Potential of Tannin and Nano-encapsulated Unsaturated Fatty Acids to Reduce Methane Production and Ruminal Biohydrogenation in The Rumen

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***Abstract*—**Methane formation and fatty acid biohydrogenation in the rumen are two undesirable phenomena due to these negative effect on environment and human health. Tannin and unsaturated fatty acid (UFA) are group of additives that has gained a progressive interest to modulate fermentation in the rumen. However, there is little information about their systemic and synergistic effect due to limited research on these additives. The present review attempt to describe the impact of tannin and/ or nano-encapsulated unsaturated fatty acid on methane production in the rumen and fatty acid profile in ruminant products. It was found that tannin and protected UFA blended in the diet have potential to enhance the accumulation of *trans*-11 18:1 (VA, vaccenic acid) in the rumen and reduce methane formation by synergistic mechanism. Tannin would suppress *Butyrivibrio fibrisolvens,* a bacteria which cause biohydrogenation, protozoa, and methanogen. UFA could also be protected from biohydrogenation by encapsulated it, resulting in low methane production and high accumulation of VA and conjugated linoleic acid (CLA) in the animal products.

***Keywords*—** Tannin, Nano-encapsulation, unsaturated fatty acid, methane production, ruminal biohydrogenation.

I. Introduction

Integrated future livestock system goals are expanded into improving productivity, providing health food sources to human, and mitigating greenhouse gas (GHG) emission particularly methane emission (CH_4). Ruminants through their fermentation process in the rumen, produce about 80 million tonnes CH_4 annually, or accounts for 28% of the total global anthropogenic GHG emissions [1]. Methane is produced in the rumen as a part of the normal anaerobic fermentation process of feed digestion. From this mechanism, about 2-12% of gross energy losses [2].

Another concern issue related to rumen fermentation is biohydrogenation mechanism which converts most of unsaturated fatty acids (UFA) to saturated fatty acid (SFA) resulting in accumulation of SFA in meat or milk so that more risk to cancer [3]. A rapidly interest toward enhancing the content of UFA in the animal-food products has been noticed by researcher over the world due to its benefit to human health. Conjugated Linoleic Acid (CLA) and other n-3 long chain fatty acids; e.g. docosahexaenoic (DHA, 22:6 n-3), docosapentaenoic (DPA, C22:5 n-3) and eicosapentaenoic acids (EPA, C20:5 n-3) could decrease risk to cardiovascular disease, cancer and immune system disorders [3].

The efforts to reduce CH_4 production and improve fatty acid profile in meat and milk have been done through dietary manipulation. Tannins have been suggested, in the diet, could reduce CH_4 production in the rumen [4] and alter ruminal biohydrogenation of dietary linoleic acid by enhancing UFA content in dairy or meat products [5]. Vegetable oils rich in UFA have recognized could decrease CH_4 production and altered fermentation patterns due to biohydrogenation and toxic effects of medium-chain or polyunsaturated fatty acids [6]. The use of oil sources rich in UFA or tannin additives individually in ruminant diet has been extensively investigated in the last few years [7]–[8]. Studies the combination effect of tannins and vegetable oils on ruminal biohydrogenation also was conducted even in limited number [9]–[10].

For our best knowledge, there is no evidence whether combination of tannin and protected unsaturated fatty acid have synergistic effect could modulate biohydrogenation and methanogenesis processes in the rumen. Given the fact that both of the additives had positive effect on methane emission and fatty acid profile in the ruminant products, we postulated that combining tannin and protected UFA using nano-encapsulation technology would be a new strategy to promote efficient production system in the ruminants. Accordingly, developing nutritional strategies for optimizing the respective mechanism is needed. The present review provides explanation the possible synergistic effect caused by tannin and nano-encapsulated UFA to maintain optimal rumen function and to deplete negative effect of methane emission and biohydrogenation in the rumen. Since the respective compounds are abundantly available in various plants and feedstuffs, further research is needed to be discussed

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particularly its potential applicability to improve ruminant-food products.

