Effects of Tannins on Conjugated Linoleic Acids (CLA) Accumulation in Ruminant Products

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Summary

Conjugated linoleic acid (CLA) is considered to be of high importance for human health that exerts important physiological effects, including anticarcinogenic effects, prevention of cholesterol-induced atherosclerosis, enhancement of the immune response, reduction in fat accumulation in body, ability to enhance growth promotion, antidiabetic effects and improvement in bone mineralization. CLA requirement for human should more than 400 mg/d for good health. The predominant CLA in ruminant fats is the *cis*-9, *trans*-11 CLA isomer, which accounts for more than 80% of total CLA isomers in dairy products and 75% of those in beef fats. The presence of CLA in ruminant products relates to the biohydrogenation of unsaturated fatty acids by rumen bacteria. The CLA concentrations in ruminant products can be increased by nutritional and management practices that facilitate higher fore-stomach output of CLAs and vaccenic acid (*trans*-11 C18 : 1) for absorption and incorporated into animal tissues. The ability of plant extracts including tannins to modify the fatty acid composition of ruminant products has received great attention recently. There is very limited information pertaining to the effects of tannins on ruminal biohydrogenation process and CLA content in milk and meat. The condensed tannins have been shown to inhibit the growth of many ruminal bacteria including bacteria associated with ruminal biohydrogenation. It has been reported that the terminal step of ruminal biohydrogenation process was inhibited by condensed tannins, thus leading to an accumulation of vaccenic acid in ruminants's tissue. This selective activity of tannins to rumen bacteria could be beneficial nutritionally by altering the ruminal biohydrogenation process and hence enhancing CLA content of ruminant-derived products.

Keywords: condensed tannin, Conjugated linoleic acid, biohydrogenation, Meat CLA, Milk CLA

Introduction

In recent decades milk and dairy products have often been claimed to have detrimental effects on human health, because their consumption has been associated with high levels of coronary heart disease (CHD) (Cabiddu, et al., 2009). In general, food products from ruminant are high in saturated fatty acids (SFA) and low in polyunsaturated fatty acids (PUFA) (Tanaka, 2005) because of the extensive fatty acid biohydrogenation carried out by the rumen microbial population (Morimoto, Van Eenennaam, DePeters, & Medrano, 2005). Normally, the consumption of SFA has been associated with an increased serum low-density lipoprotein (LDL) cholesterol level, which is a risk factor for CHD (Tanaka, 2005). This has been explained by the connection between the intake of PUFA and the risk of CHD. Due to increased consumer awareness of the link between diet and health, recent research has focused on altering the fatty acid composition of cow's milk (Benchaar, & Chouinard, 2009) or sheep's milk (Cabiddu, et al., 2009) or beef (Chullanandana, 2007). Although, the fatty acid composition of milk and beef are highly resistant to manipulation because of ruminal biohydrogenation of dairy PUFA (Tanaka, 2005). Most of dominant PUFA from milk and dairy products is conjugated linoleic acid (CLA), which is believed to have anti-carcinogenic, anti-atherogenic, inhibit lipogenesis, anti-diabetic, bone density loss and immune function modulating properties (McGuire, & McGuire, 2000).

CLA is unique among the naturally occurring anticarcinogens and presents in foods from ruminant animals. On the other hand there is little doubt that maximizing omega-3 fatty acid in ruminant production benefits human nutrition and health (Morimoto, et al., 2005). reported that CLA requirement for human should more than 400 mg/d for good health, but in the present time human receives, on the average, less than 200 mg CLA/d. In addition, increasing the proportion of health fatty acids in ruminant products is interesting, mainly milk and meat because they are major dietary sources of CLA for human (Chullanandana, 2007). Thus, increasing CLA content or the proportion of PUFA in milk or ruminant products could have significant human health benefits and enhance the consumption of dairy products.

The plant secondary compounds are known to have pronounced and significantly differing effects on ruminal microbes. Two main groups of plant secondary compounds frequently found to affect rumen microbes and ruminal fermentation are saponins and tannins. Condensed tannins (CT) have been reported to modify ruminal biohydrogenation process (Khiaosa-ard, et al., 2009). The aim of this review is to investigate the potential of condensed tannins to increase the output of CLA and vaccenic acid from the rumen, and to enhance the concentrations of these unsaturated fatty acids in ruminant-derived food products.

What are Tannins?

