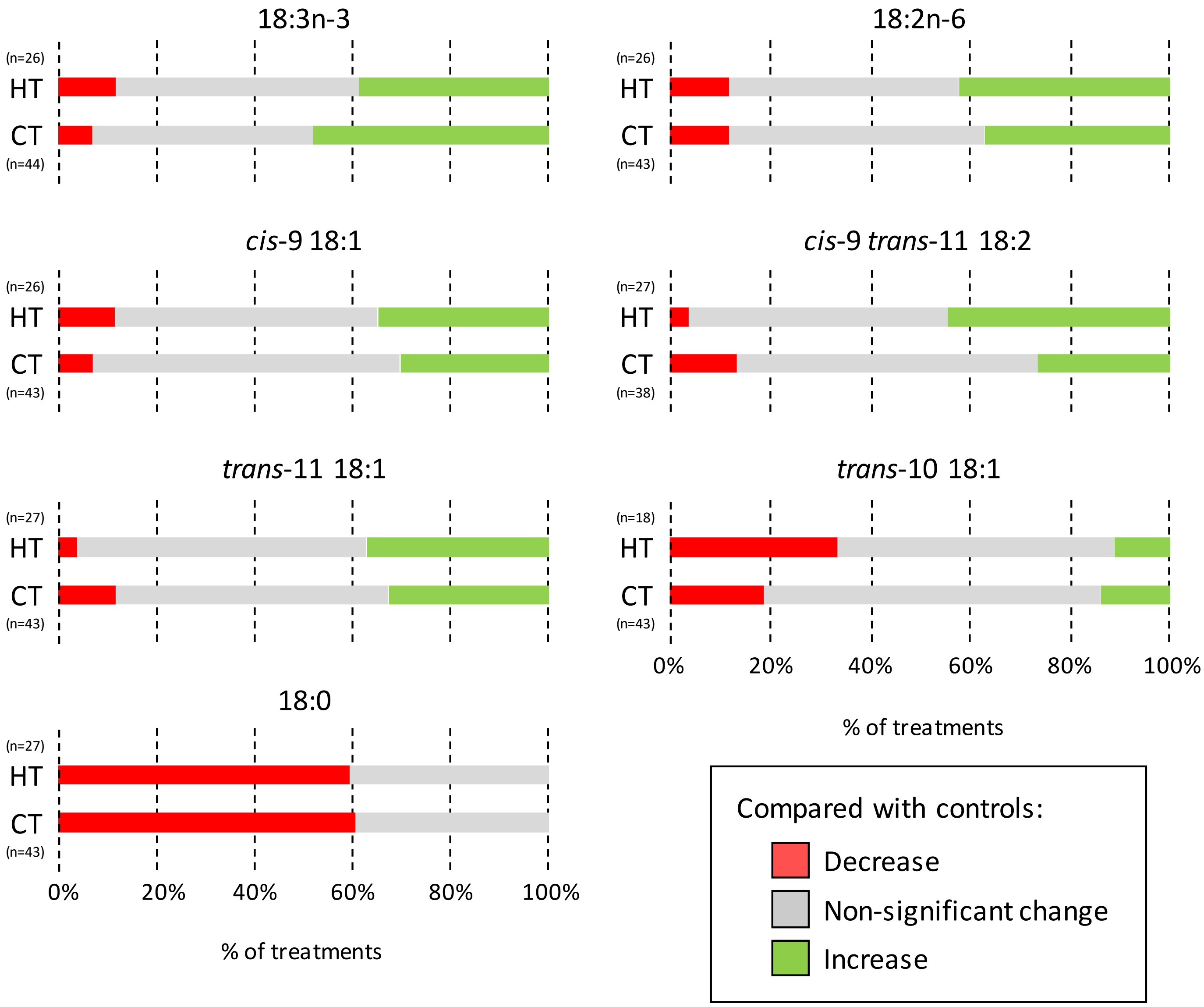
↓↓ 18:0
HYDROGENATION
oxo-18:0
hydroxy-18:0
18:0

