IJAS Iranian Journal of Applied Animal Science

A Review on Biohydrogenation and Effects of Tannin on It

Review Article

R. Valizadeh Yonjalli[1], F. Mirzaei Aghjehgheshlagh[1*], B. Navidshad[1] and S. Karamati Jabehdar[1]

[1] Department of Animal Science, University of Mohaghegh Ardabili, Ardabil, Iran

Received on: 20 Apr 2017
Revised on: 27 Aug 2017
Accepted on: 16 Sep 2017
Online Published on: Jun 2018

*Correspondence E-mail: f_mirzaei@uma.ac.ir

ABSTRACT

The process called biohydrogenation occurs mainly in ruminant animals and during it, unsaturated fatty acids, and particularly poly-unsaturated ones (linoleic and linolenic) coverts to a saturated form of stearic acid. For many years, the beneficial effects of biohydrogenation intermediate fatty acids like cis-9 trans-11 linoleic acid, the main natural isomer of conjugated linoleic acids (CLA), and the isomer of trans-9 trans-11 CLA, especially in preventing cancer, has been proved. Many researches tried to use different components to interference biohydrogenation and increase the mediator substrates of CLA (e.g. vaccenic acid (VA)). Recently, due to the effects on rumen microorganism population, so do on biohydrogenation, tannin, a poly phenolic compound, is in the center of considerations. It is well known that tannins, specially condensed tannins, affect the bacteria population involved in biohydrogenation. Consequently, reduction in biohydrogenation via dietary inclusion of tannin is a useful tool to change the milk fatty acid profile toward health promoting fatty acids.

KEY WORDS fatty acid, human health, milk composition, plant metabolites.

INTRODUCTION

Biohydrogenation (BH) is a process that happens in the rumen and it's the cause of disappearance of dietary polyunsaturated fatty acids (PUFA) (e.g. Linoleic: C18:2 and Linolenic acid: C18:3) in the rumen. Biohydrogenation converts the unsaturated fatty acids (USFA) to saturated fatty acids (SFA) by isomerization of unsaturated fatty acids to trans fatty acid intermediates, then hydrogenation of the double bonds happens (Harfoot and Hazlewood, 1988). The biohydrogenation by ruminal microorganism results in small amount of unsaturated fatty acids in ruminants meat and milk. The disappearance rate of linoleic and linolenic acids in rumen has reported 85 and 93%, respectively (Chilliard *et al.* 2007). During the BH intermediate fatty acids are produced.

Conjugated linoleic acid (CLA) is one of the intermediates, formed by BH of certain PUFA by ruminal microorganisms. This fatty acid is considered to be health-promoting (Carreno *et al.* 2015). Dietary CLA have been shown in many studies to be effective in preventing cancer, decreasing atherosclerosis, improving immune response, and altering protein or energy metabolism (Jenkins *et al.* 2008; Benchaar *et al.* 2009). Moreover, only a small amount of the rumenic acid (RA) (C18:2 Cis9 trans-11) could be present in meat and milk. RA is produced to a larger extent in muscle and mammary glands by the action of Δ^9-desaturase on C18:1 trans-11 (vaccenic acid (VA): another intermediate of ruminal BH). Increasing the levels of these fatty acids through nutritional strategies by inhibiting ruminal BH of dietary PUFA or promoting the accumulation of precursors of CLA in the rumen, such as trans-11

18:1 VA is reported (Lock and Bauman, 2004; Toral *et al.* 2013). The occurrence of any disease such as cardiovascular disease has been increased due to the industrial and unhealthy food regimes. Moreover, due to consumers' concerns for safe and healthy animal origin foods, ruminant nutritionists are making considerable efforts to develop products that are safe and potentially health promoting. Such efforts have been made to increase the unsaturated fatty acids and especially CLA, in milk and its products. Furthermore, in animal nutrition practical efforts have been conducted for increasing the VA accumulation in the rumen for increasing the levels of CLA in meat or milk. Supplementation of ruminant diets with linoleic acid-rich oils, like sunflower oil, has shown to increase milk CLA content, possibly via the increasing ruminal VA content (Palmquist *et al.* 2005). Another way to increase the VA amount is through inhibiting the final step of rumen BH by using the marine lipids such as fish oils and algae (Shingfield *et al.* 2006) or, according to recent studies (Khiaosa-Ard *et al.* 2009; Toral *et al.* 2013; Buccioni *et al.* 2015) using plant secondary metabolites e.g. poly phenolic compounds such as tannins.

Tannins are defined as polyphenolic compounds that bind to protein. Therefore, they can alter dietary protein digestion in rumen and affect ruminal microbial population. Tannins may also result in other beneficial effects, including increased milk production and improved animal growth performance (Mlambo and Mapiye, 2015), higher propionate proportions, lower protozoa numbers (Makkar *et al.* 1995a; Makkar *et al.* 1995b), decreasing methane emissions (Puchala *et al.* 2005; Soltan *et al.* 2013), inhibition of ruminal BH (Vasta *et al.* 2009; Buccioni *et al.* 2015; Carreno *et al.* 2015), anti-helminthic effects, etc. This review aimed to represent general information about ruminal BH, and their interactions.

Biohydrogenation

What is biohydrogenation?

The action of BH is carried out by ruminal microorganisms. Biohydrogenation of UFA is known as the ruminal function on dietary lipids, and free fatty acids are the substrates for this process. As a consequence, the amounts of biohydrogenated fatty acids are always lower than the primary amount for hydrolyze by lipase, and factors that affect fatty acids hydrolyze may also affect BH. In addition, the rate of rumen BH of fatty acids typically increases by increasing the amount of unsaturation of fatty acids (Bauman *et al.* 2003). Biohydrogenation is happening extensively in the rumen. In most of the diets the percentage of BH for linoleic and linolenic acid is 70-95% and 85-100%, respectively. Primary pathways of BH were established by pure cultures of rumen microorganisms (Figure 1).

The following will represent the classic pathway of BH in the rumen (Lourenco *et al.* 2010).

Biohydrogenating ruminal bacteria

Bacteria have the main role in fatty acid BH (Jenkins *et al.* 2008). BH is considered as a mechanism of protection against the toxic effects of PUFA, which involves only a few species of the rumen bacteria (Lock and Bauman, 2004).