Tannins are found in many plants such as forages crops or forages, cereals, grains, legumes, shrubs and trees, browse leave and fruits. Plant develops condensed tannins (CT) as protection against microorganism attacks such as fungi and against insect, and also apparent to ruminant as bitterness which affects palatability of the forage to animals (Muir, 2011). General effects of tannins, for example, decrease in *in vivo* nutrient utilization and in particular protein utilization, decrease in growth, decrease in palatability and feed intake (Toral, Hervas, Bichi, Belenguer, & Frutos, 2011) or decrease in various enzyme activities, etc. Since tannins are considered as "antinutritional factor" effort in the past was to improve their nutritional value and to reduce the negative effect on animal performance. Some of feeds (plants tannin-rich, such as species of *Acacia*, *Dichrostachys*, *Dorycnium*, *Hedysarum*, *Leucaena*, *Lotus*, *Onobrychis*, *Populus*, *Rumex* and *salix*) can produce useful benefits in ruminants, such as better utilization of dietary protein, faster growth rates of liveweight or wool, higher milk yields, increased fertility, and improved animal welfare and health through prevention of bloat and lower worm burdens (Mueller-Harvey, 2006; Min, et al., 2003) In addition, tannins are considered to both adverse and beneficial effects depending on their concentration, types of tannin and nature besides other factors such as animal species, physiological state of the animal and composition of the diet (Poungchompu, 2008; Min, Barry, Attwood, & McNabb, 2003). Mueller-Harvey, (2006) demonstrated that dietary concentrations of <50 g CTs kg^{-1} are beneficial. While, high forage CT concentrations (>55 g CT/kg DM) normally reduce voluntary feed intake and digestibilities, and depress rates of body and wool growth in grazing ruminants (Min, et al., 2003). Attempts have been made to deactivate tannins with the use of polyethylene glycol (PEG), a non-nutritive synthetic polymer which has a greater binding affinity than proteins, makes tannins inert by forming tannin-PEG complexes (Cabiddu, et al., 2009).

The tannins (4% Hydrolysable tannin, HT on a DM) positively affected the silage quality by reducing proteolysis in silage and could improve protein utilization, with a slight depression in organic matter digestibility (Tabacco, et al., 2006). Min, et al. (2003) reported that forage legumes contain diverse types and amounts of CT, which may modify the digestibility of dietary protein and structural carbohydrates in animal feeds. The positive effect of forage CT on Gastro-intestinal tract (GIT) parasite infestation, including gastro-intestinal nematode (GIN) and bacterial suppression in goats, has been widely documented (Muir, 2011; Alonso-Diaz, et al., 2010).

Chemistry and Structure of Tannins

Tannins are polyphenolic substances with various molecular weights and variable complexity. These are chemically not well-defined substance but rather a group of substance with the ability to bind protein in aqueous solution. Their multiple phenolic hydroxyl groups lead to the formation of capacity of complexes mainly with proteins, and to a lesser extent with metal ions, amino acid and carbohydrates (Patra, & Saxena, 2011). Based on their structure and chemical properties, tannins are divided into two groups (Bhatta, et al., 2009; Patra, & Saxena, 2011) hydrolysable tannins (HTs) and condensed tannins (CTs).

The HTs are complex molecules with a polyol as a central carbohydrate core, such as glucose, glycitol, quinic acids, quercitol and shikimic acid, which is partially or totally number of phenolic carboxylic acids are bound by esters of gallic acid (gallotannin) or ellagic acid (ellagitannins) (Figure 1 and 3). Hydrolysable tannins are susceptible to hydrolysis by acids, bases or esterase yielding polyol and the constituent phenolic acids.

The CTs or proanthocyanidins, are mainly polymers of the flavan-3-ol (epi) catechin and (epi) gallocatechin units (Figure 1 and 2) which are linked by C4-C8 and C4-C6 interflavonoid linkages. The CTs are degraded to form monomeric anthocyanidins (cyanidins and delphinidins) pigment (Figure 1) upon treatment with acid butanol reaction. CTs are secondary plant compounds that are stored in the vacuoles of plant cells and in tanniferous forages which are present as unbound or bound to protein or fiber. The CTs can react by hydrogen bonding with plant protein to from stable and insoluble fraction. Tannin-protein complexes are stable between pH 3.5-7.0 and dissociate below pH 3.0, and that ruminal pH values of approximately pH 6.5 are optimal for complexing of CT and fraction 1 leaf protein (Cabiddu, et al., 2009).