II. METHODOLOGY

The review was conducted by using qualitative method. Relevant papers were identified by searching for peer-reviewed articles that were published in English using online database from Google Scholar, Science-Direct (www.sciencedirect.com), Scopus (www.scopus.com), and PubMed (www.ncbi.nlm.nih.gov/pubmed). Publications were retrieved using keywords “methanogenesis”, “rumen biohydrogenation”, “tannin in the rumen”, “encapsulated unsaturated fatty acids”. Of the papers that were retrieved, only those that satisfied the predetermined inclusion criteria were included in the analysis.

The criteria of published articles used in the current review were (1) publication in English in a peer-reviewed journal, (2) use negative control and treatments to determine the effect of the treatment groups, (3) use tannin, tannin and/ or unsaturated fatty acids, encapsulated unsaturated fatty acids, respectively, (4) methane production and/ or fatty acids profile were measured directly either in *in vitro* or *in vivo* studies. Peer-reviewed studies were only used due to its high quality studies.

Data collected and extracted then analysed qualitatively by grouping respective parameters. Comparative among treatments also were analysed to generalize finding from elaborative data. Finally, conclusion and a possible future innovative prospect were making from generalization of analysis result.

III. RESULT AND DISCUSSION

A. Methanogenesis As Influenced by Tannin and Unsaturated Fatty Acid

Tannins are bioactive compounds that represent an important class of plant secondary metabolites produced by the plants in their intermediary metabolism. Based on their molecular weight, tannins are classified to hydrolysable tannins (HT; polyesters of gallic acid and various individual sugars) and condensed tannins (CT; polymers of flavonoids) [11]. In respective with nutritional properties on ruminants, tannins have considered their adverse and beneficial effects [12]–[13]. The negative effect of tannins include reduction of feed intake, digestibility on fiber and nitrogen, and animal performance [12] in special condition. However, there had also well recognized that tannins also have beneficial effect including prevent bloat, enhance protein utilization, control parasites, induce improvements in growth performance, and improve milk production [13]–[14]. Moreover, several studies also shown that tannins are also have antioxidant activity [15].

In the ruminants, tannins are considered a promising bioactive compounds for decreasing enteric CH_4 emissions. Across a large number of experiments, a meta-analysis of in vitro and in vivo experiments with tannins was conducted that reported a relatively close relationship between dietary tannin concentration and CH_4 production per unit of digestible OM. Increasing the level of dietary tannins leads to a clear decrease in ruminal CH_4 emissions [4]. Through meta-analysis study, it was to note that the variation in CH_4 production/digestible OM in vivo was very high at low levels of dietary tannins of <20 g/kg DM, whereas variability clearly decreased with increasing tannin concentrations. This might explain why experiments using low levels of tannins led to inconsistent results in terms of effects on CH_4 emissions [4]. It was explained from several studies that tannins would inhibit protozoa and methanogen population in the rumen, hydrogen-producing bacteria, or indirect decreased fibre digestion, thus producing CH_4 decreased [16]–[17].

Moreover, investigation on methane reduction using such matter rich in PUFAs or UFAs also resulted in positive effect. However, reported efficacy of oils on rumen methanogenesis varies dramatically among studies, depending upon on the concentration, type, and fatty acid composition of the fats used, as well as the nutrient composition of the diets [1]. Oil-rich supplements used as methane mitigation option because oils not only increase dietary energy density, but also decrease rumen CH_4 emissions by several mode of action. Oils in the rumen would involve in reductions in organic matter fermentation, rumen ciliate protozoa numbers, methanogen activity and the use of hydrogen for biohydrogenation [18].