In early microbiological studies by Polan *et al.* (1964), *B. fibrisolvens* was discovered to have a role in BH of fatty acids and forming CLA and VA, as intermediates of linoleic acid BH (Polan *et al.* 1964; Kepler *et al.* 1966). Later studies discovered other bacteria capable the biohydrogenation of fatty acids, but they did not provide much information about their mechanism of action (Lourenco *et al.* 2010). *Fusocillus* spp. was identified as stearate forming organism (Kemp *et al.* 1975).

Later, Van de Vossenberg and Joblin (2003) isolated a bacterium from the rumen fluid of a grazing cow which was phenotypically similar to '*Fusocillus*' and by analyzing they indicated that it was phylogenetically close to *Butyrivibrio hungatei*.

Afterwards, the species named *Clostridium proteoclasticum* was introduced as a stearate producer with morphological and metabolic properties that was differ from the *Fusocillus* (Wallace *et al.* 2006). Based on the metabolic pathways, the BH bacteria have been classified into two groups: group A which hydrogenate PUFA to trans 18:1 fatty acids, and group B which hydrogenate the trans 18:1 fatty acids to stearic acid (SA) (Harfoot and Hazelwood, 1997) (Figure1).

Thus, generally the whole BH reactions of linoleic and linolenic acid convert to SA don't carry out by a single species (Kemp *et al.* 1975). Finally, saturated free fatty acid that reaches the small intestine is the consequence of the hydrolysis and BH in the rumen (Lourenco *et al.* 2010).

Patra and Saxena (2010) noted that many ruminal bacterial species of the genera *Butyrivibrio*, *Ruminococcus*, *Treponema-Borrelia*, *Micrococcus*, *Megasphaera*, *Eubacterium*, *Fusocillus* and *Clostridium* are known to be associated with ruminal BH.

Butyrivibrio spp. are most active species among the group A bacteria, which form CLA from linoleic acid, while a few species of bacteria such as *Fusocillus* spp. and *Clostridium proteoclasticum* (group B) convert VA to SA. Nowadays, two main genera of *B. fibrisolvens* and *B. proteoclasticus* populations are well known for their role and sensitivity in BH (Vasta *et al.* 2010). Therefore, it has been suggested that inhibition of group B bacteria without any effect on group A bacteria may result in more vaccinic acids and CLAs (Lourenco *et al.* 2010).

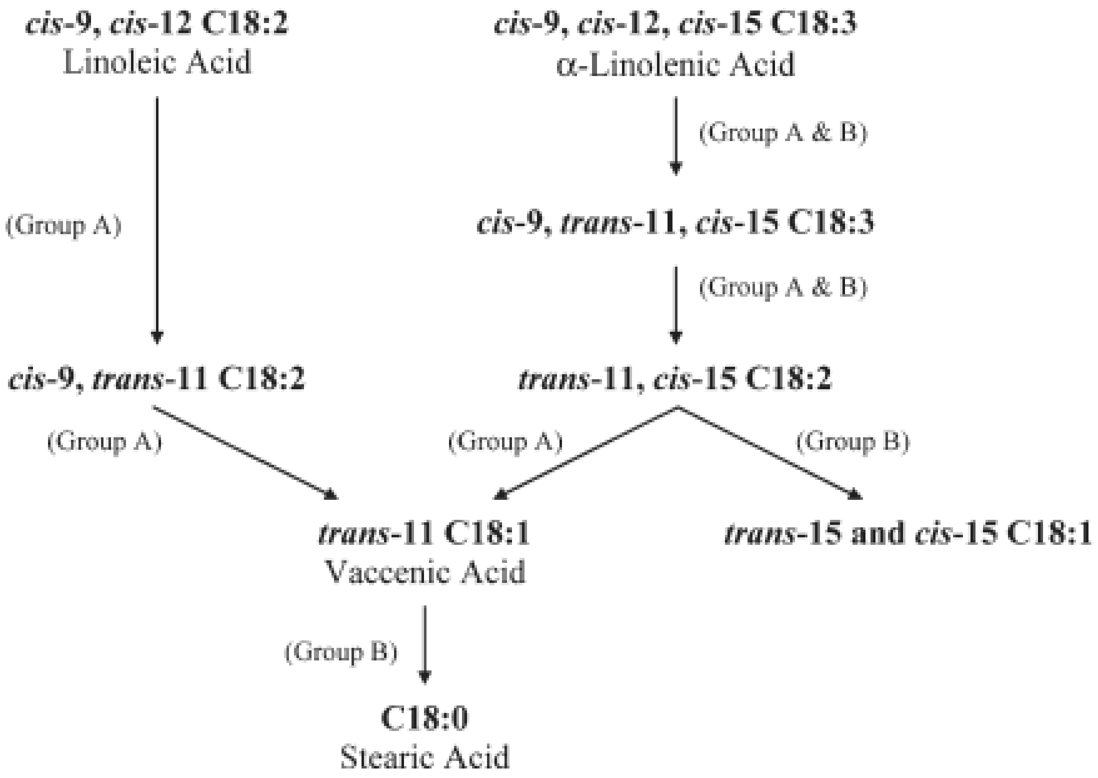


Figure 1 Biohydrogenation process for linoleic and linolenic acids in the rumen
Adapted from (Harfoot and Hazelwood, 1997)

Role of ciliate protozoa in biohydrogenation

Protozoa seem to include more than half of the rumen microbial microorganisms (Williams and Coleman, 1992) and protozoa could have about three quarters of the microbial fatty acids present in the rumen (Keeney, 1970). Therefore, protozoa could be considered as an important source of CLA and VA. Wright (1960) concluded that both bacteria and protozoa are involved in BH, Dawson and Kemp (1969) doubt on this conclusion because of extensive ingestion of bacteria by protozoa, but Girard and Hawke (1978) and Singh and Hawke (1979) suggested the minor participation of protozoa in ruminal BH due to their activity in ingestion or associating bacteria. However, protozoa lipids contain proportionally more UFA than the bacterial lipids (Harfoot and Hazlewood, 1997). Later, it was reported that these UFA (CLA and VA) confirms the role of protozoa in the formation of health-promoting fatty acids in the rumen.

