

# Review: Modulating ruminal lipid metabolism to improve the fatty acid composition of meat and milk. Challenges and opportunities

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*Growth in demand for foods with potentially beneficial effects on consumer health has motivated increased interest in developing strategies for improving the nutritional quality of ruminant-derived products. Manipulation of the rumen environment offers the opportunity to modify the lipid composition of milk and meat by changing the availability of fatty acids (FA) for mammary and intramuscular lipid uptake. Dietary supplementation with marine lipids, plant secondary compounds and direct-fed microbials has shown promising results. In this review, we have compiled information about their effects on the concentration of putative desirable FA (e.g. c9t11-CLA and vaccenic, oleic, linoleic and linolenic acids) in ruminal digesta, milk and intramuscular fat. Marine lipids rich in very long-chain n-3 polyunsaturated fatty acids (PUFA) efficiently inhibit the last step of C18 FA biohydrogenation (BH) in the bovine, ovine and caprine, increasing the outflow of t11-18:1 from the rumen and improving the concentration of c9t11-CLA in the final products, but increments in t10-18:1 are also often found due to shifts toward alternative BH pathways. Direct-fed microbials appear to favourably modify rumen lipid metabolism but information is still very limited, whereas a wide variety of plant secondary compounds, including tannins, polyphenol oxidase, essential oils, oxygenated FA and saponins, has been examined with varying success. For example, the effectiveness of tannins and essential oils is as yet controversial, with some studies showing no effects and others a positive impact on inhibiting the first step of BH of PUFA or, less commonly, the final step. Further investigation is required to unravel the causes of inconsistent results, which may be due to the diversity in active components, ruminant species, dosage, basal diet composition and time on treatments. Likewise, research must continue to address ways to mitigate negative side-effects of some supplements on animal performance (particularly, milk fat depression) and product quality (e.g. altered oxidative stability and shelf-life).*

**Keywords:** biohydrogenation, fish oil, plant secondary compounds, probiotics, ruminant

## Implications

Much research effort in ruminant nutrition is focussed on meeting current consumer demand for healthier foods. This review summarises the literature on nutritional strategies to manipulate ruminal lipid metabolism with the goal of enhancing the concentration of potentially beneficial fatty acids (FA) in milk and meat (e.g. use of marine lipid supplements or plant secondary compounds). Additional studies are, however, required to unravel the causes of some inconsistent results in the literature. Associated side effects on animal performance and product quality represent challenges that need to be addressed before practical application of some promising treatments in animal production.

## Introduction

Although improvements in living standards and availability of food are taken for granted in developed and newly industrialised countries, changes in lifestyle and eating habits have been accompanied by a higher incidence of chronic cardiovascular, metabolic and degenerative diseases (Salter, 2013; Parodi, 2016). Consumers are now increasingly aware of the multiple links between diet and well-being, giving rise to a growing market for foods with demonstrated or perceived beneficial effects on health. The concentration of a number of health-promoting FAs in milk and meat can be effectively increased through livestock feeding strategies (Dewhurst and Moloney, 2013; Shingfield *et al.*, 2013). In recent years, multiple studies have shown that ruminal lipid metabolism is a key point in determining the content of many desirable FA in ruminant products, such as c9t11-CLA,

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$t11\text{-}18:1$  (vaccenic acid),  $c9\text{-}18:1$  (oleic acid),  $18:2n\text{-}6$  (linoleic acid) and  $18:3n\text{-}3$  ( $\alpha$ -linolenic acid) (Chilliard *et al.*, 2007; Scollan *et al.*, 2014). This review summarises the available literature on the manipulation of the rumen environment to modulate milk and meat FA profile. Reference is also made to recent research on the associated positive and negative effects on animal performance and product quality. No information is provided on the use of ionophores (for which evidence of modulatory effects on biohydrogenation (BH) exists; Chilliard *et al.*, 2007) because their use for non-medicinal purposes is banned in some places (e.g. European Union).

### Metabolic origin of lipids in ruminants: the importance of ruminal metabolism

Ruminant-derived products are characterised by the presence of a broad variety of FA that are not found in the diet of livestock but derive largely from ruminal metabolism of its fat. Under conventional feeding conditions, the major lipid sources for ruminants are forages, cereal grains and oilseeds, which contain high proportions of unsaturated FA (mainly  $18:2n\text{-}6$  and  $18:3n\text{-}3$ ; Jenkins *et al.*, 2008; Buccioni *et al.*, 2012). On entering the rumen, dietary lipids are extensively metabolised (Jenkins *et al.*, 2008), starting with the release of free FA by the action of lipases. Recent data highlight the role of endogenous plant factors in this process, which may enable its manipulation (Lee *et al.*, 2009; Buccioni *et al.*, 2012). Lipolysis is followed by BH, a process consisting of sequential FA isomerisation and saturation performed by some bacteria to reduce the toxicity of unsaturated lipids for microbial growth (Jenkins *et al.*, 2008). Molecular biology techniques have revealed that earlier explanations of rumen microbial BH based mainly on *in vitro* cultures of *Butyrivibrio* strains were rather weak. Instead, it is now accepted that a number of uncultured bacterial species play a key role in the *in vivo* process (e.g. Huws *et al.*, 2011; Toral *et al.*, 2016). The contribution of fungi to BH in the rumen seems to be marginal and that of protozoa would derive from associated bacteria (Jenkins *et al.*, 2008).