Dietary inclusion of lipids containing high levels of PUFAs in the diet of ruminants has being promising according to the result from several studies. Decreased in CH_4 production was reported when linseed oil [19], mixtures of sunflower oil and fish oil [6], and mixture coconut oil and fish oil [20] was included in the diets. Increasing doses of coconut oil and fish oil quadratically decreased concentrations of CH_4, but hydrogen concentrations were only increased quadratically by CO. Both oils linearly decreased dry matter and neutral detergent fiber digestibility of feeds but did not affect the concentration of total volatile fatty acids (VFAs). Previous studies also suggest that unsaturated fatty acids in oils or fats may modify ruminal microbes and ecosystem, and rumen fermentation as well. By these finding, both tannin and UFA could effectively reduce CH_4 emissions from the ruminants.

B. Tannin and Unsaturated Fatty Acid and its Effect on Biohydrogenation Pattern

Growing interest of using tannins in the diet of ruminants have bring to the understanding that tannins could enhance fatty acid composition in animal products [21]. Utilization of plants or plant extracts rich in tannins in ruminant diets conduces to an increase in the levels of health-beneficial FA in meat and milk and improves the oxidative stability of meat. However, the possible adverse effects of tannins on animal performance and organoleptic properties of products constitute a major restriction on the practical application of this nutritional strategy [22].

A large number of studies regarding on the effect of using tannins to fatty acid profile were conducted both in meat [24]–

[25], and milk [26]–[28]. Fatty acid (FA) profile in the intramuscular fat changed when lambs fed with diet containing carob. Increasing of carob level from 24% to 35%, similar to tannins level increase from 3.4 to 4.5 g/kg DM reduced SA and total SFA, n-6/n-3 ratio, and increased level of PUFA, Rumenic Acid (RA), Linoleic Acid (LA), and Linolenic Acid (LNA) compared to control treatment [30], thus leading to the production of meat with a FA profile that is considered more beneficial to health.

In relation to milk FA profile, it was observed that no effect to minor effect of diet supplementationwith quebracho (*Schinopsis balansae*) tannins extract on the milk FA composition of dairy cows [31]–[32]. Moreover, addition of mixture of quebracho (*Schinopsis lorentzii*) and chestnut tannin extracts to ewes' diets supplemented with sunflower oil had a minor effect on FA composition of milk [26]. Lack of increasing FA profile in milk if tannin give individually is key point to searching other additive sources that, in combination or individually, could promote desirable FA.

Developed of well knowledge that level of UFA, PUFA, or CLA in the animal products mainly affected by nutritional factor, using feed rich in UFA has been intensively doing by researcher over the world. Inclusion of UFA from the forage and or oilseeds and their effect on FA profile in meat and milk has been well documented. Feeding dietary PUFA supplements including plant oils/oilseeds, which are rich in oleic acid (OA, cis-9 18:1; e.g., canola and safflower), linoleic acid (LA, 18:2n-6; e.g., sunflower, soybean and corn oil), ALA (e.g., fresh grass and flaxseed/linseed), and marine lipids such as algae and fish rich in DHA and EPA [33] increase the proportions of VA in beef. The increase in the proportions of PUFA and their biohydrogenation products including VA in beef, however, varies with levels of dietary PUFA fed. Overall, the proportion of t-18:1 isomers in beef increases linearly with dietary levels of PUFA up to 80 g/kg DM intake beyond which rumen function is impaired [34].

Meta-analysis conducted of published data on supplementation of dairy cows with oilseed lipids showed that the content of total C18 fatty acids in milk fat was increased quadratically by all supplements according to added lipid, that almost all supplements resulted in significant increases in cis and trans C18:1, and that physical protection of the supplement greatly improved the LA and ALA content of milk fat [33]. Other studies also showed that feeding forages in combination with high levels of PUFA can also have synergistic effects on the t-18:1 isomeric profile of beef with responses to PUFA being greater than that for forages [33].

