Influence of Tannin Concentration in Ration on Fermentation Parameters of Rumen Simulation Technique (RUSITEC): A Meta-analysis

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Abstract The objective of this study was to summarize and to quantify the effects of tannin on fermentation parameters of rumen simulation technique (RUSITEC) through a meta-analysis approach. Experiments reporting tannin concentration and rumen fermentation using the RUSITEC system were integrated into a database, and comprised of 6 studies and 25 treatments. Parameters recorded were nutrient digestibility, gas production, methane (CH_4) emission, short-chain fatty acid (SCFA) profiles, pH, ammonia (NH_3) and microbial population (bacteria and protozoa). The analysis of the data assembled in the database was based on mixed model methodology in which different studies were treated as random effects whereas tannin concentration was treated as fixed effects. Results revealed that *in vitro* crude protein digestibility (CPD), neutral detergent fiber digestibility (NDFD) and acid detergent fiber digestibility (ADFD) decreased linearly as the tannin concentration increased with the *P*-value of 0.047, 0.005 and 0.004, respectively. Comparing the magnitude of reduction in CP and fiber digestibility by the influence of tannin, the compound appeared to cause higher negative effect on CP digestibility than that of fiber as indicated by the slopes. However, the overall OMD did not significantly decrease although the slope remained negative. Methane emission tended to decrease at higher tannin concentration when expressed per unit of substrate (*P*=0.066) and significantly decreased when expressed per unit of total gas produced (*P*=0.005). It can be concluded that tannin is a potential compound for mitigating ruminal methane emission but its use on the other hand also decrease nutrient digestibility.

Keywords Tannin, Rumen, Fermentation, Rusitec, Meta-analysis

1. Introduction

Tannin is among plant secondary compounds produced by plants in their intermediary metabolism. It is polyphenolic compound with diverse structure (such as between hydrolysable and condensed tannin) and molecular weight but has similar property: it binds and precipitates protein [1]. With respect to ruminant nutrition, tannin is considered to have both beneficial and detrimental nutritional effects. Some of the beneficial effects of tannin are better utilization of dietary protein, faster growth rate, higher milk yield and improved animal health through prevention of bloat and nematode infection. Negative effects of tannin have been associated with its toxicity to rumen microbes and the animals especially when present at high concentration in ration (>50 g/kg dry matter) [2].

Research on tannin in relation to ruminant nutrition has been conducted under various experimental conditions, either *in vivo* (directly to the animal), *in sacco* (fistulated animal) or *in vitro* (laboratory equipment that mimic rumen fermentation). The latter method is divided into two groups, i.e. *in vitro* batch and *in vitro* continuous culture. Research synthesis of tannin effect on rumen digestion and fermentation based on *in vivo* and *in vitro* batch studies across various ruminant species has been previously performed through a meta-analytical approach [3]. However, the studies based on *in vitro* continuous culture like in rumen simulation technique or RUSITEC [4] have not been summarized. In the present study, therefore, a meta-analysis was conducted to summarize and to quantify the effects of tannin concentration on fermentation parameters of RUSITEC.

2. Materials and Methods

RUSITEC experiments reporting tannin concentration and rumen fermentation were integrated into a database (comprised of 6 studies and 25 treatments). The studies were Sliwinski et al. [5], Hess et al. [6-7], Tiemann et al. [8], Bekele et al. [9] and Khiaosa-ard et al. [10] (Table 1). Parameters recorded were nutrient digestibility, gas production, methane (CH_4) emission, short-chain fatty acid (SCFA) profiles, pH, ammonia (NH_3) and microbial population (bacteria and protozoa). Tannin forms were either from non-extracted or extracted tannins of plant origins, and constituted of different tannin types, i.e. hydrolysable, condensed or unspecified or mixed tannins. Such different tannin types were not a main point of interest but rather the amount or concentration of the tannin in the ration. Therefore, they were not stated as a categorical variable and not included in the statistical model. Studies reporting treatments with addition of polyethylene glycol (PEG) were excluded from the database since the substance may neutralize the effects of tannins under rumen environment.

The analysis of the data assembled in the database was made by a statistical meta-analysis approach [11]. Studies were treated as random effects whereas tannin concentration was treated as fixed effects using MIXED procedure of SAS version 9.2. The following statistical model was employed:

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

where, Y_{ij} = dependent variable, B_0 = overall intercept from all studies (fixed effect), B_1 = linear regression coefficient of Y on X (fixed effect), X_{ij} = value of the continuous predictor variable (dietary tannins), s_i = random effect of study i, b_i = random effect of study i on the regression coefficient of Y on X in study i, and e_{ij} = the unexplained residual errors. The study variable was declared in the CLASS statement since it does not contain any quantitative information. Data were weighted by the number of replicates each study and scaled to 1 to take into consideration of unequal variance among studies. Microbial population data were transformed into their logarithmic units to allow linear relationships with the independent variable. Model statistics presented are *P*-value and coefficient of determination (R^2).