Since BH of dietary FA is usually incomplete, numerous intermediate metabolites reach the duodenum and, after absorption, are available for incorporation into tissues and milk fat (Shingfield *et al.*, 2013). Therefore, the manipulation of microbial lipid metabolism to enhance the outflow of beneficial FA from the rumen represents a major challenge, and also an opportunity, for ruminant nutritionists (Chilliard *et al.*, 2007; Scollan *et al.*, 2014).

Rumen microbial fermentation has a further impact on the lipid composition of milk and meat by providing precursors (volatile FA) for *de novo* FA synthesis in the mammary gland and intramuscular lipid (Bernard *et al.*, 2008; Shingfield *et al.*, 2013). This metabolic pathway usually yields saturated fatty acid (SFA) of up to 16-carbon atoms, which can subsequently serve as substrates for desaturases and, in some tissues, elongases (Bernard *et al.*, 2008; Shingfield *et al.*, 2013).

### Improving the fatty acid composition of meat and milk through modulators of ruminal lipid metabolism

Replacing SFA with monounsaturated FA and polyunsaturated fatty acid (PUFA), respectively, in ruminant derived-products has been a major aim for animal nutritionists, although this may result simplistic due to specific biological effects of individual FA within each group (Shingfield *et al.*, 2013; Parodi, 2016). A wide variety of feeding strategies has been tested with varying success, due in part to the complexity of rumen microbial responses (Jenkins *et al.*, 2008; Huws *et al.*, 2011; Toral *et al.*, 2016). Much attention has been paid to the effects of supplementation with vegetable lipids (Shingfield *et al.*, 2013; Scollan *et al.*, 2014).

On the contrary, less complete information is available on the modification of the FA profile of meat and milk FA by using specific modulators of the ruminal environment, such as marine lipids rich in very long-chain n-3 PUFA, plant secondary compounds (e.g. tannin extracts or essential oils (EO)) and direct-fed microbials (Vasta and Luciano, 2011; Shingfield *et al.*, 2013; Apás *et al.*, 2015). In the present review, we have compiled information on the effects of these materials on the concentration of the putative desirable  $c9t11\text{-}CLA$ ,  $t11\text{-}18:1$ ,  $c9\text{-}18:1$ ,  $18:2n\text{-}6$  and  $18:3n\text{-}3$  in digesta, milk and meat in bovine, ovine and caprine (Tables 1 and 2 and Supplementary Tables S1 to S7). Variations in ruminal disappearance of dietary unsaturated FA complement this information and provide a good proxy for the extent of the first BH step by avoiding confounding effects due to differences in dietary FA supply. The accumulation of  $18:0$  is reported as an indication of the overall extent of BH or inhibition of its terminal step, while increases in the proportion of  $t10\text{-}18:1$  may reflect a shift towards alternative pathways.

#### Marine lipids rich in very long-chain n-3 polyunsaturated fatty acids

The efficacy of marine lipids for modulating BH of C18 FA and increasing VA and subsequently CLA in ruminant derived products has received considerable interest in recent years (Chilliard *et al.*, 2007; Noci *et al.*, 2007). Two n-3 PUFA, specifically  $20:5n\text{-}3$  (EPA) and  $22:6n\text{-}3$  (DHA), appear to be the main FA responsible for the well-known inhibitory effect of these lipids on the conversion of  $t11\text{-}18:1$  to  $18:0$  (Shingfield *et al.*, 2012; Toral *et al.*, 2017). Consistent responses in the FA profile of digesta, milk and meat lipids have been reported for dietary use of EPA- and DHA-rich sources (Table 1 and Supplementary Tables S1 to S3), including fish oil (e.g. tuna, sardine, herring, mackerel and salmon oils), microalgae biomass and oil (from *Schizochytrium* sp. and *Isochrysis* sp.), and protected marine supplements and fish meal (e.g. Kitessa *et al.*, 2004; Shingfield *et al.*, 2013; Scollan *et al.*, 2014).

As shown in Supplementary Table S1, ruminal responses to marine lipids followed similar trends in the bovine, ovine and caprine (Huws *et al.*, 2011; Shingfield *et al.*, 2012;

**Table 1** Main changes in digesta, milk and meat concentrations of selected fatty acids (compared with the control diet) in response to diet supplementation with marine lipids rich in n-3 polyunsaturated fatty acids (PUFA)

	Concentration <sup>1</sup>						
	18:0	c9-18:1	t11-18:1	t10-18:1	c9t11-CLA	18:2n-6	18:3n-3
Fish oil							
Digesta	--	++/ =	+++	++++/ =	++++/ =	- -/ =	- -/ =
Milk	-- -	- -/ =	+++	++++/ =	+++	- -	=
Meat	- / =	- -/ =	+++/ =	na	++/ =	- -/ =	- -/ =
Marine algae							
Digesta	-- -	++/ =	+++	+++	+++/ =	- -/ =	- -/ =
Milk	-- -	- -/ =	+++	+++	+++	- -/ =	- -/ =
Meat	=	=	(++)/ =	(- -)	++/- -/ =	=	- -/ =
Pure n-3 PUFA							
Digesta	-- -	+++/ =	+++/ =	+++/ =	+++/ =	+ / = / - -	+ / = / - -
Protected marine lipids							
Milk	- -/ =	- -/ =	+++	++/ =	+++	- -/ =	+ / =
Meat	=	- -/ =	+ / =	na	+ / =	+ / - -/ =	++
Fish meal							
Milk	- -/ =	=	+ / =	(++)	+ / =	- -/ =	=
Meat	=	=	na	na	na	- -/ =	=

Data derived from individual studies reported in Supplementary Tables S1 to S3, corresponding to 123 dietary treatments in bovine, 55 in ovine and 22 in caprine.