Devillard *et al.* (2006) reported the fatty acid profile of protozoa species. They noted that, except holotrich, larger species including *Ophryoscolex caudatus* contain higher concentrations of CLA and VA, while small species such as *Entodinium nannelum* and *Isotricha prostoma* contain lower concentrations of these fatty acids (Lourenco *et al.* 2010).

Manipulating ruminal biohydrogenation

Several factors influence the concentration of fatty acids in ruminant products. In this concept, the quantity and composition of dietary lipids have a main effect because of the fatty acids that escapes ruminal metabolism. Inhibiting BH is a direct manipulating effect of dietary fatty acids in the rumen. Because fatty acids metabolism is related to the other aspects of ruminal metabolism, BH can be affected indirectly too, for example through H_2 metabolism or the microbial species of other metabolic pathways (Lourenco *et al.* 2010).

One of the main manipulators is lipid supplements. Fats in ruminant diets have two main roles: (i) increasing the energy content of the diet (ii) manipulating ruminal fermentation because of their antimicrobial effect (Lourenco *et al.* 2010). The antimicrobial effect of dietary lipids is associated with the degree of unsaturation of the fatty acids present (Zhang *et al.* 2008; Yang *et al.* 2009). Poly-unsaturated fatty acids are shown to have more toxic potential on biohydrogenating bacteria than di- or monoenoic fatty acids (Maia *et al.* 2010), thus oils containing PUFA such as linolenic acid would be expected to have a greater effect on rumen BH and the fermentation process than oils rich in linoleic acid or oleic acid (Bu *et al.* 2007).

It has been reported that unsaturated oilseeds like linseed, soybean and sunflower and their products (oil, Ca salt and amides), could enhance trans-18:1 fatty acids production (Glasser *et al.* 2008). Oils rich in linoleic acid (sunflower and soybean) found to be more effective in enhancing milk CLA than oils rich in linolenic acid (Lourenco *et al.* 2010).

Some researchers used lipase as a regulator of BH. Inhibitory act of lipase could be a strategy to scape PUFA from ruminal BH, and different studies have been conducted based on this idea (Van Nevel and Demeyer, 1996; Krueger *et al.* 2009). However, it is reported that long-chain PUFA might be considered to have inhibitory effects on BH itself (Fievez *et al.* 2007), and the bacteria responsible for the last step in the conversion of monoenoic fatty acids to fully saturated fatty acid are sensitive to the toxic effects of PUFA (Maia *et al.* 2007). But, if the concentrations of PUFA can be increased, possibly by increasing lipase activity, VA metabolism may be inhibited; causing more VA and CLA leaves the rumen and an end result of higher CLA in ruminant products (Lourenco *et al.* 2010).

Defaunation which is considered as an approach in increasing ruminal function has been discussed for many years. Lourenco *et al.* (2010) reported the presence of UFA and higher concentrations of CLA and VA in ciliate protozoa. Therefore, a defaunation could result in increasing BH and SFA. Yanez-Ruiz *et al.* (2007) reported the higher ratio of SFA/PUFA in muscle of defaunated lambs.

Additionally, Different nutritional strategies against BH have been used, e.g. forage feeding, and dietary inclusion of oilseeds, protected fat sources, vegetable oils and marine products. Furthermore, an antimicrobial material such as ionophores like monensin are shown to inhibit the growth of gram-positive bacteria that produce hydrogen therefore reduce methane production and interfere with BH. However, the use of ionophores as a feed antibiotic has been banned in livestock production in certain counties (e.g. EU). Therefore, plant secondary metabolites such as essential oils (EO), saponins and tannins have been recommended as a suitable replace and a potential approach to manipulate bacterial populations involved in ruminal BH and modify the fatty acid composition of ruminant-derived products especially milk and meat (Ishlak *et al.* 2015).

Conjugated linoleic acids

A mixture of positional and geometric isomers of octadecadienoic acid with conjugated double bonds is called Conjugated linoleic acid (Lock and Bauman, 2004). The presence of CLA in milk fat was reported for the first time by Booth *et al.* (1935), at the University of Reading, United Kingdom, working on milk fat from cows that were grazing spring pasture.

Parodi (2003) identified cis-9, trans-11 CLA as milk fatty acids containing a conjugated double bond pair and because of its relationship to ruminants, the name of “rumenic acid” selected for this isomer. The other isomers of CLA were identified in ruminant fat by improving analytical techniques (Lock and Bauman, 2004).

The main source of CLA in human diets is ruminant-derived food and dairy products that contribute about 75% of the total amount of CLA (Lock and Bauman, 2004). Different health beneficial effects, e.g. reducing the incidence of cancer (Ip *et al.* 1991), diabetes (Houseknecht *et al.* 1998), and atherosclerosis (Lee *et al.* 2005) has been reported for the cis-9, trans-11 CLA (rumenic acid). It should be mentioned that the CLA content of meat and milk is strongly in deal with ruminal BH of linoleic (cis-9, cis-12 C18:2) and linolenic acid (cis-9, cis-12, cis-15 C18:3) (Vasta *et al.* 2009).

Griinari and Bauman (1999) proposed that endogenous synthesis could be an important source of cis-9, trans-11 CLA in milk fat, with synthesis involving the enzyme Δ^9-desaturase and VA as the substrate.