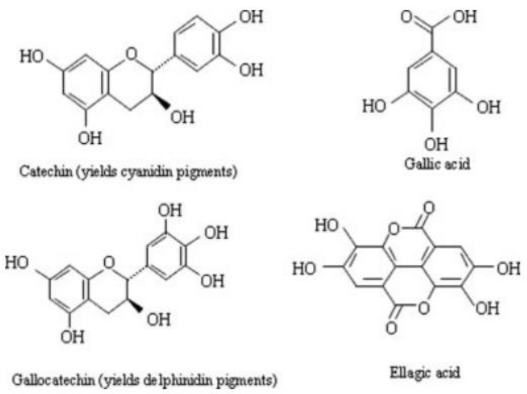

Figure 1 Monomeric units of condensed (catechin and gallcatechin) and hydrolysable tannins (gallic and ellagic acid) (Patra, & Saxena, 2011)

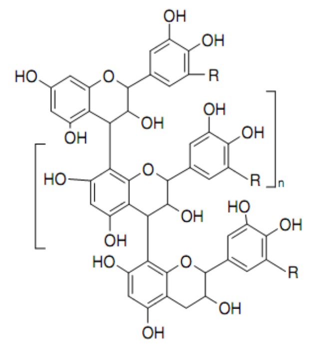

Figure 2 Structures of condensed tannins from fodder legumes (Mueller-Harvey, 2006).

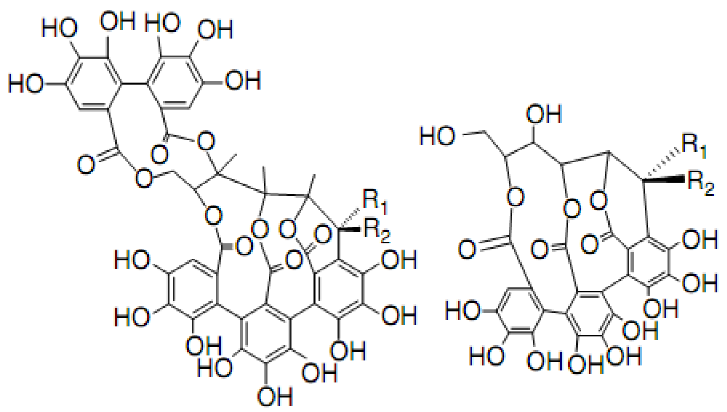

Figure 3 Structures of hydrolysable tannins (ellagitannins) from chestnut wood (Mueller-Harvey, 2006).

Effect of Tannins on Rumen Fermentation

Tannins are generally regarded as antinutritional substance, certain tannins at low concentrations alter ruminal fermentation and microbial protein synthesis. Tannins also reduce ruminal CH4 production by shifted microbial populations toward bacteria at cost of protozoa, when included either as temperate legume or as purified tannins extracts (Khiaosa-Ard, et al., 2009). Tannin sources containing both HT and CT were more potent in suppressing methanogenesis than those containing only HT (Bhatta, et al., 2009).

Min, et al. (2003) suggested that low concentrations of CT (20-45 g CT/kg DM) reduce rumen forage protein degradation due to reversible binding to these proteins and to reducing the populations of proteolytic rumen bacteria. The effects of CT on the growth of rumen bacteria and on microbial proteolysis have been recently described by Min, et al. (2005), who clearly showed the reduction of the rate of proteolysis and the inhibition of the growth of proteolytic rumen microorganisms. Patra, & Saxena, (2011) has been confirmed that besides the formation of tannin-protein complexes, reduction in proteolysis might be attributed to direct effects of CTs on microbial proteolytic enzyme activity or to indirect effects on rumen metabolite concentration that can regulate proteolytic activity in some bacteria. In agreement with Cabiddu, et al. (2009) who found that tannins in forage legume reduced ruminal microbial activity, as shown in Figure 4 Fiber and crude protein degradations were reduced and ammonia concentration in incubated fluid-feed containing tannin was lowered (Khiaosa-Ard, et al., 2009). In addition, Bhatta, et al. (2009) found that tannins increased in vitro pH, reduced total volatile fatty acids (VFA) and increased propionate concentrations. However, quebracho CT extracted (150 g/d) exerted negligible effects on digestion, rumen microbial fermentation and protozoa numbers (Benchaar, et al., 2008).

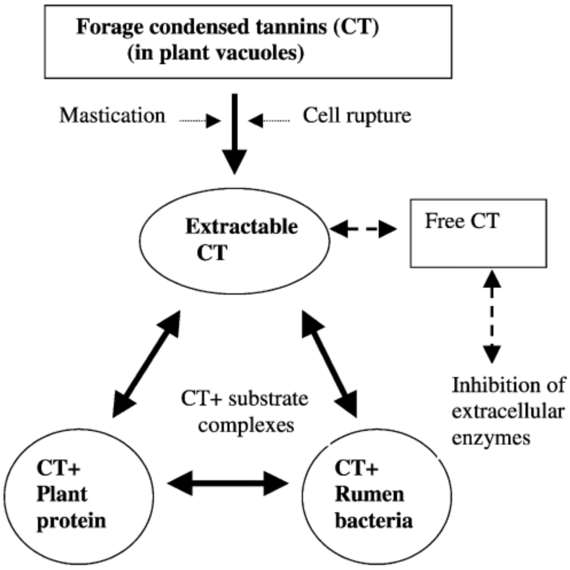

Figure 4 Proposed effect of condensed tannins (CT) on rumen bacteria and plant protein in the rumen and their interactions (Min, et al., 2003).