<sup>1</sup>For each marine lipid, predominant responses (i.e. those observed in more than 25% of cases) in digesta, milk or meat are codified as positive (+), negative (-) or not significantly different (=) compared with the control; 'na' denotes non-available data. This 25% cut-off value was arbitrarily established to counteract the high between-study variability and facilitate the identification of major trends. For positive and negative responses, the mean percentage of variation is reported as: + (<15% increase), ++ (15% to 50% increase), +++ (50% to 100% increase), ++++ (>100% increase), - (<15% decrease), -- (15% to 50% decrease), --- (>50% decrease). For example, '+ / = ' would indicate that in more than 25% of cases the response to the marine lipid was a significant increase of 15% to 50% in the fatty acid, while in other >25% there was no statistical difference. Other responses were either not observed or observed in <25% of cases. Parentheses are used for variables with a single datum.

Toral *et al.*, 2016), and were broadly reflected in their milk FA composition (Boeckaert *et al.*, 2008; Bernard *et al.*, 2015; Frutos *et al.*, 2017). Direct interspecies comparisons support these findings (Toral *et al.*, 2015, 2016 and 2017). Besides hampering ruminal t11-18:1 saturation, marine lipids may also partly inhibit *cis*-18:1 and *trans* 18:2 hydrogenation, as suggested by increases in the accumulation of c9-18:1 and c9t11-CLA in digesta (Table 1 and Supplementary Table S1; Huws *et al.*, 2011; Toral *et al.*, 2017). Decreases in ruminal 18:2n-6 and 18:3n-3 would indicate more extensive metabolism of C18 PUFA with marine lipid supplements, presumably by bacterial groups with low or no sensitivity to their toxic effect. The commonly observed higher content of t10-18:1, derived from alternative BH pathways, may be promoted more by DHA than EPA (Toral *et al.*, 2017), which seems consistent with the frequently observed greater concentration of t10-18:1 in digesta and milk due to supplementation with DHA-rich algae compared with EPA-rich fish oils (Boeckaert *et al.*, 2008; Shingfield *et al.*, 2012; Vahmani *et al.*, 2013; Supplementary Table S1).

Inhibition of the terminal BH step induces a shortage of ruminal 18:0, the major substrate for mammary stearoyl-CoA desaturase (SCD), which explains the lower milk concentration of c9-18:1, in cows, sheep and goats fed marine lipids (Table 1; Chilliard *et al.*, 2007; Frutos *et al.*, 2017). Down-regulation of the SCD gene by FA in marine lipids or BH intermediates might also contribute to this response (Ahnadi *et al.*, 2002; Carreño *et al.*, 2016), although data on *in vivo*

SCD activity are inconclusive (Faulconnier *et al.*, 2018). Conversely, because most c9t11-CLA in the bovine, ovine and caprine derives from endogenous synthesis via SCD, its concentration in milk and meat increases with the higher ruminal outflow of t11-18:1 (Bernard *et al.*, 2008; Scollan *et al.*, 2014).

There is also evidence that marine lipids decrease the milk concentration of some undesirable SFA (12:0, 14:0 and 16:0), but with lower effectiveness than plant lipids (Shingfield *et al.*, 2013; Vahmani *et al.*, 2013; Bernard *et al.*, 2015).

Compared with lactating ruminants, there are fewer studies with growing ruminants and often the intramuscular FA profile reported is not as comprehensive (Supplementary Table S6). While supplementation with fish oil can cause sizeable changes in the n-6:n-3 PUFA ratio (whose implications for human health are under some debate; Salter, 2013), it generally does not increase the PUFA : SFA ratio in intramuscular lipid above the 0.1 to 0.15 normally observed. Noci *et al.* (2007) reported that increasing the level of fish oil fed to cattle led to a linear increase in the concentration of t9- and t11-18:1, c9t11-CLA, EPA and DHA, and a decrease in the n-6 : n-3 ratio in intramuscular lipid. Parvar *et al.* (2017) reported increases in EPA and DHA proportions in intramuscular lipid for lambs fed 3% fish oil but CLA or *trans*-18:1 isomers were not reported. With respect to algae, inclusion of DHA-rich *Schizochytrium* biomass in a lamb ration increased the concentration of EPA and DHA and decreased the n-6 : n-3 PUFA ratio in intramuscular lipid

## Changing the fatty acid profile of meat and milk

**Table 2** Main changes in digesta, milk and meat concentrations of selected fatty acids (FA) and rumen disappearance of c9-18:1, 18:2n-6 and 18:3n-3 (compared with the control diet) in response to diet supplementation with plant secondary compounds<sup>1</sup>

