PATTERN OF POLYUNSATURATED FATTY ACID BIOHYDROGENATION AS INFLUENCED BY DIETARY TANNIN

Anuraga Jayanegara
Department of Nutrition and Feed Technology, Faculty of Animal Science
Bogor Agricultural University, Jl. Agatis Kampus IPB Dramaga Bogor 16680, Indonesia
E-mail: anu_jayanegara@yahoo.com

Abstract

Large amounts of polyunsaturated fatty acids undergo transformation processes in the rumen through microbial biohydrogenation to form fatty acids with higher saturation degree. The respective process explains the high content of saturated fatty acids in products of ruminants and the potential risk of consumers' health by consuming such products. Various nutritional approaches have been attempted to modulate biohydrogenation process in order to obtain healthier fatty acid profile from consumers' perspective. The present paper is aimed to review the influence of dietary tannin, a naturally produced plant secondary compound, on the pattern of polyunsaturated fatty acids biohydrogenation occurring in the rumen. The effect of tannin on some key fatty acids involved in biohydrogenation process is presented together with the underlying mechanisms, particularly from up-to-date research results. Accordingly, different form of tannin as well as different level of the application are also discussed.
Key words: tannin, biohydrogenation, fatty acid, ruminant

Introduction

Current goals of livestock production systems have expanded towards a wider context, i.e. from merely focusing on improving productivity of the animals (increasing daily weight gain, milk production, etc.) towards a more integrated approach by considering other aspects related to the systems. For instance, an important issue is regarding the role of livestock in minimizing the impact of global warming due to greenhouse gasses accumulation in the atmosphere, particularly methane emission (Cottle et al., 2011). Another current important issue that has to be taken into account in relation to livestock production is concerning the role of livestock to promote human health through the production of animal-source foods with a particular dietetic quality. This attribute has been positively assigned to polyunsaturated fatty acids (PUFA), particularly to *n*-3 fatty acids (Barcelo-Coblijn and Murphy, 2009), and conjugated linoleic acid (CLA; Benjamin and Spener, 2009), but has been negatively assigned to saturated fatty acids (SFA). Unfortunately, large amounts of PUFA undergo transformation processes in the rumen through microbial biohydrogenation to form fatty acids (FA) with higher saturation degree (Chilliard et al., 2007). The respective process explains the high content of SFA in products of ruminants and the potential risk of consumers' health by consuming such products. Therefore, the biohydrogenation process needs to be controlled in order to obtain better FA profiles in the products of animals. Accordingly, developing nutritional strategies for optimizing the respective process while maintaining optimal rumen function and fermentation is highly desirable.

The present paper is aimed to review the influence of dietary tannin, a naturally produced plant secondary compound, on the pattern of PUFA biohydrogenation occurring in the rumen. Since the respective compound is abundantly available in various tropical plants, therefore, it is of importance to be further discussed especially regarding its potential applicability to improve the quality of animal-source foods. An overview about lipid digestion in ruminants is presented in the next section, followed by some nutritional

measures that have been applied to modify FA biohydrogenation in the rumen. Finally, the influence of tannin on such biohydrogenation process is presented.

Lipid digestion in ruminants: lipolysis and fatty acid biohydrogenation

Dietary lipids, which mainly consist of triglycerides, phospholipids and galactolipids, undergo transformation processes in the digestive tract of ruminants, i.e. through lipolysis and biohydrogenation by rumen microorganisms. Lipolysis, which is a precondition prior to the next process, results in the release of free FA, followed by biohydrogenation to saturated FA which is the reduction of the number of double bonds in the carbon chain of the FA (Jenkins, 1993). The transformation processes explain the differences in the tissue profiles of FA between ruminants and non-ruminants, in which the former is more saturated than the latter (Doreau et al., 2011). During lipolysis, hydrolysis of lipids is conducted mainly by microbial lipases, although there is also the action of plant lipases. The main species of rumen bacteria responsible for lipolysis is *Anaerovibrio lipolytica*. This species produces two hydrolitic enzymes, i.e. cell-bound esterase and extracellular lipase, which have activity on triglycerides and esterified fatty acids (Jenkins et al., 2008). However, *A. lipolytica* lacks the capability of hydrolyzing phospho- and galactolipids, and for these substrates, the hydrolysis is done by *Butyrivibrio* spp (Lourenco et al., 2010). In addition to bacteria, protozoal species such as *Epidinium ecaudatum* also play a considerable role in lipolysis and may contribute up to 40% of the hydrolytic activity (Lourenco et al., 2010).