Effect of Tannins on Ruminant Nutrition and Performance

Tannins have negative effects on ruminant nutrition because they reduce voluntary feed intake (VFI), protein and structural carbohydrate digestibility, and intestinal enzyme activity or they cause illness (Poungchompu, 2008). Reduction in diet digestion is attributed to the formation of stable complexes between CT and protein or/and carbohydrates, even though the great diversity of tannin in nature means that it is difficult to generalize about their effects on rumen microbes. It has been reported that inhibition increases with increasing the degree of tannin polymerization. The Schematic representation of the effects of tannins on ruminal metabolism and ruminant performance are show in Figure 5

The detrimental effect of tannin or tropical tannin-rich plant on animal production have been described, in contrast, their potential benefits have long been neglected. In addition, CT in several forage plants have been shown to offer advantages for ruminants, and have resulted in increased milk production, wool growth, ovulation rate, and lambing percentage, as well as reduced bloat risk and internal parasite burdens (Min, et al., 2003). This is possibly related to action of the CT in increasing essential amino acids (EAA) absorption from the small intestine; in the case of internal parasites their inactivation by CT is also involved. Alonso-Diaz, Torres-Acosta, Sandoval-Castro, & Hoste, 2010. pointed out that tropical tannin-rich plants have a positive effects on small ruminants particularly goats either as source of feed or as nutraceuticals with anthelmintic properties.

The low dose of CT and HT at 10 g/kg diet DM had no effect on ruminal fermentation and animal performance (Toral, et al., 2011). Krueger et al. (2010) found that CT and HT had no effects on animal performance, rumen fermentation or carcass and non-carcass traits in finishing beef steers with supplementing 14.9 g/ kg DM of commercial tannins extract, however DMI was immediately reduced upon supplementing CT and HT at 20 g/ kg diet DM. Similarly, reported by Min, et al. (2003), high forage CT concentrations (>55 g CT/kg DM) generally reduce voluntary feed intake and digestibilities, and depress rates of body and wool growth in grazing ruminants. Milk production and composition did not changed with supplementing of CT at 150g/d in dairy cows (Benchaar, et al., 2008), but milk fat was high in dairy sheep grazed tannin forage lugumes (Molle, et al., 2009).

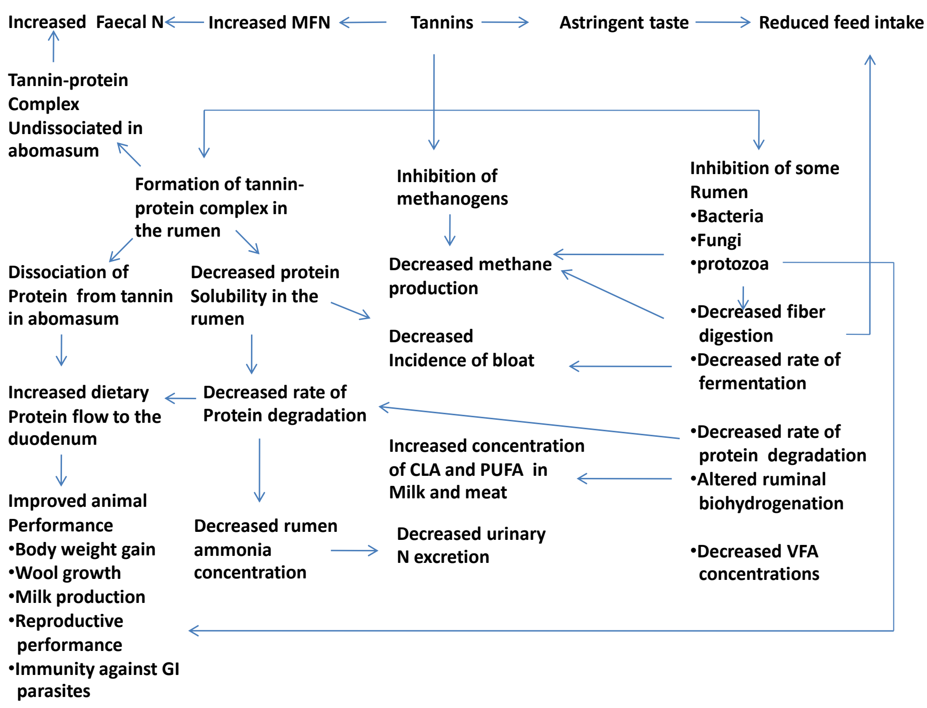

Figure 5 Shematic representation of the effects of tannins on ruminal metabolism and ruminant performance (Patra, & Saxena, 2009).