Tannin

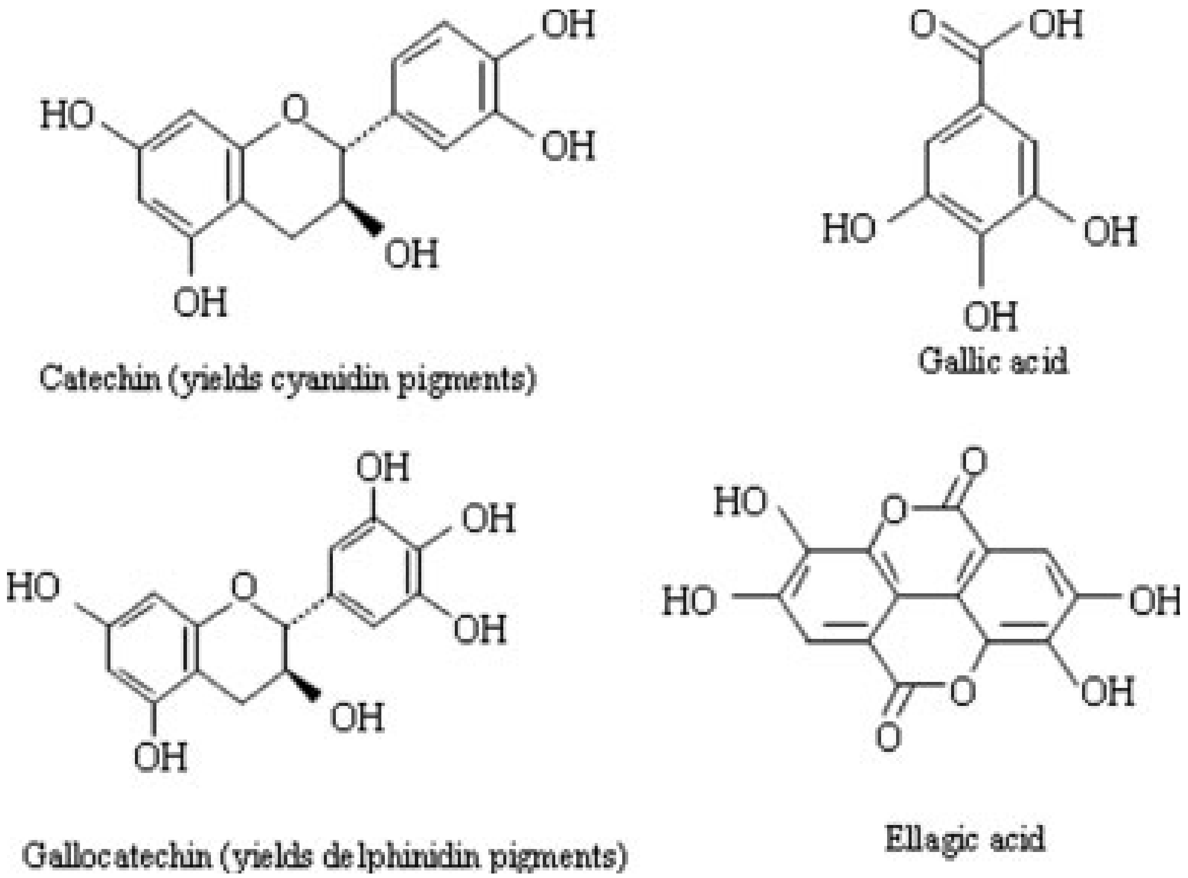
Chemical structure

Tannins are water-soluble polyphenolic polymers with high molecular weight; due to the presence of a large number of phenolic hydroxyl groups they have the capacity to bind mainly with proteins, carbohydrates ions and form complexes (Patra and Saxena, 2010). Tannins are usually divided into two groups: hydrolysable (HTs) and condensed tannins (CTs). The HTs are complex molecules with a polyol as a central core, such as glucose, glucitol, quinic acids, quercitol and shikimic acid, which is partially or totally esterified with a phenolic group, i.e. gallic acid (3,4,5-trihydroxy benzoic acid; gallotannins) or gallicacid dimer-hexa hydroxyl diphenic acid (ellagitannins) (Haslam, 1989) (Figure 2, adapted from Patra and Saxena, 2010).

Hydrolysable tannins possibly can be hydrolyzed by acids, bases or esterases resulting in polyol and the constituent phenolic acids (Haslam, 1989).

The CTs, or proanthocyanidins, are formed by polymerization of flavan-3-ol (epi) catechin and (epi) gallocatechin units, which are linked by C4-C8 and C4-C6 interflavonoid linkages (Hagerman and Bulter, 1989; Ferreira *et al.* 1999). Other monomers of CTs, such as profisetinidins, probinetidins and proguibortinidins, are reported by Haslam (1989).

The number of monomeric is different and it determines the degree of polymerisation therefore produces different oligomers with different chemical structures and biological characteristics (Waghorn, 2008).



Catechin (yields cyanidin pigments)

Gallic acid

Gallocatechin (yields delphinidin pigments)

Ellagic acid

Figure 2 Monomeric units of condensed (catechin and gallocatechin) and hydrolysable tannins (gallic and ellagic acid)
Adopted from (Patra and Saxena, 2010)

Generally, different parts of the plants, i.e. new leaves and flowers include higher concentrations of tannin and the concentration of tannin in these plants could be affected by various factors such as temperature, light intensity, water and nutrient stress, soil quality and topography (Patra and Saxena, 2010).

Both of HT and CT might be presented in the same plant, but it is possible that HT is present in some plants while other species contain CT (Haslam, 1989).

Effects of tannins

Tannins present in many feeds such as fodder legumes, browse the leaves and fruits (Hedqvist *et al*. 2000). Some of these plant species (e.g. *Acacia*, *Dichrostachys*, *Dorycnium, Hedysarum*, *Leucaena*, *Lotus*, *Onobrychis*, *Populus*, *Rumex*, *Salix* and *Vitis vinifera*) are used in animal nutrition. In non-ruminants tannins are mostly considered as an anti-nutritional factor, because these animals are more sensitive than ruminants (Table 1).

Detrimental effects of tannins are related to their toxic effects. Higher concentrations of tannins might result in reduced feed intake by decreasing the palatability of feed (Patra and Saxena, 2010).

Moreover, tannins have the ability to bind feed particles that make them less digestible and results in lower digestibility of feed constitutes like protein, carbohydrate, starch, and cell wall (Mueller-Harvey, 2006). As a consequence, these poly phenols are possible to the lower animal performance. In some cases interaction between tannins and digestive enzymes such as trypsin, causing decrease in food utilization is probable. Gallotannin has been shown to produce hepatic necrosis in humans and grazing animals (Macáková *et al*. 2014).