After being liberated as free FA, the unsaturated FA start to enter biohydrogenation pathways. Fatty acids present in forages, cereals and oil seeds mainly contain 18 C-atoms, i.e. oleic acid (c18:1 *n*-9), linoleic acid (C18:2 *n*-6) and α-linolenic acid (C18:3 *n*-3). Biohydrogenation transforms these FA into various isomers of PUFA and monounsaturated fatty acids (MUFA), particularly *trans*-FA and CLA, and they end up as stearic acid (C18:0) which is the final product of the process (Jenkins et al., 2008). This has been demonstrated by the huge disappearance proportion of C18:2 *n*-6 and C18:3 *n*-3 in the rumen with the values of 85% and 93%, respectively (Doreau and Ferlay, 1994), as well as C18:0 accumulation (Fievez et al., 2007). Among the main biohydrogenation intermediates are rumenic acid (*c*9,*t*11 C18:2), a CLA isomer, and vaccenic acid (*t*11 C18:1). The initial step of biohydrogenation is the isomerization by moving the *cis*-12 bond to either the C11 or the C13 position, followed by hydrogenation of the double bonds. Although there are several biohydrogenation pathways of C18:2 *n*-6, the main pathway is: *c*9,*c*12 C18:2 → *c*9,*t*11 C18:2 → *t*11 C18:1 → C18:0. Similarly, the main biohydrogenation pathway of C18:3 *n*-3 is: *c*9,*c*12,*c*15 C18:3 → *c*9,*t*11,*c*15 C18:3 → *t*11,*c*15 C18:2 → *t*11 C18:1 → C18:0 (Chilliard et al., 2007).

The stepwise biohydrogenation in the rumen is carried out by different groups of microorganisms, i.e. bacteria, protozoa and anaerobic fungi. *Butyrivibrio fibrisolvens* is a main bacteria species that performs biohydrogenation from C18:2 *n*-6 to *c*9,*t*11 C18:2 and *t*11 C18:1, but not to C18:0, the ultimate biohydrogenation product. From the phylogenetic tree based on 16S rRNA sequence analysis, the bacterial species that is capable of producing C18:0 from C18:2 *n*-6 is *Clostridium proteoclasticum* (Jenkins et al., 2008). Protozoa are considered to contribute significantly to FA metabolism in the rumen since about three quarters of the microbial FA originate from protozoa, considering that up to half of the rumen microbial biomass is also from protozoa. However, it has been mentioned that there was only a slight decrease in biohydrogenation after defaunation, and the presence of protozoa was not essential for the process (Lourenco et al., 2010). In the case of anaerobic fungi, especially also *Neocallimastix frontalis* is able to metabolize

C18:2 *n*-6 to form *c*9,*t*11 C18:2, but the activity is very small compared to *B. fibrisolvens* (Jenkins et al., 2008).

Nutritional measures for manipulating fatty acid biohydrogenation in the rumen

Increasing the contents of PUFA especially of omega-3 FA in ruminant products is desirable. This is due to, in contrast to the SFA, the presumed health benefits of omega-3 FA such as reducing the risk of cardiovascular disease in humans and lowering plasma cholesterol level (Barcelo-Coblijn and Murphy, 2009). Promoting the level of *c*9,*t*11 C18:2, which is produced from partial biohydrogenation of C18:2 *n*-6 in the rumen, is of interest since it has been shown to prevent cancer proliferation, decreased atherosclerosis and improved immune response (Palmquist et al., 2005). In relation to the respective CLA isomer, *t*11 C18:1 is also desirable since it can be converted in the tissues of ruminants to *c*9,*t*11 C18:2 via the action of the enzyme Δ-9 desaturase (stearoyl-CoA desaturase) by adding a *cis*9-double bond (Chilliard et al., 2007). Therefore, any nutritional measure for manipulating biohydrogenation of FA in the rumen is directed towards these objectives. Feeding forages to ruminants is associated with an improvement of FA profiles in the products since, in general, lipids in forages are naturally rich in PUFA (Lourenco et al., 2008). Doreau et al. (2011), for instance, mentioned that feeding grass-based diets resulted in higher levels of C18:3 *n*-3 in muscle as compared to concentrate-based diets. Another study observed that feeding of grass silage to fattening bulls led to higher C18:3 *n*-3 in adipose tissue than that of maize silage (Staerfl et al., 2011). Sources of variation in the rates and extents of ruminal biohydrogenation of dietary FA from forages as well as their transfer to products are forage species, cultivar, conservation method and level of inclusion (Dewhurst et al., 2006).