Effect of Tannins on Ruminal Biohydrogenation

CT extract diet led to a different FA profile in the incubation fluid effluent and was found effective in modifying ruminal biohydrogenation. Khiaosa-Ard, et al. (2009) observed that incubation fluid effluent and feed residues of feed supplemented with CT extract (7.9% of DM) showed a considerable proportion of the 3 biohydrogenation intermediates, *cis*-9, *trans*-11, *cis*-15, C18:3, *trans*-11, *cis*-15 C18:2 and *trans*-11 C18:1, which did not occur in the initial feeds, and was a distinct point of action, namely the inhibition of the terminal step, thus leading to an accumulation of *trans*-11 C18:1 in incubation fluid effluent (Figure 6). The incubation with ruminal fluid of alpine forage (1.8 CT g/kg DM) increased vaccenic acid in the incubation fluid effluent, and indicated that alpine forage may have an inhibitory effects on the last step of ruminal biohydrogenation (Khiaosa-Ard, et al., 2011). It has been possibly achieved by suppressing protozoa and enhancing the bacterial population, thus removing potential microbes involved in biohydrogenation and increasing competition between bacteria involved in biohydrogenation and others.

Patra, & Saxena (2005). Reported that many ruminal bacteria species of the genera *Butyrivibrio*, *Ruminococcus*, *Treponema-Borrelia*, *Micrococcus*, *Megasphaera*, *Eubacterium*, *Fusocillus* and *Clostridium* are known to be associated in ruminal biohydrogenation. *Butyrivibrio* spp. are most active species among the group A bacteria, which form CLA from linoleic acid, while few species of bacteria such as *Fusocillus* spp. and *Clostridium proteoclasticum* (group B) convert vaccenic acid to stearic acid. Therefore, it has been suggested that selective inhibition of group B bacteria without affecting group A bacteria may provide more vaccenic acids and CLAs (show in Figure 6). There is very limited information concerning to the effects of tannins on ruminal biohydrogenation process and CLA content in meat and milk.

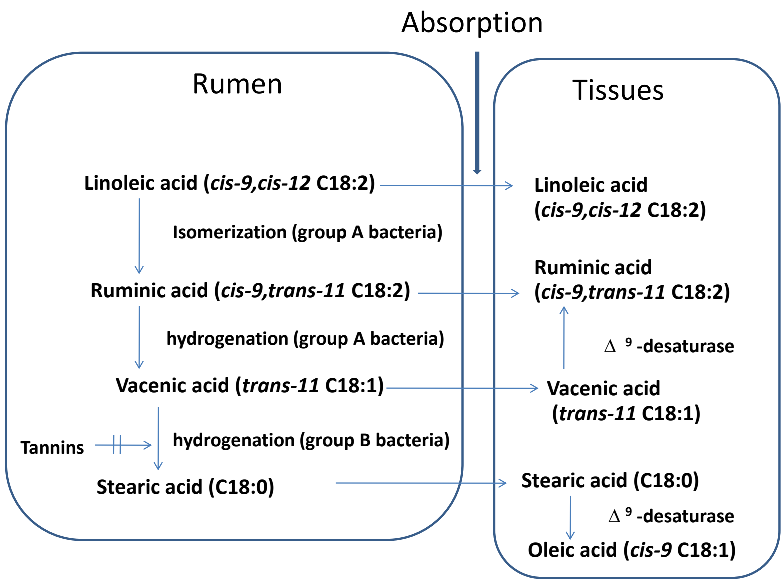

Figure 6 Biohydrogenation process in the rumen and tissues. Specific inhibition of group B bacteria by tannins may increase concentrations of polyunsaturated fatty acids and conjugated linoleic acid in tissues. (modified from Patra, & Saxena, 2011).

The plant extracts including CTs have been shown to inhibit the growth of many ruminal bacteria including bacteria associated with ruminal biohydrogenation (Patra, & Saxena, 2005; Khiaosa-Ard, et al., 2011). From the study of Durmic, et al. (2008) demonstrated that 37 plants extracts inhibited the growth of *C. proteoclasticum*, of which 10 did not affect the growth of *Butyrivibrio fibrosolvens*. Vasta, et al. (2010) found that quebracho tannin (9.6% of DM intake) in the diet for sheep increased the population of *B. fibrosolvens*, while the growth of *C. proteoclasticum* reduced. This selective activity of tannins to rumen bacteria could be beneficial nutritionally by altering the ruminal biohydrogenation process and hence enhancing CLA content of ruminant-derived products.