	Concentration <sup>2</sup>							Rumen disappearance <sup>2</sup>		
	18:0	c9-18:1	t11-18:1	t10-18:1	c9t11-CLA	18:2n-6	18:3n-3	c9-18:1	18:2n-6	18:3n-3
Polyphenol oxidase										
Digesta	++/=	+++/=	+++/+=	++/=	++/=	+++	+++	--	-/=	-
Milk	=	=	-	++/=	-/=	++	+++			
Meat	=	-/=	- -/=	=	=	++/=	++/=			
Oxygenated FA										
Digesta	-/=	na	+ / = / -	na	-/=	+ / =	na	(-)	na	
Milk	(++)/=(+ + +)(=)(-)	(+ + +)(=)(-)	+ / (=)	na	+++	+++	(+ + +)(=)(-)			
Tannins and other phenols										
Condensed tannin extracts										
Digesta	- -/=	++/=	+++/=	- - -/=	+++/+=	+++/=	++/=	=	- -/=	- -/=
Milk	=	+ / =	++/=	=	=	++/=	+ / =			
Meat	-/=	-/=	=	+++	+++/=	++/=	+++/=			
Hydrolysable tannin extracts										
Digesta	-/=	++/=	++/=	- -/=	+++/=	+++/=	+++/=	(=)	(=)	(=)
Milk	-/=	-/=	++/=	=	=	++/=	+ / (=)			
Tannin-containing forages										
Digesta	- -/=	++/=	=	=	=	++/=	+++/=	(=)	(- -)	(- -)
Milk	=	- -/=	++/=	(- -)	=	++/=	+++/=			
Meat	=	+ / =	na	na	+++/=	- - / + / =	+++			
Others and mixes										
Digesta	=	=	=	=	=	=	++/=	--	-/=	- -/=
Milk	=	- -/=	+++/=	=	+++/=	+ / =	+ / =			
Meat	=	=	++/=	=	+++/=	+ / =	+++/=			
EO and their constituents <sup>3</sup>										
Garlic oil										
Digesta	- -/=	++/=	=	++/+=	=	+++/+=	+++/+(+ + +)	na	na	na
Milk	+	(+ + + +)	+	na	+++	na				
Cinnamol oil										
Digesta	- -/=	=	- - -/=	=	+++/=	+++/=	+++/=(=)(-)(=)	(=)	(- - -)(=)(- -)(=)	
Milk	(++)/(-)	(+ + + +)(=)	(=)	(=)	(+ + + +)(=)	(=)	(-)(=)			
Clove + eucalyptus oil										
Digesta	- -/=	++/=	- - -/=	=	=	=	=	na	(=)	(=)
Milk	=	=	=	=	=	=	=			
Other EO										
Digesta	- -/=	- -/=	- - / =	(=)	=	=	=	(- -)	(=)	(=)
Milk	+ / =	+++/=	=	=	=	=	+++/+=			
Meat	=	=	+++/=	na	+++/=	+ / =	=			
Saponins										
Digesta	=	(+)/=	=	=	=	=	=	(=)	=	=
Milk	=	=	(-)	(=)	(=)	=	=			
Meat	=	+	=	=	=	+ / =	=			

EO = essential oil.

Data derived from individual studies reported in Supplementary Tables S4 to S6, corresponding to 176 dietary treatments in bovine, 119 in ovine and 62 in caprine.

<sup>1</sup>Detailed information about their source of origin and dose, if available, and results from other unclassified products and mixes of different types of plant secondary compounds are individually reported in Supplementary Tables S4 to S6.

<sup>2</sup>For each plant secondary compound, predominant responses (i.e. those observed in more than 25% of cases) in digesta, milk or meat are codified as positive (+), negative (-) or not significantly different (=) compared with the control; 'na' denotes non-available data. This 25% cut-off value was arbitrarily established to counteract the high between-study variability and facilitate the identification of major trends. For positive and negative responses, the mean percentage of variation is reported as: + (<15% increase), ++ (15% to 50% increase), +++ (50% to 100% increase), + + + (>100% increase), - (<15% decrease), -- (15% to 50% decrease), - - (>50% decrease). For example, '+ + /=' would indicate that in more than 25% of cases the response to the compound was a significant increase of 15% to 50% in the FA, while in other > 25% there was no statistical difference. Other responses were either not observed or observed in <25% of cases. Parenthesis are used for variables with a single datum.

<sup>3</sup>Subcategories include treatments with compounds identified as the main active components in garlic oil (diallyl sulphide and propyl propane thiosulfinate), cinnamon oil (cinnamaldehyde) and clove and eucalyptus oil (eugenol). Responses to EO in meat have been summarised in the 'other EO' category due to the few available data for growing ruminants.

(Hopkins *et al.*, 2014). Increasing the level of inclusion of a similar algal product in a beef ration led to a linear increase in the concentration of t11-18:1, c9t11-CLA (quadratic), EPA and DHA and a linear decrease in the n-6 : n-3 ratio in intramuscular lipid (Phelps *et al.*, 2016).

A common feature of the effect of marine lipids is the increment in milk t10-18:1, which seems less pronounced in goats than in cows and ewes (Shingfield *et al.*, 2013; Toral *et al.*, 2015; Supplementary Table S2). There is no information available for meat. The implication of the higher t10-18:1 concentration for consumers is still unclear because of the uncertain involvement of ruminant *trans* FA

in cardiovascular disease (Salter, 2013). In addition, although the association between t10-18:1 and milk fat depression (MFD) is also equivocal, it has been considered as a biomarker of altered BH pathways associated with this syndrome (Shingfield *et al.*, 2013). Furthermore, there is a dearth of knowledge about the impact on human health and animal performance of other rumen-derived FA that increase after marine lipid supplementation, such as oxo-FA and several 16-, 20- and 22-carbon intermediate metabolites (Kairenus *et al.*, 2015; Toral *et al.*, 2015), which highlights the need for further investigation in this field.