Also, Feeding tannin could result in useful and beneficial effects. Better utilization of dietary protein, higher growth rates or wool, higher milk yields, improved fertility, and improved animal welfare and health through prevention of bloat and lower worm burdens are examples as well as environmental effects by reducing methane emission and nitrogen excretion (Mueller-Harvey, 2006). These effects refer to both types of tannin (condensed and hydrolysable), but some researchers believed in more beneficial effects of condensed tannin.

Figure 3 is a schematic graph for beneficial effects of condensed tannin in animal nutrition which is adopted from Mlambo and Mapiye (2015).

Table 1 Literature review of tannin effects on biohydrogenation (BH)

Reference	Tannin amount	Tannin source	*In vitro/in vivo*	Fat supplement	Results
Sivakumaran *et al.* (2004)	100, 200 and 300 mg/L	*Dorycnium rectum*	*In vitro*	-	Inhibition of the growth of *C. proteoclasticum* at concentrations of 100, 200 and 300 mg/L of *in vitro* medium; inhibiting the growth of *B. fibrisolvens* by low and medium molecular weight fractions of tannin at these concentrations; high molecular weight fraction stimulated the growth of *B. fibrisolvens* at the concentration of 100 mg/L
Durmic *et al.* (2008)	1 mg/mL	Extract from different plant species	*In vitro*	-	Plants selectively inhibited targeted ruminal bacteria involved in BH; some plant species could inhibit saturation of linoleic acid (LA), conjugated linoleic acids (CLA) and vaccenic acid (VA) *in vitro*
Benchaar and Chouinard (2009)	150 g/d	*Schinopsis balansae* (tree)	*In vivo*	-	Low potential of quebracho condensed tannin extract to alter ruminal BH and modify the fatty acid profile of milk fat at levels used in this study
Cabiddu *et al.* (2009)	Grazing	Sulla (*Hedysarum coronarium*)	*In vivo*	-	Tannin content of Sulla reduced ruminal microbial activity. Condensed tannins in this plant at flowering led to lower cis-9, trans-11 CLA and trans-11 C18:1 but also lower total trans FA, $\omega6/\omega3$ ratio and higher linoleic and linolenic acid in milk
Khiaosa-Ard *et al.* (2009)	7.9% of DM	*Acacia mearnsii*	*In vitro*	Linseed oil	Plant containing condensed tannin reduced the BH of α-linoleic acid 20% that might be resulted from reduction in organic matter degradability rather than inhibiting BH Higher concentrations of BH intermediates, cis-9, trans-11, cis-15 C18:3, trans-11, cis-15 C18:2, and trans-11 C18:1 in CT treatments.
Vasta *et al.* (2009)	0.6 and 1 mg/mL of BRF	*Ceratonia siliqua*, *Acacia cyanophylla*, *Schinopsis lorentzii*	*In vitro*	-	Reduction in amount of VFA and branched-chain fatty acids in the rumen; accumulation of VA and reduction in SA concentration; without effect on CLA isomers in BRF
Vasta *et al.* (2009)	8.93% on the DM	Quebracho (*Schinopsis lorentzii*) tannin	*In vivo*	-	Tannins supplementation reduced the biohydrogenation of the PUFA in the rumen; reduced the concentration of C18:0 and increased VA of the ruminal fluid; increase in concentration of PUFA and lower SFA in *Longissimus dorsi* muscle of lambs
Vasta *et al.* (2010)	9.57% of DM	Quebracho tannins	*In vivo*	-	Increased VA concentration in the rumen compared to the tannin-free diet without effecting the concentration of stearic acid; lower ratio of SA/VA; lower population of *B. proteoclasticus* in tannin groups than *B. fibrisolvens* and protozoa
Khiaosa-Ard *et al.* (2011)	Grazing	Alpine forage	*In vitro-in vivo*	-	Total phenols of forage of alpine were about twice compared to the lowland forages; alpines wards caused a change in the fatty acid BH process by enhancing VA in ruminal fluid *in vivo*, but not when incubated *in vitro*
Liu *et al.* (2011)	10 or 30 g of CT/kg of diet	Chestnut	*In vivo*	Coconut oil	Decreasing the concentration of total VFA in the rumen fluid; CO decreased counts of *Fibrobacter succinogenes*; no significant effect on *Ruminococcus flavefaciens*, or *Ruminococcus albus* population
Toral *et al.* (2011)	10g/kg DM	Quebracho condensed tannins and chestnut hydrolysable tannins	*In vivo*	Sunflower oil	No effect on concentrations of the major fatty acids classes in milk (i.e., saturates, MUFA and PUFA); very limited effects on the proportion of particular fatty acids, and was not able to enhance milk VA and rumenic acid (RA)
Rana *et al.* (2012)	1.06 g/kg and 3.18 g/kg of body weight	Terminalia chebula extract	*In vivo*	-	Increasing total MUFA and PUFA content in muscle; reducing SFA; improving the Δ^9-desaturase activity as desaturation index; enhancement of total CLA content (58.73%) in muscle
Kälber *et al.* (2013)	*Ad-libitum*	Buckwheat (*Fagopyrum esculentum*) and phacelia (*Phacelia tanacetifolia*, chicory (*Cychorium intybus*), ryegrass (*Lolium multiflorum*)	*In vivo*	-	No effects on levels of CLA in milk fat; VA in milk fat was slightly elevated with buckwheat and phacelia but decreased by chicory; This study could not confirm the hypothesis that mature forage dicotyledons enhance the recovery rates of alpha-linolenic acid (ALA) and LA in milk via increased concentrations of phenolic compounds.
Toral *et al.* (2013)	20g/Kg of DM	Extract of quebracho tannins	*In vivo*	Sunflower oil	Dietary tannins increased the milk concentration of some 18:1 and 18:2 isomers and decreased branched-chain FA. Significant differences between treatments in trans-11 18:1 and cis-9, trans-11 CLA were only observed on day three. Unable to modify milk fatty acid composition over long term
Ghaffari *et al.* (2014a)	12%	Pistachio by-products (PBP)	*In vivo*	-	No effects of pistachio by-products inclusion in the diet on the relative abundance of *Butyrivibrio fibrisolvens* and *Butyrivibrio proteoclasticus* (main involved bacteria in BH)
Ghaffari *et al.* (2014b)	30%	Pistachio by-products	*In vivo*	Fish oil	Capability of pistachio by-products to modify fatty acid profile of milk in dairy goat; higher concentrations of C16:0 and trans-C18:1 isomer in propolis-based products (PBP) treatments milk fat; no effects on the concentrations of saturated, monounsaturated, and PUFA in milk; higher amounts of trans fatty acids in PBP groups
Minieri *et al.* (2014)	49 g/kg DM	Quebracho tannins extract	*In vitro*	Soybean or linseed oil	Quebracho tannins may be an effective method for reducing in solid associated bacteria (SAB) the BH of LA and linolenic acid (LNA); no effect on rumen accumulation of cis-9, trans-11 18:2, and trans-11 18:1fatty acids
Rahimi *et al.* (2014)	30%	Pistachio hull	*In vivo*	-	Effect on ruminal BH in goats; higher concentrations of rumenic and vaccenic acid; increasing long-chain fatty acids (LCFA), PUFA, MUFA and decreasing saturated and short chain fatty acids
Sedighi-Vesagh *et al.* (2014)	32%	Pistachio by-products	*In vivo*	-	Change in the proportions of the milk fatty acids by PBP supplementing; decrease in 8:0 to 16:0 FA and increasing proportions of cis-9, trans-11 18:2 and trans-11 18:1, polyunsaturated and long-chain fatty acids; lower proportions of short, medium and saturated fatty acids