C. Potential of Nano-encapsulated UFA-Tannin Mixture on Modulation of Ruminal Fermentation

In relation to the expanded goal of ruminants in providing eco-friendly environment and healthy food source, researchers are intensively exploring various technologies and policies to mitigate enteric CH_4 emissions and enhance CLA in meat and milk. However, most of the dietary interventions adversely affect fermentation in the rumen and animal productivity [33]. In these respective purposes, tannin and UFA sources have potentially to be combined in order to modulate ruminal fermentation and resulting better FA profile and less CH_4 as well. Combination of tannin and UFA sources have studied in limited number [35]–[37].

A significant change in the FA profile of ewes'milk by inclusion of quebracho (*Schinopsis lorentzii*) or chestnut (*Castanea sativa*) tannins extracts (24 g CT/kg DM and 40 g HT/kg DM, respectively) in diets supplemented with soybean oil [27]. Conversely, the tannin and fish/ soybean oil mix treatment did not modify the proportions of majority of the unsaturated FA in milk but decreased CH_4 production. This phenomenon could be attributed to a lack of effects of the both provided supplements [35]. These inconsistent finding may be caused by moderate biohydrogenation of UFA in oil sources. Therefore, protection of UFAs and PUFAs from ruminal biohydrogenation seems to be more effective way to modulate ruminal fermentation.

Study on the effect of nano-encapsulated CLA against in vitro ruminal biohydrogenation resulted increased CLA content in vitro and decreased the population of *B. fibrisolvens*. CLA in animal products is associated with beneficial effect on decreasing risk of cancer and has becoming parameter of meat or milk quality. These results indicate that nano-encapsulation could be applied to enhance CLA levels in ruminants by increasing the stability of CLA without causing adverse effects on ruminal fermentation [36]. Furthermore, Lipid-Encapsulated CLA supplementation also increased the duodenal flows and the proportions of C18:2 trans-10, cis-12 and C18:2 cis-9, trans-11, reflecting a calculated protection of 15–16% from rumen biohydrogenation [37].

So far, to our best knowledge, there is no research conducted to investigate the synergistic effect between tannin and nano-encapsulated UFA or CLA on either CH_4 production or FA profile in meat and milk. According to the resulted from a large number of studies above, there is still need to searching the strategy to protect UFA against biohydrogenation and in the other hand also decrease CH4 from ruminal fermentation. It is because not most of studies has a minor effect in enhancing FA profile in milk. When tannins combine with nano-encapsulated UFA, tannins will reduce both CH4 and population of *Butirivibrio fibrisolvens*, a major bacteria caused biohydrogenation. By this mechanism, more UFA protected against biohydrogenation resulting on greater proportion of CLA in animal-food products.

IV. Conclusion

Tannin and oilseeds rich in unsaturated fatty acid (UFA) could decrease CH_4 and enhance FA profile in animal-food products either individually or in mixture. However, many studies showed less effective in increasing FA-beneficial profile. Nano-encapsulated UFA or CLA combined with tannin have a great potential to produce more healthy animal food sources and to decrease more CH4 emissions. So far, there is no research conducted to investigate the synergistic effect between tannin and nano-encapsulated UFA or CLA on either CH_4 production or FA profile in meat and milk. Future challenges include enhancing protection characteristics of existing rumen-protected oil sources, and development and commercialization of novel protection strategies.

APPENDIX

GHG : Greenhouse Gas
FA : Fatty Acids
UFA : Unsaturated Fatty Acids
PUFA(s) : Polyunsaturated Fatty Acids
SFA : Saturated Fatty Acids
CLA : Conjugated Fatty Acids
DHA : Docosahexaenoic
EPA : Eicosapentaenoic acids
DPA : Docosapentaenoic
CT : Condensed Tannins
HT : Hydrolyzable Tannins
OM : Organic Matter
DM : Dry matter
CO : Coconut oil
VFA : Volatile Fatty Acids
RA : Rumenic Acid
LA : Linoleic Acid
LNA : Linolenic Acid

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