Another aspect that warrants additional research is the interaction with basal diet composition, which influences the BH responses to marine lipids through changes in the rumen microbiota (Jenkins *et al.*, 2008; Buccioni *et al.*, 2012). For example, in cows fed fish oil, the higher the proportion of concentrate the higher the milk content of  $t10\text{-}18:1$  at the expense of  $t11\text{-}18:1$ , with more adverse effects on  $t11\text{-}18:1$  in diets based on corn silage than on grass silage (Shingfield *et al.*, 2005). The method of silage making is also relevant, as incremental levels of fish oil induced quadratic responses in the concentration of 18:0,  $c9\text{-}18:1$  and 18:3n-3 in intramuscular lipid from steers fed unwilted grass silage, but a linear decrease was found in those offered wilted silage and no interaction was detected for  $t11\text{-}18:1$  (Noci *et al.*, 2007). In goats, fish oil probably induced a more pronounced inhibition of the last BH step in diets rich in starch from extruded wheat than from barley (the former being more rapidly degradable), increasing  $c9t11\text{-}CLA$  in milk, but without affecting the low  $t10\text{-}18:1$  level (Bernard *et al.*, 2016).

Finally, it is worth mentioning the limited transfer of dietary EPA and DHA into final products and the variable success of protection technologies (Ahnadi *et al.*, 2002; Kitessa *et al.*, 2004). Apparent transfer efficiency to milk seems lower in cows and goats ( $\approx 4\%$  to  $5\%$ ) than in ewes ( $\approx 9\%$ ; Chilliard *et al.*, 2007; Bichi *et al.*, 2013). Whether this is due to differences in diet composition, lipid dosage or actual interspecies variation in ruminal or post-ruminal metabolism (e.g. duodenal absorption, blood transport or mammary FA uptake and secretion) is unclear given the lack of direct comparative studies between species. Dietary inclusion of fish oil in growing ruminants can lead to an increase in the concentration of EPA and DHA in intramuscular lipid (maximum achieved is  $\sim 15$  mg EPA + DHA/100 g muscle), but muscle from cattle fed fish oil does not generally reach the concentrations defined by the European Food Safety Authority (2009) to permit labelling as a 'source' of n-3 PUFA (40 mg/100 mg muscle).

#### *Plant secondary compounds*

Research on the role of plant secondary compounds in ruminant nutrition was initially driven by their negative effects on diet utilisation, but some studies revealed favourable modulation of rumen BH (Lourenço *et al.*, 2008; Vasta *et al.*, 2009). A wide variety of compounds has been examined, including tannins, EO, saponins, polyphenol oxidase (PPO) and oxygenated FA (Benchaar and Chouinard, 2009; Buccioni *et al.*, 2012; Ramos-Morales *et al.*, 2016). However, results are often inconsistent (Table 2 and Supplementary Tables S4 to S6), due to the diversity in active components, dosage, experimental approaches (*in vivo v. in vitro*) and ruminant species.

Replacing grass by forage legumes, in particular red clover, usually decreases the overall extent of BH, which has been attributed to the inhibition of lipolysis by the higher PPO activity in legumes (Vanhalatalo *et al.*, 2007; Buccioni *et al.*, 2012). Ruminal responses to most other compounds (e.g. oxygenated FA, terpenes and tannins) may be largely

mediated by their impact on the microbiota (Carreño *et al.*, 2015; Ramos-Morales *et al.*, 2016). Both types of effect could be additive or synergistic, as shown with the combined use of PPO- and tannin-rich forages (Campidonico *et al.*, 2016).

Tannins have been the focus of several studies, mainly in small ruminants, but their ability to modulate BH is still controversial (Khiaosa-ard *et al.*, 2009; Vasta and Luciano, 2011; Carreño *et al.*, 2015). Different publications, using either condensed or hydrolysable tannin extracts or tannin-rich forages, report a slowdown of initial PUFA metabolism in the rumen (Campidonico *et al.*, 2016; Alves *et al.*, 2017), rather than the specific inhibition of  $t11\text{-}18:1$  saturation that was initially suggested (Khiaosa-ard *et al.*, 2009; Vasta *et al.*, 2009). A review on the use of EO supports a similar mechanism, with promising effects of cinnamon and garlic oils and their purified active components on decreasing the first step of BH of PUFA and, less commonly, the terminal step (Lourenço *et al.*, 2008; Doreau *et al.*, 2017). Deviation from major BH pathways has been reported in some cases (e.g. for garlic oil and the tanniferous bush *Cistus ladanifer*, Lourenço *et al.*, 2008; Alves *et al.*, 2017; Doreau *et al.*, 2017), but a lower  $t10\text{-}18:1$  concentration in digesta is often found (Khiaosa-ard *et al.*, 2009; Carreño *et al.*, 2015), which would be potentially advantageous. On the contrary, for other products such as plant saponins, quercetin or eugenol-rich EO, very little or no influence on the ruminal FA profile has been observed to date (Lourenço *et al.*, 2008; Khiaosa-ard *et al.*, 2009).

The inconsistent effects of similar types of tannins or EO (Supplementary Table S4) may be partly due to different experimental approaches (*in vivo v. in vitro*) and, more importantly, to the variation in their structural features and reactivity (depending on extraction methods, plant varieties and parts, etc.; Mueller-Harvey, 2006; Vasta and Luciano, 2011; Khalif *et al.*, 2012). Dosage could also be important: for example, Carreño *et al.* (2015) reported that low and moderate levels of grape tannin extract (2% to 4% diet dry matter (DM)) favoured the accumulation of dietary PUFA (18:2n-6 and 18:3n-3) in digesta (in an *in vitro* system), whereas a higher dose (8%) enhanced  $t11\text{-}18:1$  concentration. Using high levels of tannins, EO or other plant extracts is interesting from a research perspective (Khiaosa-ard *et al.*, 2009; Vasta *et al.*, 2009), but would be impractical under farm conditions because of their cost and risk of toxicity. No study seems to have investigated variations among ruminant species in the modulatory effects on BH, but differences might be speculated due to their known different tolerances to some secondary metabolites, such as tannins (Mueller-Harvey, 2006).