FIGURE 2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HT
CT
(n=44)
18:2n-6
(n=26)
HT
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cis-9 18:1
(n=26)
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cis-9 trans-11 18:2
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trans-11 18:1
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18:0
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CT
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80%
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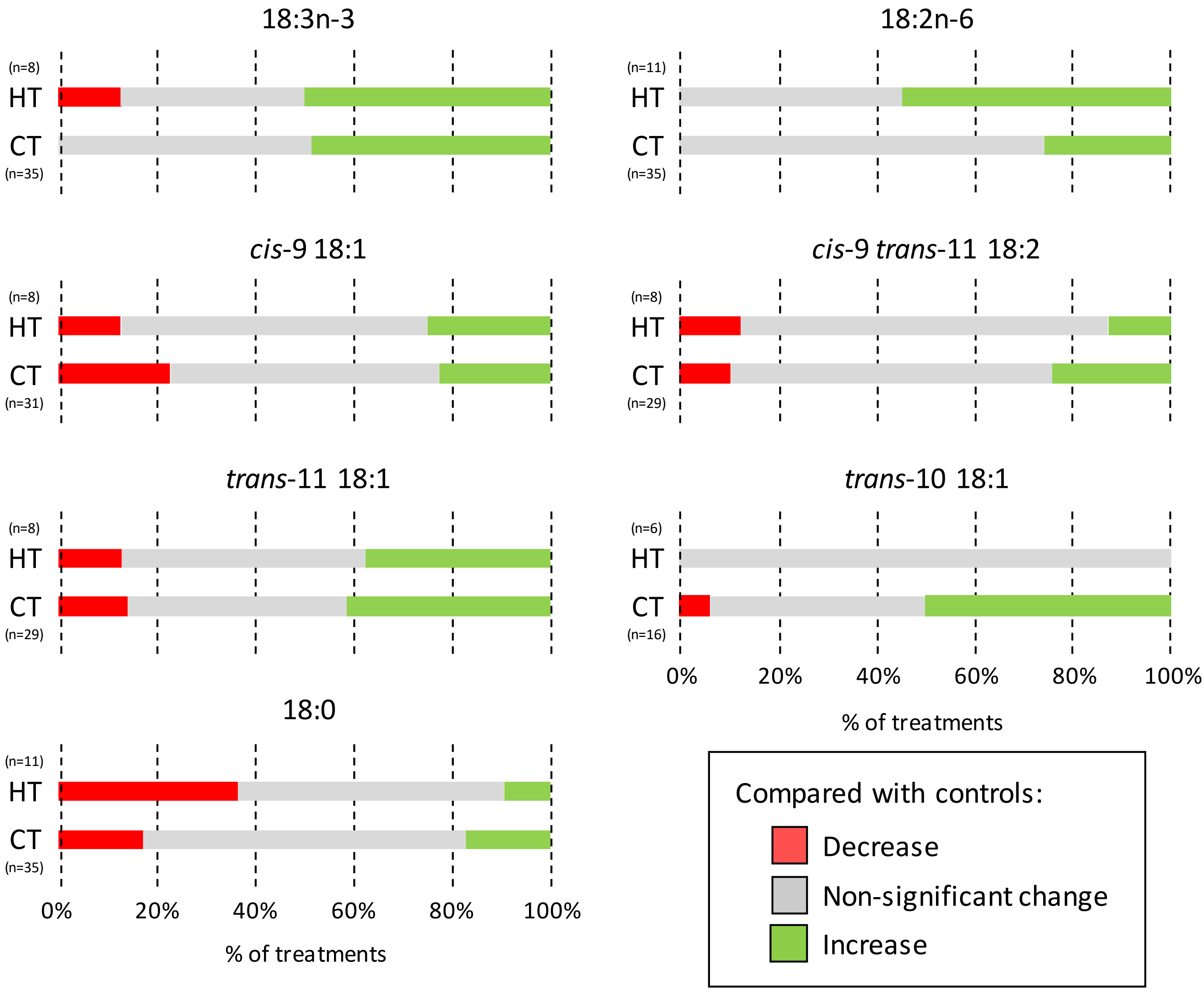
% of treatments
Compared with controls:
Decrease
Non-significant change
Increase