DM: dry matter.

Continued Table 1 Literature review of tannin effects on biohydrogenation (BH)

Reference	Tannin amount	Tannin source	*In vitro/in vivo*	Fat supplement	Results
Buccioni *et al*. (2015)	52.8 g/kg of DM	Chestnut and quebracho tannin extract	*In vivo*	Soybean oil	More efficiency of quebracho tannin in effecting rumen BH of PUFA, increasing concentrations of cis-9, trans-11 CLA and VA in milk fat; disturbance effect of tannin extracts on rumen BH, and on microbe metabolism which was achieved by the relative abundance of *B. fibrisolvens* and *B. proteoclasticus* populations and by VFA molar proportion results
Carreno *et al*. (2015)	20, 40, 60 and 80 g/kg diet DM	Chestnut, oak, quebracho and grape	*In vivo*	-	The oak tannin extract, at a dose of 20 g/kg DM, increased total PUFA, 18:3 n-3, 18:2 n-6 and trans-11 18:1, and decreased trans-10 18:1 and 18:0 without any negative effect on ruminal fermentation
Heidarian Miri *et al.* (2015)	1.27% and 2.53% of DMI	Cumin (*Cuminum cyminum*) seed extract (CES)	*In vivo*	-	No effect on molar proportion of VFA; increase in the ruminal concentration of RA and VA; higher linoleic acid and linolenic acid and lower SA amounts in CSE-fed goats; inhibition of BH By CES. Decrease in the growth of some main species of the rumen bacteria involved in BH but not *Butyrivibrio* sp
Ishlaka *et al.* (2015)	100 g/kg of DM	Quebracho condensed tannin extract	*In vitro*	-	Effect on BH; decreased concentration of C18:0; abundance of *Butyrivibrio* population DNA; increased VA concentration in the rumen fluid
Grosse Brinkhaus *et al.* (2016)	223, 30.3 g of CT/kg of DM	*Medicago Sativa* (AL), *Onobrychis viciifolia* (SF) and *Lotus corniculatus* (BT)	*In vivo*	-	No effect on milk fatty acid except greater proportion of 18:1n-9 with SF than with AL; tendency of 18:3 n-3 proportions to be higher with SF than BT but 20:4n-6 tended to be higher with AL and low with BT; the proportions of 22:6 n-3 and 22:5 n-3 were lowest with SF and highest with BT, and AL
Guerreiro *et al*. (2016)	6 mg	*Cistus ladanifer* (rockrose)	*In vitro*	-	CT was the most effective fraction of the plant in influencing rumen BH; increasing accumulation of trans-11-18:1, which was achieved not by inhibition of last step but by promoting first step of BH
Jafari *et al.* (2016)	0, 10, 15, 25%	Papaya leaf	*In vitro*	-	Significant decrease in BH rate of C18:2 n-6 (LA) and C18 PUFA; increase in production of rumen BH intermediates like CLA; decreasing saturated fatty acid in the rumen fluid after 24 h of incubation; increase in total bacteria and *Butyrivibro fibrisolvens* population
Shakeri *et al.* (2016)	-	Pistachio by-products	-	-	Reducing ruminal VFA; abundance total ruminal bacteria; a useful strategy for increasing CLA in dairy goat milk
Szczechowiak *et al.* (2016)	4.5 g/kg of DM 4.83 g/kg DM of total diet	*Vaccinium vitis idaea*	*In vitro-in vivo*	Fish-soybean oils blend	the potential of the MIX treatment to modulate fatty acids levels in both *in vitro* and *in vivo* experiments on VA accumulation in the rumen; without effect on milk UFA, especially C18:2 cis-9- trans-11concentration; unable to effect BH
Toral *et al.* (2013)	3.5%	Alfalfa and sainfoin	*In vitro*	Sunflower oil	Ability of two tannin-containing legumes to inhibit ruminal BH; no effects on accumulation of cis-9 trans-11 conjugated linoleic acid; greater concentrations of 18:2n-6, *cis*-9 18:1 and total PUFA in sainfoin hay cultures; variations in trans-11 18:1 proportions
Alves *et al*. (2017)	50, 200 g/kg DM	*Cistus ladanifer*	*In vivo*	Soybean:linseed oils blend	Combining high levels of *C. ladanifer,* as source of condensed tannins, and vegetable oils in the diet of lambs affects the BH of C18 fatty acids, mainly by inhibiting the completeness of BH and consequently increasing the accumulation of C18 BH intermediates in rumen, abomasum and plasma

DM: dry matter and DMI: dry matter intake.

Generally, the detrimental effects of tannin are mostly dose related and they depend on different factors like animal breed, plant species, and management as well production condition. Controlling these factors will help in the possibility of reducing harmful or negative effects of tannins in animals. For example, Krueger *et al.* (2010) mentioned that supplementation of condensed and hydrolysable tannins at low doses do not bring negative effects on animal performance or economical traits. The performance of small ruminants fed trees and shrubs is indicated to depend on animal and plant species and the breed (Papanastasis *et al.* 2008).