Conjugated Linoleic acid (CLA) in Ruminant Products

Cis-9 *trans*-11 C18:2 CLA is the predominant isomers, representing 75-90% of the total CLA in ruminant fat, *trans*-7, *cis*-9 CLA is the second most prevalent isomer at 3-16% of the total CLA (Tanaka, 2005). The name of *cis*-9 *trans*-11 CLA isomer is rumenic acid because of its unique relationship to ruminants (Kramer, et al., 1998). CLA is mixture of geometric and positional isomers of linoleic acid with conjugated double bonds. CLA in ruminant fat are intermediates in the biohydrogenation of polyunsaturated fatty acids (PUFA) by ruminal microorganisms (Toral, et al., 2011). The originate CLA from absorption after escaping incompleted biohydrogenation in the rumen (route 1 from Figure 8) and the major source is endogenous synthesis of CLA from *trans*-11 C18:1 vacenic acid (VA) by desaturation in tissue (route 2 from Figure 8). Conversion of FA to *cis*-9 *trans*-11 C18:2 CLA by the Δ^9-desaturase in mammary gland and body tissue has been reported to be a major source of *cis*-9 *trans*-11 C18:2 CLA in ruminant products rather than the direct result of absorption of *cis*-9 *trans*-11 C18:2 CLA escaping from the rumen (Khiaosa-Ard, et al., 2009).

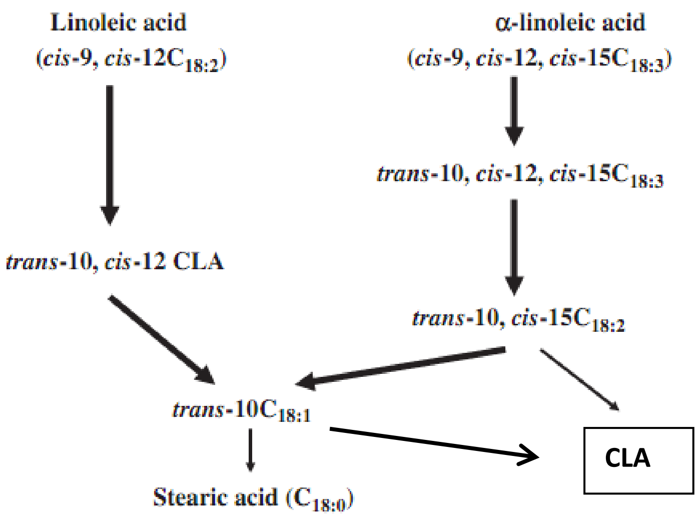

Figure 7 Pathways of ruminal hydrogenation of linoleic and α- linolenic acid (Tanaka, 2005)

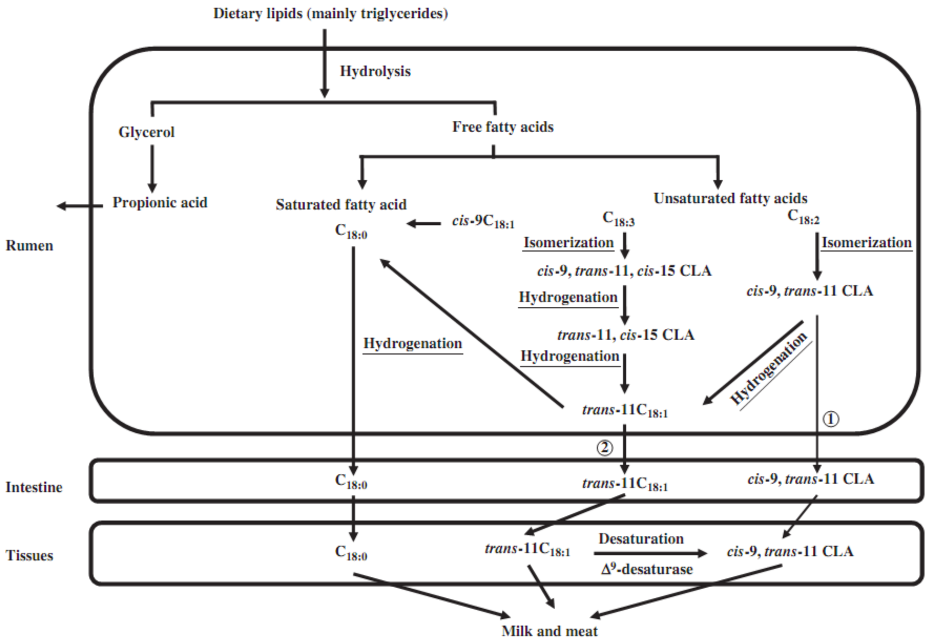

Figure 8 Lipid metabolism in the rumen and the origins of conjugated linoleic acid in ruminant products (Tanaka, 2005).