FIGURE 3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% of treatments
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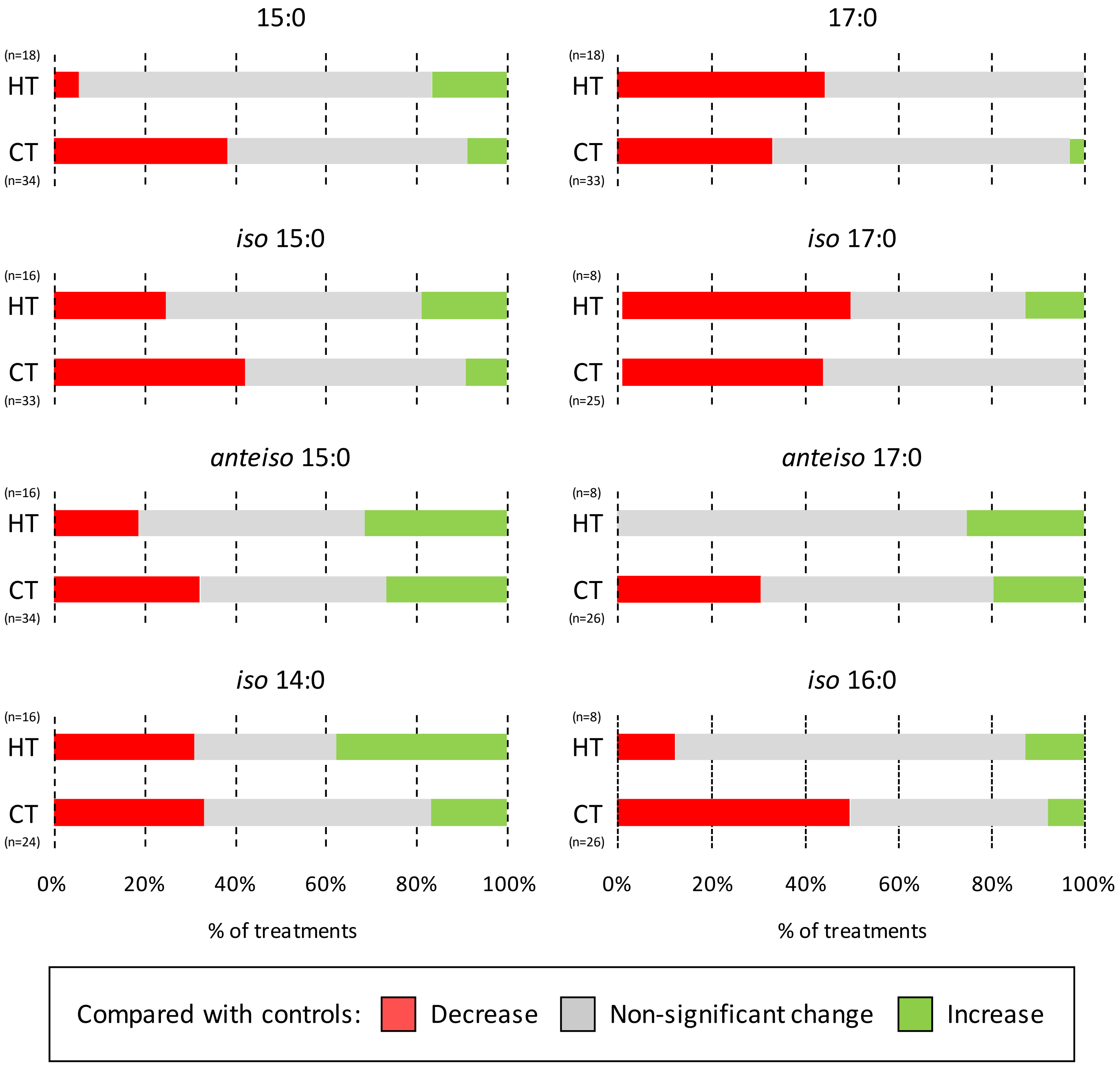
% of treatments
Compared with controls:
Decrease
Non-significant change
Increase

FIGURE 4

To be done

FIGURE 5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20%
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80%
100%
% of treatments
Compared with controls:
Decrease
Non-significant change
Increase