There is even less information on the influence of dietary secondary compounds on milk FA than on rumen BH (Supplementary Table S5) and interspecies comparisons are therefore more challenging because post-ruminal lipid metabolism may also vary among species. The positive impact of forages rich in PPO and oxygenated FA on rumen FA (e.g. increasing PUFA concentrations) has been confirmed in cow and ewe milk, respectively (Addis *et al.*, 2005;

Vanhalto *et al.*, 2007), but promising *in vitro* effects of tannin extracts and EO are not always confirmed in lactating animals, probably due to the lower levels of inclusion used *in vivo* (Benchaar and Chouinard, 2009; Toral *et al.*, 2013). In any event, some EO (e.g. from garlic) and tannins (e.g. in sulla) appear to improve milk FA profile under practical conditions (Buccioni *et al.*, 2012; Khalif *et al.*, 2012). With regard to  $t10\text{-}18:1$ , although little or no changes are generally observed, caution should be taken because very few studies report intermediates from alternative BH pathways.

In meat animals, care should be taken to ensure that interpretation of the effect of rumen modifiers is not confounded by changes in intramuscular fat concentration since this can result in changes in the FA profile *per se*. Intramuscular lipid from cattle or lambs grazing a red clover-rich pasture had a higher 18:3n-3 concentration than that from similar animals grazing perennial ryegrass (but similar to that from the white clover-rich pasture) (Scollan *et al.*, 2014). Replacing grass silage with a mixture of grass and red clover silage increased the deposition of n-3 PUFA in intramuscular lipid of finishing cattle (Lee *et al.*, 2009). There is a general tendency for cattle grazing botanically diverse pastures that supply a range of plant secondary compounds, to have higher n-3 and total PUFA in intramuscular fat compared with cattle grazing predominantly ryegrass pastures (Moloney *et al.*, 2008), despite both pastures having a similar FA profile.

More specifically, including quebracho tannins in lamb rations increased 18:3n-3 and total PUFA concentrations in muscle, supporting their ruminally active properties, but only improved  $t11\text{-}18:1$  and  $c9t11\text{-}CLA$  levels when included in a concentrate diet (Vasta *et al.*, 2009). Although additional information on the interaction between basal ration and plant secondary compounds is limited, dietary inclusion of a source of condensed tannins (*C. ladanifer*) had a minor effect in lambs when the basal diets had no oil but increased  $t11\text{-}18:1$  and  $c9t11\text{-}CLA$  in muscle neutral lipids when it contained a blend of plant oils (Jeronimo *et al.*, 2010). These results suggest that a cautious approach should be adopted before implementing a specific treatment in practical farming, and that it should be tested in advance in the particular conditions of each production system.

Dietary inclusion of EO improved the FA profile of intramuscular lipid from goats (increasing  $t11\text{-}18:1$ ,  $c9t11\text{-}CLA$  and 18:2n-6 concentrations; Mandal *et al.*, 2014) but not from bulls (Prado *et al.*, 2016). As well as the species variations, the different results for the two studies may reflect the different sources of EO (clove and castor/cashew oils, respectively) and basal diet composition (sunflower oil was included in the former study).

#### *Direct-fed microbials*

Data on the effects of direct-fed microbials, also known as probiotics, on ruminal BH and the FA profile of milk and meat are reported in Supplementary Table S7. Information is still very limited, but encouraging results (i.e. increased milk  $t11\text{-}18:1$  and  $c9t11\text{-}CLA$  concentration) have been shown in

goats fed strains of *Butyrivibrio fibrisolvens* (Shivani *et al.*, 2016), *Lactobacillus plantarum* (Maragkoudakis *et al.*, 2010) and a mixture of *Lactobacillus reuteri*, *Lactobacillus alimentarius*, *Enterococcus faecium* and *Bifidobacterium bifidum* (Apás *et al.*, 2015). Greater milk 18:2n-6 and 18:3n-3 proportions were also found in the two latter studies. Conversely, a multi-strain product containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum* and *E. faecium* slightly decreased  $t11\text{-}18:1$ ,  $c9t11\text{-}CLA$  and 18:2n-6 in ovine milk (Payandeh *et al.*, 2017), and available data in lactating cows and beef (using mostly *Propionibacterium* spp. and *Saccharomyces cerevisiae*) do not seem very positive (Supplementary Table S7). Nevertheless, given that cows fed *Propionibacterium* spp. showed greater milk  $c9t11\text{-}CLA$  in a high-starch diet and no changes in a low-starch ration (Philippeau *et al.*, 2017), interactions with basal diet composition should be explored. Microbiology studies to identify and cultivate active biohydrogenating bacteria to be used as probiotics are also required.

#### *Persistency of changes*

Responses of rumen microbiota to BH modulators may vary over time, with some inconsistent effects being probably due to interspecies differences. For instance, in cows fed a ration based on corn silage, fish oil-induced decreases in milk 18:0 and  $c9\text{-}18:1$  concentrations were transient, and the large enhancements in milk  $t11\text{-}18:1$  and  $c9t11\text{-}CLA$  found during the first days on treatment subsequently declined (Shingfield *et al.*, 2006). However, the effects of marine lipids in grazing cows (AbuGhazaleh, 2008) were similar to those in ewes (Bichi *et al.*, 2013), with quick and persistent enrichments in milk  $t11\text{-}18:1$  and  $c9t11\text{-}CLA$  that suggest major changes in the rumen microbiota at the beginning of treatment and relative stability afterwards. Nevertheless, slower responses in milk  $t10\text{-}18:1$  suggest that changes in microorganisms involved in alternative BH pathways may take longer (Shingfield *et al.*, 2006; Boeckaert *et al.*, 2008; Carreño *et al.*, 2016).