Table 1. Studies included in the meta-analysis of the effect of tannin concentration on fermentation parameters of rumen simulation technique (RUSITEC)

Study no.	Reference	Basal feed	Tannin source	Tannin level (g/kg DM)
1	Sliwinski et al. (2002a)	Grass hay, silage and barley	Chestnut	0 to 2.5
2	Hess et al. (2006)	Koronivia grass	*Cratylia argentea* , *Calliandra calothyrsus*	0 to 135
3	Hess et al. (2008)	Koronivia grass	*Leucaena leucocephala*, *Flemingia macrophylla*, *Calliandra calothyrsus*	0 to 62.2
4	Tiemann et al. (2008a)	Koronivia grass	*Vigna unguiculata, Calliandra calothyrsus*	0 and 71
5	Bekele et al. (2009)	Koronivia grass	*Samanea saman*, *Acacia angustissima*, *Sesbania sesban, Cajanus cajan*	0 to 45
6	Khiaosa-Ard et al. (2009)	Grass-clover hay	*Onobrychis viciifolia*, *Acacia mearnsii*	0 and 78.9

3. Results and Discussion

In vitro crude protein digestibility (CPD), neutral detergent fiber digestibility (NDFD) and acid detergent fiber digestibility (ADFD) decreased linearly as the tannin concentration 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 tannin, the compound appeared to cause higher negative effect on CP digestibility than that of fiber as indicated by the slopes. An increase of tannin concentration 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. However, these figures might change if the slopes were corrected by the intercepts due to substantial different digestibility between CP and fiber at dietary tannins equal to 0 g/kg. All of these relationships had high R^2, i.e. higher than 0.4. The results support a theory that tannin may form complexes with some nutrients such as protein and carbohydrate and, therefore, may reduce their digestibility in the digestive tract of ruminants [1-2, 12]. However, the overall OMD did not significantly decrease although the slope remained negative. This was also the case for the total gas production.

Methane emission tended to decrease at higher tannin concentration 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. Explanation of the methane decrease due to tannin appears to be because of the decrease in digestibility of nutrients, particularly fiber, which decreases H_2 production as a substrate for methanogenesis as well as direct inhibition on methanogen population; the latter occurs since tannin is toxic to some groups of rumen microbes including the methanogen [3, 13]. Tannins had almost no effects on all SCFA variables, except that the substance linearly decreased C_4 (*P*=0.013, R^2=0.403). Dietary tannin had also no significant effects on ruminal pH, NH_3, bacteria and protozoa population.

Table 2. Equations for linear regression of the effect of tannin concentration on fermentation parameters of rumen simulation technique (RUSITEC)

Response parameter	n	Parameter estimates						R^2
		Intercept	SE intercept	*P* intercept	Slope	SE slope	*P* slope	
OMD (mg/g)	25	441.9	36.81	<0.001	–0.672	0.3918	ns	0.178
CPD (mg/g)	14	644.2	76.19	0.014	–2.921	1.2914	0.047	0.407
NDFD (mg/g)	25	323.6	26.56	<0.001	–1.231	0.3801	0.005	0.411
ADFD (mg/g)	14	277.1	20.42	<0.001	–1.549	0.4102	0.004	0.543
Gas (ml/g)	14	81.1	28.69	ns	–0.170	0.1298	ns	0.220
CH_4 (ml/g)	25	10.9	1.89	0.002	–0.0255	0.01301	0.066	0.231
CH_4 (ml/l gas)	14	170.4	47.73	0.07	–0.582	0.1631	0.005	0.677
Total SCFA (mmol/l)	23	83.5	10.89	0.002	–0.0003	0.04986	ns	0.000
C_2 (% total SCFA)	23	63.5	1.84	<0.001	0.0002	0.00929	ns	0.000
C_3	23	22.2	1.83	<0.001	0.0155	0.01129	ns	0.140
C_4	23	10.9	1.99	0.005	–0.0139	0.00501	0.013	0.403
iso-C_4	19	0.72	0.229	0.052	0.0007	0.00117	ns	0.042
C_5	19	2.75	0.894	0.054	–0.0007	0.00166	ns	0.019
iso-C_5	19	0.89	0.307	0.063	–0.0011	0.00101	ns	0.126
iso-SCFA	19	1.61	0.349	0.019	–0.0005	0.00166	ns	0.009
C_2/C_3	23	2.93	0.234	<0.001	–0.0011	0.00166	ns	0.036
pH	25	7.00	0.050	<0.001	0.0001	0.00035	ns	0.010
NH_3 (mmol/l)	25	6.44	1.862	0.018	–0.0285	0.01822	ns	0.155
log bacteria (10^9/ml)	23	9.11	0.313	<0.001	0.0008	0.00063	ns	0.114
log protozoa (10^4/ml)	23	3.58	0.127	<0.001	0.0004	0.00127	ns	0.008

ADFD, *in vitro* acid detergent fiber digestibility; C_2, acetate; C_3, propionate; C_4, butyrate; CPD, *in vitro* apparent crude protein digestibility; n, number of treatment; NDFD, *in vitro* neutral detergent fiber digestibility; OMD, *in vitro* organic matter digestibility; R^2, coefficient of determination; SCFA, short-chain fatty acids; SE, standard error.

4. Conclusion

Tannin is a potential natural compound for mitigating ruminal methane emission but its use on the other hand also decrease nutrient digestibility. Further investigation is therefore required to determine an optimum concentration of tannin in ration in which it mitigates methane emission and simultaneously supports rumen digestion and fermentation.

5. References

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