Supplementation of lipid rich feeds, either in the form of extracted lipids or whole oil seeds, is another strategy for modulating ruminal biohydrogenation as well as for increasing energy contents of the diets. In addition to high PUFA contents of many lipid sources (e.g., linseed, sunflower, soybean oils and seeds, fish oil), it has been known that dietary lipids may cause antimicrobial effects on biohydrogenating bacteria especially lipids with higher degree of unsaturation. Lipids rich in either C18:2 *n*-6 or C18:3 *n*-3 are both contributing to the increase of CLA contents in milk (Lourenco et al., 2010). Fish oil, which is rich in long-chain PUFA, particularly eicosapentaenoic acid (EPA, C20:5 *n*-3) and docosahexaenoic acid (DHA, C22:6 *n*-3), has been shown to inhibit the last step of biohydrogenation, i.e. the conversion of *t*11 C18:1 to C18:0, which may be due to its influence on *B. fibrisolvens*, *Ruminococcus albus* and *Selenomonas ruminantium* (Potu et al., 2011). Alternatively to fish oil, microalgae also contain long-chain PUFA and its supplementation increased C22:6 *n*-3, CLA and *t*11 C18:1 levels in milk (Boeckaert et al., 2008). Several oils that are rich in MCFA such as coconut (rich in lauric acid, C12:0, and myristic acid, C14:0) and palm oils (rich in palmitic acid, C16:0) are considered not to have benefited FA composition in ruminant products (Lourenco et al., 2010). Administration of lipids in the form of whole seeds usually (but not always) results in a lower biohydrogenation than when given as extracted oils. The extent of this reduction depends on the hardness of the hull, particle size and mastication for breaking the oilseed hulls (Doreau et al., 2011). Prior to supplementation, lipids may also be protected through various treatments. The ideal criteria of lipid protection technology have been proposed by Jenkins and Bridges Jr. (2007) as (1) consistent and predictable enhancement of UFA flow to the duodenum, (2) adequate release and absorption of the UFA in the intestines, and (3) minimal adverse effects on ruminal fermentation. Some protection techniques that have been implemented are, for instance, encapsulation of emulsified oils into a matrix of

formaldehyde-treated proteins (Sterk et al., 2010), formation of calcium salts (Theurer et al., 2009) and formation of fatty acyl amides (Lundy et al., 2004).

Plant secondary compounds may alter ruminal biohydrogenation of FA as well. Studies of Leiber et al. (2004 and 2005) showed that C18:3 *n*-3 levels in milk were to some extent independent of their intake. Accordingly, the results then led to a hypothesis that plant secondary compounds may play a role to the elevated contents of C18:3 *n*-3 and/or *c*9,*t*11 C18:2 observed in milk and milk products originating from cows grazing on alpine pastures compared to those from intensive lowland production systems. These compounds may include polyphenol or tannin (will be discussed in the next section), saponin and essential oil. Regarding saponin, it has been reported that a saponin-rich extract from *Yucca schidigera* inhibited the growth of *B. fibrisolvens* more than other bacteria species (Wallace et al., 1994), suggesting that there is a potential of saponins in modifying FA biohydrogenation. However, further research needs to be undertaken to ascertain this since direct evidence so far has shown lack of effects of saponins in altering FA composition in ruminal fluid (Khiaosa-ard et al., 2009), milk (Benchaar and Chouinard, 2009) and meat (Brogna et al., 2011). Essential oils have an activity against several bacteria that are involved in ruminal biohydrogenation. Their effects in the rumen are quite variable, depending on nature of the compounds, level of applications, basal diet and adaptation time of ruminal microbes in the presence of the compounds (Benchaar et al., 2008). Some plant extracts and essential oils from Australian plants have been reported to have a selective inhibitory effect on *C. proteoclasticum* without affecting *B. fibrisolvens*, and some can inhibit the saturation of C18:2 *n*-6, *c*9,*t*11 C18:2 and *t*11 C18:1 *in vitro* (Durmic et al., 2008). On the contrary, feeding of cinnamaldehyde, the main component of cinnamon bark (*Cinnamon cassia*) essential oil to dairy cows revealed no effects on milk fatty acid profile thus suggesting that the potential of this essential oil to alter ruminal biohydrogenation process is low (Benchaar and Chouinard, 2009).