It can be concluded that the negative effects of tannins in ruminant are not highlighted, because these animals are not much sensitive to this phenolic compound.

Moreover the positive effects of tannins are greater than the harmful effects. Finally, it needs to mention that under the controlled conditions and by considering effective factors such as plant species, animal breed, type of tannin and suitable dosage; tannins are candidate to be used as a supplement in ruminant nutrition.

Effects of tannins on ruminal biohydrogenation

Despite toxic and detriment effects of tannins on ruminant animal's performance, it is now recognized that these phenolic compounds can be beneficial depending on type and chemical structure, consumption amount and animal species (Makkar, 2003; Mueller-Harvey, 2006). While some *in vitro* experiments such as Khiaosa-Ard *et al.* (2009); Vasta *et al.* (2009) and Carreno *et al.* (2015) showed positive effects of tannin on rumen VA accumulation, results of *in vivo* studies based on the tannin nature and source, dosage and other factors, seems to be inconsistent. Some *in vivo* studies suggest no significant or even negative effects (Benchaar and Chouinard, 2009; Vasta *et al.* 2009; Toral *et al.* 2011; Toral *et al.* 2013). In contrast, some others proved the positive effects of tannin (Cabiddu *et al.* 2009; Vasta *et al.* 2010; Buccioni *et al.* 2015; Carreno *et al.* 2015).

Sivakumaran *et al.* (2004) demonstrated that all three fractions (i.e. low, medium and high molecular weight) of proanthocyanidins from *Dorycnium rectum* forage at different concentrations (of 100, 200 and 300 mg L^{-1}) inhibited the growth of *C. proteoclasticum* at *in vitro* medium.

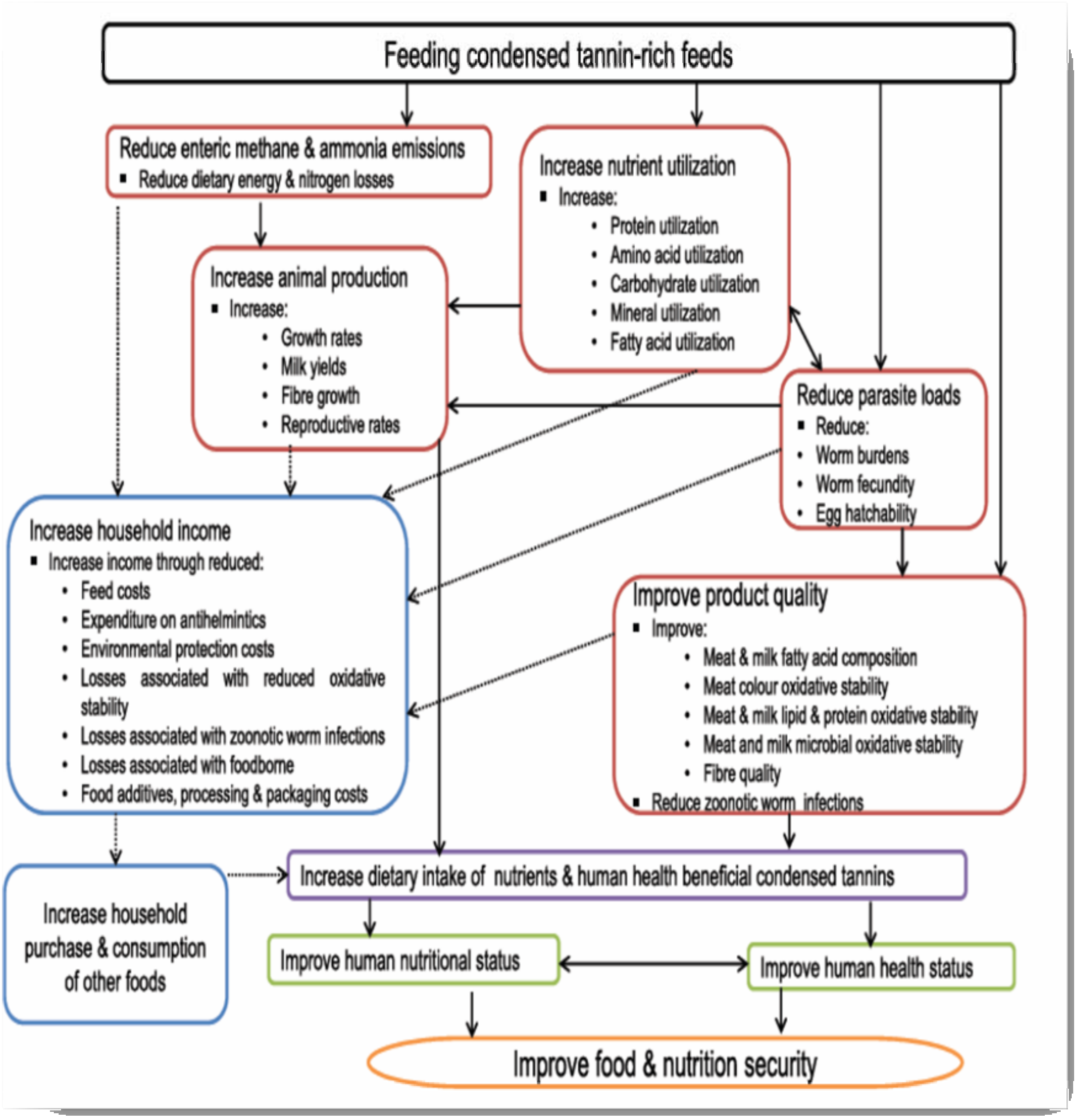


Figure 3 A summary of potential benefits of feeding condensed tannins to ruminant animals
Adapted from (Mlambo and Mapiye, 2015)

Low and medium molecular weight fractions inhibited the growth of *Butyrivibrio fibrisolvens* at all three studied concentrations, but the higher molecular fraction stimulated the growth of this bacterium at the concentration of 100 mg L^{-1}. Kronberg *et al.* (2007) reported that quebracho CT (200 g kg^{-1} of flaxseed) reduced BH of C18:3 in flaxseed in an *in vitro* batch culture. However, feeding tannin-treated flaxseed to beef cattle in the same study did not increase the concentrations of C18:3 and 18:5 in plasma neutral lipids.