Variation with time also seems to occur with probiotics, where some changes in milk FA composition seem clearer after the 1<sup>st</sup> month on treatment (Maragkoudakis *et al.*, 2010; Apás *et al.*, 2015), and some others may be reversed in the long term (3 months; Shivani *et al.*, 2016). The impact of many plant secondary compounds on BH may also be compromised by time because of the adaptation of the microbiota. For example, in ewes, the positive effect of quebracho tannins on milk  $t11\text{-}18:1$  and  $c9t11\text{-}CLA$  was only transient, and was subsequently displaced by a gradual increase in  $t10\text{-}18:1$  (Toral *et al.*, 2013).

There are no studies on the persistency of changes in the FA profile of intramuscular lipid due to dietary inclusion of marine oil or other ruminally active compounds. However, when bulls that had grazed a ryegrass pasture were housed and offered a concentrate ration for 168 days before slaughter, the proportion of 18:3n-3 in muscle was still higher than in bulls that had never been to pasture and were slaughtered at the same

carcass weight (Mezgebo *et al.*, 2017), demonstrating that a perturbation due to diet can persist. While the difference in FA is not relevant to human nutrition, it could be relevant to meat flavour (Mezgebo *et al.*, 2017). Long-term supplementation might not be necessary to have a detectable effect on meat FA, which would be particularly advantageous if the supplement is expensive.

### Associated positive and negative side effects in ruminant animals

Besides the impact on the FA profile of intramuscular lipid and milk, BH modulators can have a broad range of other effects (e.g. on fertility, as anti-parasitic treatments or to reduce the environmental footprint of livestock), depending on the type of product. Nevertheless, this section will only focus on potential side effects on animal performance and product quality.

#### *Effects on animal performance*

There is a widespread perception that marine lipids detrimentally affect ruminal fermentation and feed intake, with consequent reductions in milk production (e.g., Ahnadi *et al.*, 2002; Boeckaert *et al.*, 2008). However, small doses of marine lipids ( $\leq 1.5\%$  DM) can efficiently modify BH while maintaining rumen function and milk yield, but unfortunately MFD is a common feature of this feeding strategy (Bichi *et al.*, 2013; Shingfield *et al.*, 2013). This syndrome represents the main effect of marine lipids on animal performance and a major concern due to potential economic losses, especially in the small ruminant sector, as their milk is mostly processed into cheese.

Although ewes and goats appear to be less prone than cows to marine lipid-induced MFD, this condition has been described in the three species (Ahnadi *et al.*, 2002; Shingfield *et al.*, 2013; Toral *et al.*, 2015). Algae and both free and protected fish oils cause this syndrome (Kitessa *et al.*, 2004; Chilliard *et al.*, 2007; Boeckaert *et al.*, 2008), which seems to be associated with the action of EPA and DHA on rumen microbiota, favouring alternative BH pathways that produce antilipogenic metabolites (Shingfield and Gruinari, 2007). The shift towards the formation of  $t10$ -18:1 at the expense of  $t11$ -18:1 has received a great deal of attention as a marker of altered rumen function, but specific BH intermediates that could explain marine-lipid-induced MFD have not been well characterised yet (Shingfield and Gruinari, 2007; Kairenus *et al.*, 2015). Recent studies suggest a contribution of little known candidate inhibitors, such as metabolites of 18:3n-3 and very long-chain PUFA, and oxylipids of ruminal origin (e.g.  $t10c15$ -18:2,  $t10$ -containing C20 and C22 FA and 10-oxo-18:0; Kairenus *et al.*, 2015; Toral *et al.*, 2015; Carreño *et al.*, 2016). Mammary mechanisms mediating this type of MFD have also been examined (Shingfield *et al.*, 2013; Frutos *et al.*, 2017; Faulconnier *et al.*, 2018). Molecular biology studies have shown that reductions in the mammary expression of enzymes involved in *de novo* FA synthesis

(e.g. ACACA, ACSS2 and FASM) and related transcription factors (e.g. SREBF1 and INSIG1) are common features in responses to marine lipids, whereas downregulation of genes responsible for FA uptake and triacylglycerol synthesis (e.g. LPL and GPAT4) are less frequent (Shingfield *et al.*, 2013; Carreño *et al.*, 2016; Frutos *et al.*, 2017). Nutrigenomic studies applying high-throughput technologies will likely provide new insight in this regard.

Regarding growing ruminants, since dietary inclusion of lipid must be restricted (to  $\approx 60$  g/kg DM consumed) to avoid impairment of rumen function, the capacity to manipulate the FA composition by use of ruminally available oil sources is limited. In support of this, Scollan *et al.* (2001) found no adverse effect of dietary inclusion of fish oil at 30 g lipid/kg DM in a total diet that contained 60 g/kg DM. Wistuba *et al.* (2006) added 30 g fish oil/kg, which increased the lipid content in the diet to 67 g/kg DM and intake was decreased. A similar negative impact of fish oil inclusion (30 g/kg) on intake and growth of lambs was reported by Parvar *et al.* (2017). Scollan *et al.* (2001) suggested that the negative effects of fish oil inclusion seen in some studies are likely mediated by specific BH intermediates of fish oil rather than a negative effect of fish oil on rumen function, analogous to MFD. Hopkins *et al.* (2014) observed no negative effect of dietary inclusion of DHA-rich algae in the diet of lambs but referred to a related non-peer reviewed study where a reduction in intake in lambs offered the same product was observed.