Tannin and its influence on fatty acid biohydrogenation

Tannin can be divided into two categories based on its property in binding protein, i.e. tannin phenol (or tannin) which can bind protein, and non-tannin phenol which is not able to bind protein. Generally, tannin has a much higher molecular weight than that of non-tannin phenol; the latter is simpler phenol such as catechol, pyrogallol, gallic acid, catechin and other flavanols (Scalbert, 1991). Based on its molecular structure, tannin is classified as hydrolysable tannin (HT) and condensed tannin (CT; also known as proanthocyanidin), although other tannin may occur which is combination of these two basic structures (McSweeney et al., 2001). There is a great structural diversity of tannin between different sources and even slight changes in the structure may produce measurable biological effects (Mueller-Harvey, 2006). Hydrolysable tannin contains carbohydrate (generally glucose molecule) as a central core with hydroxyl groups which are esterified with phenolic groups. Different from that, CT does not have a central carbohydrate core and are complexes of oligomers and polymers of flavanoid units linked by carbon-carbon bonds with a molecular weight of 2,000–4,000 kDa (Goel et al., 2005). Hydrolysable tannin is more susceptible to enzymatic and non-enzymatic hydrolysis than that of CT, and usually is better soluble in water. Further, based on the hydrolysis products, HT can be divided into gallotannin, which yield gallic acid and glucose, and ellagitannin, which yield elagic acid and glucose (Reed, 1995).

Multiple phenolic hydroxyl groups enable tannin to form complexes especially with protein, although complexes with polysaccharides and minerals are also occurring. With regard to interactions between tannin and protein, the strengths of the complexes depend on characteristics of both tannin and protein such as molecular weight, tertiary structure,

isoelectric point and compatibility of binding sites (Reed, 1995). Three different bonds may exist in tannin-protein complexes, i.e. hydrogen bonds through a large number of free phenolic hydroxyl groups, hydrophobic bonds through aromatic ring structures and covalent bonds through oxidative polymerization reactions due to heating, exposure to UV radiation and the action of polyphenol oxidase (Silanikove et al., 2001).

In relation to biohydrogenation of FA in the rumen, tannin has been reported to reduce PUFA biohydrogenation, accumulate *t*11 C18:1 and/or decrease concentration of C18:0 (Khiaosa-ard et al., 2009; Vasta et al., 2009; Cabiddu et al., 2010; Jayanegara et al., 2011; Jayanegara et al., 2012), to increase the transfer rate of C18:3 *n*-3 from feed to milk as a product (Kälber et al., 2011), and thus may alter FA composition in the products of animals (Doreau et al., 2011). *In vitro* incubations of tropical (Jayanegara et al., 2011) and alpine plants (Jayanegara et al., 2012) with additional linseed oil suggest the ability of plant phenolics in modulating FA biohydrogenation, i.e. by decelerating the process right from the first step. This was indicated by lower disappearance of C18:3 *n*-3 and C18:2 *n*-6 in the incubations of both tropical and alpine plants containing high phenolics in the respective studies. Such effects may be explained by the toxicity of phenolics, particularly of tannin phenolics, on bacterial species involved in FA biohydrogenation (Khiaosa-ard et al., 2009; Vasta et al., 2010). It is also possible that phenolics may inhibit the process of lipolysis which is a precondition prior to further transformation of fatty acids, i.e. biohydrogenation (Jenkins, 1993; Lourenco et al., 2010). In accordance to this, Cabiddu et al. (2010) observed a negative relationship between tannins present in *Vicia sativa* and *Trifolium incarnatum* with the biohydrogenation of C18:3 *n*-3. Other phenolics considered to be responsible for the increase found in the transfer of C18:3 *n*-3 from feed to milk (Kälber et al., 2011) when fed buckwheat, which might be rutin or fagopyrin (Leiber et al., 2012).