Effect Tannins on Milk Fatty Acids Profile

Cis-9 *trans*-11 18:2, rumenic acid (RA), is the major isomer of CLA in ruminant derived food products and is considered to be the principal component of potential human health promoting effects that have been attributed to CLA. The main source of RA is endogenous synthesis, via desaturation of *trans*-11 18:1, vaccenic acid (VA), in the mammary gland or other body tissue (Toral, et al., 2011). The presence of CLA in the milk and adipose tissue (body fat) from ruminants relates to the biohydrogenation of PUFA by rumen bacteria. Ruminant fat contains numerous isomers of CLA. *Cis*-9, *trans*-11 CLA comprises 75-90% of total CLA, and although it is an intermediate in ruminal biohydrogentation of linoleic acid, its major source is endogenous synthesis involving Δ^9-desaturase with *trans*-11 C18:1 produced in the rumen as the substrate. Thus, the keys to increase the CLA content of ruminant fat are to increase rumen output of *trans*-11 C18:1 and tissue activity of Δ^9-desaturase (Tanaka, 2005) (show in Fig.7 and 8). The elevation of VA as the precursor of CLA formed in body tissue and mammary gland is probably favorable from a human health point of view.

In *in vivo* studies, addition of quebracho tannins in the diet of sheep resulted in increased concentrations of *trans*-11 C18 : 1 in the rumen (Vasta, et al., 2010; Vasta, et al., 2009), and *cis*-9, *trans*-11 C18 : 2 and poly unsaturated fatty acids in lamb meat (Vasta, et al., 2009). Cabiddu, et al. (2009) found higher linoleic and linolenic acid in milk fat when fed dairy sheep with sulla at flowering (condensed tannins). Whereas, Benchaar, & Chouinard, (2009) reported low potential of condensed tannin from quebracho trees (150 g/d) to alter ruminal biohydrogenation process and modification of the fatty acids profile of milk fat at the feeding rates. Similarly, addition of the extract of CT and HT at 10 g of tannin/kg DM was not able to enhance milk fatty acid profile

Conclusions

Tannins are considered to both adverse and beneficial effects depending on their concentration, types of tannin and nature besides other factors such as animal species, physiological state of the animal and composition of the diet. Moderate concentrations of tannins (depending upon the type of tannins) in the diet improve body weight and wool growth, milk yields and reproductive performance, and increase the efficiency of protein digestion and thus improve animal health. The condensed tannins have been shown to inhibit the growth of many ruminal bacteria including bacteria associated with ruminal biohydrogenation, especially terminal step of biohydrogenation. This selective activity of tannins to rumen bacteria could be beneficial nutritionally by altering the ruminal biohydrogenation process and hence enhancing CLA content of ruminant-derived products (meat and milk). However, not all types of tannins produce beneficial nutritional and animal performance. The structure of tannins may influence physiological effects such as nitrogen metabolism, rumen fermentation, rumen microbial populations, intake and performance of ruminants, which has not been studied well in ruminant nutrition. Moreover, mechanism of CT effects on ruminal biohydrogenation are not understanding well. Further studies are necessary to determine effects of varying CT concentrations and types on ruminal biohydrogenation as well as to identify the bacterial species, CT-tolerant bacteria species, and their involvement and their interrelation with other species involved in biohydrogenation.

References

Alonso-Diaz, M. A., Torres-Acosta, J. F. J., Sandoval-Castro, C. A., & Hoste, H. (2010). Tannins in tropical tree fodders fed to small ruminants: A friendly foe?. *Small Rum. Res.*, 89, 164-173.

Benchaar, C., & Chouiard, P.Y. (2009). Short communication: Assessment of the potential of cinnamaldehyde, condensed tannins, and saponins to modify milk fatty acid composition of dairy cows. *J. Dairy Sci.*, 92, 3392-3396.

Bhatta, Uyeno, R. Y., Tajima, K., Takenka, A., Yabumoto, Y., Nonaka, I., Enishi, O., & Kurihara, M. (2009). Difference in the nature of tannins on in vitro ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. *J. Dairy Sci.*, 92, 5512-5522.

Cabiddu, A., Molle, G., Decandia, M., Spada, S., Fiori, M., Piredda G., & Addis., M. (2009). Responses to condensed tannins of flowering sulla (*Hedysarum coronarium* L.) grazed by dairy sheep Part 2: Effects on milk fatty acid profile. Livest. *Sci.*, 123, 230-240.

Chullanandana, K. (2007). *Accumulation of conjugated linoleic acid in beef and cows milk through supplementation of soybean oil, whole cottonseed or rumen protected conjugated linoleic acid.* Doctoral dissertation, Suranaree University of Technology, Thailand.