Concerning plant secondary compounds, some reductions in milk fat concentration have also been observed in cows and ewes fed PPO- and tannin-rich legumes and in goats supplemented with EO, but their magnitude is small and likely due to a dilution effect by higher milk production (e.g. Addis *et al.*, 2005; Kholif *et al.*, 2012). The positive response to legumes seems to be explained, at least in part, by higher feed intake, but improved nutrient utilisation could also contribute, as reported for EO (Kholif *et al.*, 2012). However, inclusion of saponins and direct-fed microbials seems to have few effects on dairy performance (e.g. Benchaar and Chouinard, 2009; Philippeau *et al.*, 2017). In lambs, the addition of quebracho to the ration decreased feed intake when included in an herbage- but not in a concentrate-diet (Vasta *et al.*, 2009), which seems to be a consistent dose-dependent finding. In contrast, condensed tannins from either grape extract or *C. ladanifer* did not affect feed intake or growth of lambs (Jeronimo *et al.*, 2010) and EO or saponins did not affect these variables in goats (Mandal *et al.*, 2014).

#### *Effects on product quality*

The sensory quality of food can be defined by its texture, flavour, including the odour (smell attributes) and aroma (sensations perceived by the retro-nasal airway) and taste. The sensory quality of dairy products can be influenced by the FA composition of milk, for example, the production of oxidised flavour at 8 days post-sampling was positively correlated with levels of 18:2n-6, 18:3n-3 and total PUFA in milk fat (Timmons *et al.*, 2001). The FA composition of milk can also influence processing characteristics whereby milk with a

high PUFA concentration is more susceptible to oxidation and therefore has a shorter shelf-life. However, milk from dairy cows supplemented with fish oil had no oxidised flavours (Nelson and Martini, 2009). Similarly, Kitessa *et al.* (2004) reported that milk from cows fed ruminally protected fish oil had similar organoleptic properties to that of a non-lipid supplemented control ration. High PUFA milk generally results in softer butter and cheese (Dewhurst and Moloney, 2013) but Jones *et al.* (2005) found no differences in flavour of butter or cheese manufactured from milk with a threefold increase in EPA + DHA, due to fish oil supplementation, compared with control milk. In contrast, while Glover *et al.* (2012) observed no effect of dietary algal supplementation on the oxidative stability of milk, that of butter was decreased. Plant secondary compounds that alter milk FA composition are likely to have a smaller effect on milk shelf-life than sources of marine oil since many of these compounds also have anti-oxidant properties that are detectable in milk. They may also be aromatic and so confer additional flavours to milk and meat but further studies are required on this topic.

Characteristics important to consumer perception of the quality or eating experience of meat may also be influenced by the FA composition of intramuscular lipid. When EPA and DHA increased in intramuscular lipid due to inclusion of protected fish oil in the diet of steers, lipid oxidation increased, resulting in a loss of shelf-life (Dunne *et al.*, 2011). A similar effect was seen due to the inclusion of microalgae in the diet of steers (Phelps *et al.*, 2016). The appropriate ratio of antioxidants to n-3 PUFA in meat to ensure lipid and colour stability during retail display remains to be determined. In this regard, combinations of plant-derived compounds that have antioxidant properties and marine lipids seem the most promising. There were few effects on product quality of unprotected fish oil or EO in the studies of Vatansever *et al.* (2000) and Prado *et al.* (2016), respectively. Wistuba *et al.* (2006) concluded that the differences found by a trained sensory panel due to fish oil inclusion in the diet of cattle were 'relatively small and would probably not be discernible to the average consumer'. Ponnampalam *et al.* (2002) similarly reported that sensory characteristics of lamb were not affected by dietary inclusion of fish meal or fish oil. This likely reflects the relatively small change in muscle PUFA in these studies. The greater concentration of EPA and DHA in intramuscular lipid with the use of ruminally protected fish oil increased the sensory score for 'abnormal' but overall liking was not affected (Richardson *et al.*, 2004). Similarly, lamb from the combined unprotected fish oil/marine algae diet had higher sensory scores for 'abnormal' and 'rancid' compared with the unprotected fish oil treatment but overall liking was not affected (Nute *et al.*, 2007). In general, secondary plant compounds have few significant effects on meat eating quality.

## Future perspectives

Manipulation of ruminal BH has proven effective in improving milk and meat FA profile, but further studies are required

to unravel the causes of some inconsistent results. In addition, to be applied under practical farm conditions, feeding strategies have to be sustainable and cost-effective so research must continue to address ways to mitigate negative side effects on animal performance (e.g. MFD). A further challenge to be addressed is increasing our understanding of *in vivo* microbiology of ruminal BH, with an urgent need to determine which populations are truly involved in the process. This, in turn, may be useful for selection of new direct-fed microbials. Equally relevant is the acquisition of more in-depth knowledge of the effects of individual FA, including BH intermediates, on human health. Providing answers to this question seems key to assessing whether modified ruminant products would actually exert measurable effects on consumer health. Finally, evaluation of alternative feed resources with potential to modulate more efficiently the FA profile of milk and meat, including by-products rich in active compounds or EPA- and DHA-containing transgenic plants, as well as advances in microalgae production, are some of the topics with significant prospects for future growth in this field.

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## Declaration of interest

None.

## Ethics statement

None.

## Software and data repository resources

Data are available in the Supplementary Tables S1 to S7.

## Supplementary material

To view supplementary material for this article, please visit <https://doi.org/10.1017/S1751731118001994>

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