Regarding the occurrence of biohydrogenation intermediates by the influence of plant phenolics, in an *in vitro* study, Khiaosa-ard et al. (2009) observed a considerable increase of *t*11 C18:1 at the expense of C18:0 when adding *Acacia mearnsii* extract (source of CT) at 79 g/kg DM to a grass-clover hay diet supplemented with linseed oil. The authors therefore suggested that the tannins inhibited the terminal step of FA biohydrogenation. The accumulation of *t*11 C18:1 in the presence of tannin phenolics was confirmed by other *in vitro* study (Vasta et al., 2009). This effect might be related to phenolic toxicity, especially of tannin, on bacterial species involved in FA biohydrogenation, through selective inhibition of cell wall synthesis (Smith et al., 2005), interaction of phenols with microbial proteins (Silanikove et al., 2001), and direct interaction between phenols and lipids (He et al., 2006). *Clostridium proteoclasticum*, a bacterial species responsible for the terminal step of biohydrogenation, i.e. the conversion from *t*11 C18:1 to C18:0 (Jenkins et al., 2008), was proved to decrease by 31% in the rumen of lambs fed by a tannin-supplemented diet compared to a control diet (Vasta et al., 2010).

Different fractions of tannin, i.e. HT and CT, contributed to the modification of biohydrogenation pattern. Jayanegara et al. (2011) found that the main class of polyphenol which prevented C18:3 *n*-3 and C18:2 *n*-6 from biohydrogenation was that of the HT, while the appearance of *c*9,*t*11 C18:2 was closer correlated with the CT. This illustrates that both types of tannins are involved in the inhibition of biohydrogenation but in different steps, namely HT in the first and CT in the second step. By contrast, it has been observed *in vitro* and *in vivo* that the addition of CT extracts to the diet caused no difference in the concentration of conjugated C18:2 in the ruminal fluid, but led to a considerable increase of *t*11 C18:1 at the expense of C18:0 instead (Khiaosa-ard et al., 2009). This indicates that inhibition of the third step of biohydrogenation took place. Thus, a differentiated and still

not always coherent influence of different tannins on the biohydrogenation pathway can be observed.

It has to be noted that the effect of tannin on FA biohydrogenation cannot be found in every study (Abbeddou et al., 2011) and it appears that the phenolic effects are minor when the concentration is below a certain threshold (Jayanegara et al., 2011; Khiaosa-ard et al., 2011). For instance, supplementation of commercially available extracts of tannins, i.e. a 1:1 mixture of *Castanea sativa* and *Schinopsis lorentzii* (quebracho, a source of CT) at a level of 10 mg/g DM to a diet containing sunflower oil did not alter the proportions of the major FA classes in milk (PUFA, MUFA and SFA) as well as the proportions of *c*9,*t*11 C18:2 and *t*11 C18:1 in milk (Toral et al., 2011). The low dose of the tannin mixture in that study was presumed to have been the reason for the lack of change in the FA in the respective study. It appears that the levels of polyphenols in diets as well as the sources from where they were obtained are among the factors of influence affecting the biohydrogenation of FA and general rumen fermentation (Jayanegara et al., 2011).

Conclusion

The pattern of PUFA biohydrogenation can be modulated in the presence of dietary tannin and, thus, may potentially modify FA composition in the products of animals. Among the main effects of tannin on biohydrogenation are reducing PUFA biohydrogenation, accumulating *t*11 C18:1 and/or decreasing concentration of C18:0. There is still a controversy whether the respective substance is able to increase *c*9,*t*11 C18:2 concentration in rumen fluid. The effect of tannin on biohydrogenation appears to be source- and dose-dependent.

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