Durmic, Z., McSweeney, C. S., Kemp, G. W., Hutton, P., Wallace, R. J., & Vercoe, P. E. (2008). Australian plants with potential to inhibit bacteria and processes involved in ruminal biohydrogenation of fatty acids. *Anim. Feed Sci. Technol.*, 145, 271-284.

Galyean, M. (1989). *Laboratory procedure in animal nutrition research.* New Mexico States University: USA.

Khiaosa-ard, R., Soliva, C. R., Kreuzer, M., & Leiber. (2011). Influence of alpine forage either employed as donor cow's feed or as incubation substrate on in vitro ruminal fatty acid biohydrogenation. *Livest. Sci.*, 140, 80-87.

Khiaosa-Ard, R., Bryner, S. F., Scheeder, M. R. L., Wettstein, H. R., Leiber, F., Kreuzer M., & Soliva, C. R. (2009). Evidence for the inhibition of the terminal step of ruminal α-linolenic acid biohydrogenation by condensed tannins. *J. Dairy Sci.*, 92,177-188.

Kramer, J. K. G., Parodi, P. W., Jensen, R. G., Mossoba, M. M., Yurawecz, M. P., & Adlof, O. R. (1998). Rumenic acid : A proposal common name for the major conjugated linoleic acid isomer found in natural products. *Lipids*, 33, 853.

Krueger, W. K., Gutierrez-Banuelos, H., Carstens, G. E., Min, B. R., Pinchak, W. E., Gomez, R. R., Anderson, R. C., Krueger, N. A., & Forbes, T. D. A. (2010). Effects of dietary tannin source on performance, feed efficiency, ruminal fermentation, and carcass and non-carcass traits in steer fed a high-grain diet. *Anim. Feed Sci. Technol.*, 159, 1-9.

McGuire, M. A., & McGuire, M. K. (2000). Conjugated linoleic acid (CLA) : A ruminant fatty acid with beneficial effects on human health. *J. Anim. Sci.*, 77, 1-8.

Min, B. R., Barry, T. N., Attwood, G. T., & McNabb, W. C. (2003). The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. *Anim. Feed Sci. Technol.*, 106, 3-19.

Molle, G., Decandia, M., Giovanetti, V., Cabiddu, A., Fois, N., & Sitzia, M. (2009). Responses to condensed tannins of flowering sulla (*Hedysarum coronarium* L.) grazed by dairy sheep; Part 1: Effects on feeding behabiour, intake, diet digestibility and performance. *Livest. Sci.*, 123, 138-146.

Morimoto, K. C., Van Eenennaam, A. L., DePeters, E. J., & Medrano, J. F. (2005). Hot Topic: Endrogenous production of n-3 and n-6 fatty acids in mammalian cells. *J. Dairy Sci.*, 88, 1142-1146.

Mueller-Harvey, I. (2006). Unravelling the conundrum of tannins in animal mutrition and health. *J. Sci. Food Agric.*, 86, 2010-2037.

Muir, J. P. (2011). The multi-faceted role of condensed tannins in the goat ecosystem. *Small Rum. Res.*, 98, 115-120.

Patra, A., & Sexena, J. (2011). Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *J. Sci. Food Agric.*, 91, 24-37.

Poungchompu, O. (2008). Effects of saponins and tannins in tropical plants on methanogenesis suppression by rumen microbes in ruminants. Doctoral dissertation, Khon Kaen University, Thailand.

Tabacco, E., Borreani, G., Crovetto, G. M., Galassi, G., Colombo, D., & Cavallarin, L. (2006). Effect of chestnut tannin on fermentation quality, proteolysis, and protein rumen degradability of alfalfa silage. *J. Dairy Sci.*, 89, 4736-4746.

Tanaka, K. (2005). Occurrence of conjugated linoleic acid in ruminant products and its physiological functions. *Anim. Sci. J.*, 76, 291-303.

Toral, P. G., Hervas, G., Bichi, E., Belenguer, A., & Frutos, P. (2011). Tannins as feed additives to modulate biohydrogenation: Effects on animal performance, milk fatty acid composition and ruminal fermentation in dairy ewes fed a diet containing sunflower oil. *Anim. Feed Sci. Technol.*, 164, 199-206.

Vasta, V., Mele, M., Serra, A., Scerra, M., Luciano, G., & Lanza, M. (2009). Metabolic fate of fatty acids involved in ruminal biohydrogenation in sheep fed concentrate or herbage with or without tannins. *J. Anim. Sci.*, 87, 2674-2684.

Vasta, V., Yanez-Ruiz, D. R., Mele, M., Serra, A., Luciano, G., & Lanza, M. (2010). Bacterial and protozoal communities and fatty acid profile in the rumen of sheep fed a diet containing added tannins. *Appl. Environ. Microbiol.*, 76, 2549-2555.