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Tannin as a feed additive for mitigating enteric methane emission from livestock: meta-analysis from RUSITEC experiments

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Abstract. A study was performed to evaluate the effect of dietary tannin on methane emission from livestock by using a meta-analysis method. A total of six rumen simulation technique (RUSITEC) experiments that composed of 25 treatments from published articles were integrated in a database. Parameters recorded were nutrient digestibility, total gas production, methane emission, volatile fatty acid (VFA) profiles, pH, ammonia concentration and microbial counts, i.e. total bacteria and protozoa. Data were statistically analyzed by using a mixed model methodology in which different studies were treated as random effects and dietary tannin was considered as fixed effect. Results revealed that methane emission decreased linearly with increasing level of dietary tannin ($P<0.01$; $R^2 = 0.677$). Tannin decreased nutrient digestibility, i.e. crude protein digestibility ($P<0.05$; $R^2 = 0.407$), neutral detergent fiber digestibility ($P<0.01$; $R^2 = 0.411$) and acid detergent fiber digestibility ($P<0.01$; $R^2 = 0.543$). Tannin generally did not alter VFA profiles, pH, ammonia concentration and microbial population in the RUSITEC system. It is concluded that increasing level of dietary tannin leads to a decrease in enteric methane emission from livestock. The decrease appears to be indirectly through reduction in digestibility of nutrients, particularly fiber, and directly through inhibition of archaea methanogen.

1. Introduction

Tannin represents a class of plant secondary metabolites and is produced by plants in their intermediary metabolism. It is a polyphenolic compound with various molecular weight and has the ability to bind protein. With regard to ruminant nutrition, tannin is considered to have both beneficial and detrimental nutritional effects. Some beneficial effects of tannin are better dietary protein utilization, higher body weight gain, higher milk production, increased fertility, and improved animal welfare and health through prevention of bloat and lower worm burden. Negative effects of tannin are associated with its toxicity to some species of rumen microbes and ruminant animals [1].

Research on mitigating methane emission from ruminants has received attention in recent years [2–4]. This is because methane is among the most important greenhouse gases in the atmosphere and contributes to global warming phenomenon [5]. Ruminants considerably contribute to the methane emission through microbial fermentation in their digestive tract, particularly in the rumen, through the action of methanogenic archaea. It has been estimated that ruminants produce approximately 80 million tonnes of methane per year. Related to this problem, tannin is considered to be a promising natural

compound to decrease such methane emission [6]. However, such methane mitigating effect of tannin appears to be inconsistent.

The present study therefore aimed to perform a research synthesis using the statistical meta-analysis in order to summarize the effects of tannin on methane emission from ruminants and its related parameters. This study focused on experiments conducted using Rumen Simulation Technique (RUSITEC).

2. Material and methods

2.1. Database development

Experiments reported tannin concentration and methane emission from RUSITEC [7] were integrated in a database. Other related parameters were also recorded in the database such as digestibility, gas production, volatile fatty acid (VFA) profiles, N retention, pH, ammonia and microbial population (bacterial and protozoal counts). A total of six RUSITEC experiments that comprised of 25 treatments were tabulated (table 1). Basal feed consisted of grass hay, silage, barley, *Brachiaria humidicola* and clover hay. Source of tannin was obtained from chestnut extract, sainfoin, *Acacia mearnsii*, *Cratylia argentea*, *Calliandra calothyrsus*, *Leucaena leucocephala*, *Flemingia macrophylla*, *Vigna unguiculata*, *Samanea saman*, *Acacia angustissima*, *Sesbania sesban* and *Cajanus cajan*. Tannin level ranged from 0 to 135 g/kg dry matter.

Table 1. Studies included in the meta-analysis of tannin and methane emission of RUSITEC system.

Reference	Basal feed	Tannin source	Level (g/kg)
Sliwinski et al. (2002)	Mixture of grass hay, silage and barley	Extract from chestnut	0 to 2.5
Hess et al. (2006)	*Brachiaria humidicola*	*Cratylia argentea* and *Calliandra calothyrsus*	0 to 135
Hess et al. (2008)	*Brachiaria humidicola*	*Leucaena leucocephala*, *Flemingia macrophylla* and *Calliandra calothyrsus*	0 to 62.2
Tiemann et al. (2008)	*Brachiaria humidicola*	*Vigna unguiculata* and *Calliandra calothyrsus*	0 and 71
Bekele et al. (2009)	*Brachiaria humidicola*	*Samanea saman*, *Acacia angustissima*, *Sesbania sesban* and *Cajanus cajan*	0 to 45
Khiaosa-ard et al. (2009)	Grass-clover hay	Sainfoin and extract from *Acacia mearnsii*	0 and 78.9

2.2. Statistical analysis

Database statistical analysis was performed according to mixed model methodology [8] in which different studies were treated as random effects whereas tannin levels were treated as fixed effects. The following model was employed:

$$Y_{ij} = B_0 + B_1X_{ij} + s_i + b_iX_{ij} + e_{ij}$$

where, Y_{ij} is dependent variable, B_0 is overall intercept from all studies (fixed effect), B_1 is linear regression coefficient of Y on X (fixed effect), X_{ij} is value of the continuous predictor variable (tannin

level), s_i is random effect of study i, b_i is random effect of study i on the regression coefficient of Y on X in study i, and e_{ij} is the unexplained residual errors.