Durmic *et al.* (2008) studied several plants for their ability to modify ruminal BH. In this study, extracts of 37 plants inhibited the growth of *C. proteoclasticum*, among them 10 plant did not affect the growth of *B. fibrosolvens*. The active components of these plants have not been reported.

Cabiddu *et al.* (2009) conducted a study to evaluate the effect of poly ethylene glycol (PEG) supplementation on the fatty acid composition of milk from Sarda sheep that were grazing Sulla (a fodder contains tannin). The results of their study showed that odd branched chain fatty acids were higher in PEG than the control group and this confirms the hypothesis that tannin in Sulla inhibited ruminal microbial activity.

Both linoleic (C18:2) and linolenic (C18:3) long chain fatty acids were lower in the milk of PEG treatment than the control group, so they concluded that feeding condensed tannins in Sulla at flowering stage resulted in lower c-9, t-11 CLA and t-11 C18:1, lower total trans fatty acids, ω6/ω3 ratio and higher linoleic and linolenic acid concentration in milk.

Khiaosa-Ard *et al.* (2009) reported that addition of CT (78.9 g kg^{-1} of DM) inhibited the last step of linolenic acid BH. This inhibition resulted in accumulation of trans-11 C18:1 in feed residues. But it was unable to have a significant effect on the reduction of C18:3 compared with the control treatment. In a study in Vasta *et al.* (2010) evaluated the effects of tannins on ruminal BH in sheep. Their results showed that tannins increased VA in the rumen, but this treatment didn't affect the concentration of stearic acid, and the SA/VA ratio was significantly lower in the tannin-fed lambs than the control group. Their results suggested that the last step of the BH was inhibited by tannins. The *B. proteoclasticus* population was lower, and *B. fibrisolvens* and protozoan populations were higher in the rumen of lambs fed the tannin than the control group. Their results suggest that quebracho tannins affected BH by changing microbial population in the rumen.

Buccioni *et al.* (2015) studied milk fatty acid composition, rumen microbial population, and animal performances in diets rich in linoleic acid and supplemented with tannins.

This study included using two different sources of tannin and based on the results the authors suggested that condensed and hydrolysable tannins have differential effects on rumen microbes, therefore, the effect of different types of tannin on rumen bacteria and BH were inconsistent. Finally, they concluded that the use of fatty acid source and a practical dose of tannin extract in the diet of dairy ewes can be an efficient strategy to improve the nutritional quality of milk.

Recently, Carreno *et al.* (2015) conducted an *in vitro* study to analyze the effect of four commercial extracts of tannins (from chestnut, oak, quebracho and grape) at four doses (20, 40, 60 and 80 g/kg diet DM) to select the best treatment in modulating BH of unsaturated fatty acids. They performed two *in vitro* experiments on rumen microorganisms. Finally, they noted that the four examined tannin extracts were able to modulate the *in vitro* BH of unsaturated fatty acids. Furthermore, the oak tannin extract, 20 g/kg diet DM, increased total PUFA, 18:3n-3, 18:2n-6 and trans-11 18:1, and decreased trans-10 18:1 and 18:0 rumen concentrations without negative effects on ruminal fermentation.

In contrast to the above, some studies have been reported none or negative effect of tannin on BH.

Vasta *et al.* (2009) also reported that the concentration of trans-11 C18:1 increased while the concentration of total CLA did not increase in an *in vitro* ruminal fluid.

Benchar *et al.* (2009) in studying the effects of plant secondary metabolites cinnamaldehyde, saponins and condensed tannin on milk fatty acid profile of dairy cows, reported that feeding cinnamaldehyde or condensed tannin didn't have a significant effect on milk fatty acid profile.

Their results showed low potential of cinnamaldehyde, condensed tannins, and saponins to affect ruminal BH and modify the fatty acid profile of milk fat.

Toral *et al.* (2011) studied tannins effects in dairy ewes fed a diet containing sunflower oil. They used tannin as a feed additive to evaluate its effects on modulating ruminal BH, effects on animal performance, milk fatty acid composition and ruminal fermentation. These researchers reported that the addition of the tannins extract to a sunflower oil containing diet did not affect the concentrations of the major fatty acids in milk (i.e., saturated, monounsaturated, and polyunsaturated), had very limited effects on the proportion of some particular fatty acids, and couldn't enhance milk VA and RA content. In conclusion, they mentioned that the addition of a commercial mixture of condensed and hydrolysable tannin extracts to a diet containing sunflower oil had no effect on ruminal fermentation and animal performance, or even an important impact on milk fatty acid profile in lactating ewes.

Later, Toral *et al.* (2013) investigated the effect of the adding quebracho tannins in a diet rich in linoleic acid on ewe performance and milk fatty acid composition. Their results showed that dietary tannins increased concentration of several 18:1 and 18:2 isomers and decreased that of branched-chain fatty acids in milk. Overall, the addition of quebracho tannins to a diet rich in linoleic acid did not alter milk fatty acid composition, especially over the long term. They recommended further researches to investigate other types of tannins, at practical doses, in modulating dietary PUFA during ruminal BH.

CONCLUSION

Conjugated linoleic acid is considered as a healthy, beneficial fatty acid in human nutrition for a long time. The main source of these fatty acids is ruminant products such as milk and meat. In these animals it is produced at the end of the first step in the ruminal BH process. Therefore, many efforts have been carried out to interfere the second step and increase the levels of produced CLA. Several feed additives have been recommended to increase the content of CLA in milk fat. Plant metabolites such as tannin are considered to be one of these additives. Tannins are well known for their beneficial effects on animal performance and production. The recent researches have been focused on effects of tannin on ruminal BH. The main approach of tannin to interfere BH is via antimicrobial activities. According to the results of different studies tannin seems to be a beneficial mean to reduce the second step of BH through inhibiting the bacteria involved in hydrogenation of VA to stearic acid, increasing VA accumulation to increase CLA levels.

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