The study variable was stated in the CLASS statement due to its non-quantitative information. Data were unweighted by number of replicates from each experiment. Outliers were identified by examining studentized residuals. Any values beyond ± 3 SD were considered as outliers and were removed from the dataset. Data reported in different measurement units were transformed into similar units in order to allow direct comparison among experiments. Microbial population data (both bacterial and protozoal counts) were transformed into their logarithmic units. Model statistics presented were P-value, root mean error square (RMSE) and coefficient of determination (R^2). Since the data were unbalance across all parameters, meta-analysis was conducted based on the available data for each parameter.

3. Results and discussion

Crude protein digestibility (CPD), neutral detergent fiber digestibility (NDFD) and acid detergent fiber digestibility (ADFD) decreased linearly as the dietary tannin level increased with the P-value of 0.047, 0.005 and 0.004, respectively (table 2). Comparing the magnitude of reduction in CP and fiber digestibility by the influence of dietary tannins, the compounds appeared to cause higher negative effect on CP digestibility than that of fiber, as indicated by the slopes. An increase of dietary tannin levels by 1 g/kg declined CPD by 2.921 mg/g. The decrease was lower for the NDFD and ADFD, i.e. 1.231 and 1.549 mg/g, respectively.

Table 2. Effect of tannin level (g/kg dry matter) on nutrient digestibility.

Parameter	n	Intercept	Slope	P-value	R^2
OMD (mg/g)	25	442	–0.672	ns	0.178
CPD (mg/g)	14	644	–2.921	0.047	0.407
NDFD (mg/g)	25	324	–1.231	0.005	0.411
ADFD (mg/g)	14	277	–1.549	0.004	0.543

OMD, organic matter digestibility; CPD, crude protein digestibility; NDFD, neutral detergent fiber digestibility; ADFD, acid detergent fiber digestibility; n, number of data; ns, non-significant; R^2, coefficient of determination.

Nutrient digestibility was clearly hampered by increasing level of dietary tannin and this occurred to both crude protein and fiber fractions. The results support a theory that tannin forms complexes with natural polymers such as protein and carbohydrate [1, 9] and, therefore, may reduce their digestibility in the digestive tract of ruminants. This binding property of tannin is resulted from a large number of free phenolic groups that form strong hydrogen bonds at multiple sites with protein [10, 11]. Tannin may also form complexes with protein through hydrophobic binding between the aromatic ring structure of tannin and hydrophobic region of the protein [12]. Additionally, covalent bonds may also be formed between protein and tannin through oxidative polymerization reaction as a result of heating, exposure to UV radiation and the action of polyphenol oxidase [13].

In agreement with McSweeney et al. [14], higher negative effect of dietary tannin on CP digestibility than that of fiber may suggest that the effect of tannin on fiber digestion is a secondary effect as compared to on protein digestion. Protein appears to have more possible binding sites with tannin than that of fiber since fiber appears to interact with tannin through only hydrogen bonds [10]; protein may also form complex with tannin through hydrophobic binding and covalent bonds as discussed above. In addition to the interaction between tannin and dietary components, the action of tannin on specific microorganisms may explain the above response as well. It may be possibly that proteolytic bacteria are more tannin sensitive than those of fiber degrading bacteria. This is perhaps supported by the work of

Min et al. [15] who observed that condensed tannin in *Lotus corniculatus* reduced the population of some proteolytic bacteria, but total ruminal microbial protein were remain unchanged.

Total gas production was not affected with dietary tannin level (table 3). Methane emission tended to decrease when expressed per unit of substrate (P=0.066), and significantly decreased when expressed per unit of total gas produced (P=0.005). The latter had a high R^2, i.e. 0.677. However, the relationship became insignificant when it was expressed as methane per unit of digestible organic matter, but again, the slope was remain negative.

Table 3. Effect of tannin level (g/kg dry matter) on gas production and methane emission.

Parameter	n	Intercept	Slope	P-value	R^2
Gas (ml/g)	14	81.1	–0.170	Ns	0.220
CH_4 (ml/g)	25	10.9	–0.026	0.066	0.231
CH_4 (ml/l gas)	14	170	–0.582	0.005	0.677
CH_4/DOM (ml/g)	25	27.0	–0.031	Ns	0.108

DOM, digestible organic matter; n, number of data; ns, non-significant; R^2, coefficient of determination.

It was obvious that methane emission decreased as the dietary tannin level increase. Part of the methane decrease appears to be due to the decrease in digestibility of nutrients, particularly fiber, which decreases hydrogen production as a substrate for methanogenesis [5, 16]. Another mechanism related to methane mitigating effect of tannin is through a direct effect in inhibiting methanogens. It has been previously demonstrated that pyrogallol, gallic acid and tannic acid, these are among the monomers of tannins, were toxic for methanogens [17]. Recent study of Bhatta et al. [18] reported that tannins suppressed the population of methanogens in vitro with an average decrease of 11.6% in hydrolysable tannin incubations and 28.6% in incubations containing both hydrolysable and condensed tannins when compared with incubations containing added polyethylene glycol-6000, an inactivating agent of tannins. Tannin apparently does not only reduce the population of methanogen, but also proved to inhibit the growth of *Methanobrevibacter ruminantium*, a common methanogen species in the rumen [16].

Dietary tannin had no significant effect on ruminal pH, ammonia concentration, bacteria and protozoa population (table 4). Dietary tannin had almost no effect on all VFA variables, except that the plant secondary compound linearly decreased butyrate concentration (P=0.013; table 5).

Table 4. Effect of tannin level (g/kg dry matter) on rumen fermentation and microbial population.

Parameter	n	Intercept	Slope	P-value	R^2
pH	25	7.00	0.0001	ns	0.010
NH_3 (mmol/l)	25	6.44	–0.0285	ns	0.155
Log bacteria	23	9.11	0.0008	ns	0.114
Log protozoa	23	3.58	0.0004	ns	0.008

n, number of data; ns, non-significant; R^2, coefficient of determination.

Table 5. Effect of tannin level (g/kg dry matter) on volatile fatty acid (VFA) profile.

Parameter	n	Intercept	Slope	P-value	R^2
Total VFA (mmol/l)	23	83.5	–0.0003	ns	0.000
C_2 (%)	23	63.5	0.0002	ns	0.000
C_3 (%)	23	22.2	0.0155	ns	0.140
C_4 (%)	23	10.9	–0.0139	0.013	0.403
isoC_4 (%)	19	0.72	0.0007	ns	0.042
C_5 (%)	19	2.75	–0.0007	ns	0.019
isoC_5 (%)	19	0.89	–0.0011	ns	0.126
C_2/C_3	23	2.93	–0.0011	ns	0.036

C_2, acetate; C_3, propionate; C_4, butyrate; C_5, valerate; n, number of data; ns, non-significant; R^2, coefficient of determination.

Lower dietary protein digestibility by the action of tannin was not confirmed by lower ruminal ammonia concentration as the final product of protein degradation in the rumen [13, 19]. It was also not confirmed by lower proportion of *iso*VFA, both *iso*C_4 and *iso*C_5. Theoretically, such *iso*VFA is produced during ruminal fermentation of branched-chain amino acids such as valine and leucine [20] and, hence, may reflect the degradation of protein. Apparently such insignificant effect of tannin on rumen fermentation profiles is related to its structural diversity in various plants [1].

Volatile fatty acid (VFA) reflects to certain extent the fermentation of dietary nutrients, especially carbohydrate fermentation [19, 21]. Along with the reduction of nutrient digestibility by increasing level of dietary tannin, total VFA concentration should have been reduced. This could be regarded to the possibly higher variation in the RUSITEC system since there are other influencing factors such as medium outflow [7], and rate of passage and absorption, while these do not occur in the in vitro batch system [22]. Dietary tannin had no clear effect on protozoa population. This is in agreement with Makkar [9] who stated that the effect of tannin on protozal count is variable. Such result might be related to a view that holotrich seem to be more susceptible to tannin than that of entodiniomorph [23] although the population of holotrich is much lower. So, the large pool of protozoa appears to be not that sensitive to the presence of tannin in the rumen. In addition to such inconsistent effect of tannin on ruminal protozoa, Patra and Saxena [24] suggested that tannin present in all types of plants is not equally effective on protozoa.

It has been known that ruminal protozoa are the host of some methanogens and may contribute to methane emission [25]. Thus a reduction in protozoal population may decrease some population of methanogen and decrease the methane emission as well. However, the present meta-analysis showed lack of effect of dietary tannin on protozoa population. Therefore, based on this meta-analysis, it seems not likely that methane reduction by increasing level of dietary tannin is related to the reduction in protozoa population. There is also uncertainty whether the methane reduction is connected to a shift in acetate to propionate proportion of the VFA. Theoretically, higher acetate leads to higher methane production since the production of acetate from pyruvate produces hydrogen. Conversely, higher propionate leads to lower methane since hydrogen is used by pyruvate to produce propionate [5]. However, the meta-analysis result does not likely to support an argument that methane reduction by increasing dietary tannin level is due to a decrease in acetate to propionate ratio.

4. Conclusion

Increasing level of dietary tannin leads to a decrease in ruminal methane emission. The decrease appears to be indirectly through reduction in digestibility of nutrients, particularly fiber, and directly through methanogen inhibition. However, based on the current meta-analysis, it seems not likely that methane reduction by increasing level of dietary tannin is related to a reduction in protozoa population and a shift in acetate to propionate ratio.

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