

Condensed Tannin Effects on Nitrogen Digestion in Ruminants: A Meta-analysis from *in Vitro* and *in Vivo* Studies

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ABSTRACT

Statistical meta-analysis approach was conducted to quantify the effect of a wide range of dietary condensed tannin (CT) concentration on nitrogen digestion in ruminants. A total of 19 studies from published papers and own previously unpublished studies comprising of 100 treatments were pooled in a database. The database was segregated into two categories based on different methods or systems where the experiments were carried out, i.e. *in vitro* (6 studies, 65 treatments) and *in vivo* experiments (13 studies, 35 treatments). Mixed model effects were applied to the data; different studies were treated as random effects whereas dietary CT (continuous predictor variable) was treated as fixed effects. The results showed that in the *in vitro* studies, organic matter digestibility (OMD) decreased linearly ($P=0.002$) as CT concentration in feed increased. Likewise, such linear decrease of OMD at increasing CT was observed in the *in vivo* studies ($P<0.001$) as well as crude protein digestibility (CPD, $P<0.001$). The variation on *in vitro* OMD was higher at lower level of CT. Nitrogen retention was not significantly affected by CT level. It was concluded that CT reduced nutrient digestibility in ruminants, but its effect on N retention was unclear from the present study.

Key words: condensed tannin, meta-analysis, nitrogen digestion, ruminant

INTRODUCTION

A major constraint of ruminant production in tropical countries like Indonesia is the poor quality of feed particularly roughage. Rice straw which contains a considerable proportion of ligno-cellulose and silica (Van Soest, 2006) is commonly fed to the ruminants especially in rural areas. Such practice is often linked to the poor performance and productivity of the animals due to inadequate nutrient supply. Supplementation with concentrate is an option to overcome the problem (Jayanegara & Sofyan, 2009). However, increasing cost and problem associated with continuous supply of the concentrate may reduce the applicability of such option. In many areas of the tropics, therefore, supplementation using shrubs and tree leaves provide a promising strategy since those roughages are generally rich in crude protein as compared to straws and grasses (e.g. Hess *et al.*, 2008; Camacho *et al.*, 2010).

In addition to its high crude protein content, many shrubs and tree leaves in the tropics contain high con-

tents of plant secondary compounds particularly tannins (Mueller-Harvey, 2006; Makkar *et al.*, 2007). Tannins are polyphenolic compounds which are able to interact with other macromolecules such as proteins and carbohydrates. They are divided into two major groups, i.e. hydrolysable and condensed tannins. Hydrolysable tannins are the polymer of gallic or ellagic acid that esterified with sugar molecule, while condensed tannins are polymer of flavonoid compounds (Goel *et al.*, 2005). Condensed tannins are considered to have both detrimental and beneficial effects, depend on the source and concentration applied (Makkar, 2003).

Although there have been a number of experiments conducted to observe the influence of CT on nitrogen digestion of ruminants, the scope was limited in a relatively narrow and limited range of dietary CT concentration. No studies so far have attempted to integrate the findings quantitatively and come out with a more generalized result. In the present study, therefore, a statistical meta-analysis approach (Sauvant *et al.*, 2008) was conducted to quantify the effect of a wide range of dietary condensed tannin concentration on nitrogen digestion in ruminants. To take into account on different methods used in assessing the effect of CT on nitrogen digestion, experiments generated by *in vitro* and *in vivo* methods were both considered in the analysis.

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MATERIALS AND METHODS

Meta-data Development

A database was constructed from experiments reporting dietary condensed tannins and measuring variables related to N digestion such as organic matter digestibility (OMD), crude protein digestibility (CPD), N retention, ammonia (NH₃) and *iso* short-chain fatty acids (*iso*SCFA) production. In addition, other related variables such as bacterial and protozoal counts were also pooled in the database. The database was initially developed to examine the relationship between dietary tannins in general (both condensed and hydrolysable tannins) and ruminal methane production (*in vitro* and *in vivo*), of which the results will be published elsewhere.

Condensed tannin forms were either from non-extracted or extracted tannins of plant origins. Studies reporting treatments with addition of polyethylene glycol (PEG) were excluded from the database since the substance may partially or completely neutralize the effects of tannins under rumen environment (Getachew *et al.*, 2001; Jayanegara & Sofyan, 2008).

A total of 19 studies from published papers and own previously unpublished studies comprising of 100 treatments were pooled in the database (Table 1). Papers reporting more than one experiment were individually encoded. The database was segregated into two categories based on different methods or systems where the experiments were carried out, i.e. *in vitro* (6 studies, 65 treatments) and *in vivo* experiments (13 studies, 35 treatments). *In vitro* studies were conducted using

Table 1. Studies included in the meta-analysis of the effect of condensed tannin concentrations in feeds on nitrogen digestion of ruminants

Study no.	Reference	System	Basal feed	CT source	CT form	CT level (g/kg DM)
1	Tavendale <i>et al.</i> (2005)	<i>In vitro</i>	- *)	<i>Medicago sativa</i> , <i>Lotus pedunculatus</i>	Non-extracted	0.2 and 99.0
2	Jayanegara <i>et al.</i> (2009)	<i>In vitro</i>	Grass hay and barley straw	<i>Rhus typhina</i> , <i>Salix alba</i>	Non-extracted	0 to 14.5
3	Bhatta <i>et al.</i> (2009)	<i>In vitro</i>	-	Mimosa, quebracho	Extracts	13.3 to 36.7
4	Own study 1	<i>In vitro</i>	-	Various tropical plants (n=27)	Non-extracted	0 to 86.0
5	Own study 2	<i>In vitro</i>	-	Various temperate plants (n=18)	Non-extracted	0 to 9.0
6	Own study 3	<i>In vitro</i>	Grass hay and concentrate	Mimosa, quebracho	Purified extract	0 to 87.7
7	Woodward <i>et al.</i> (2002)	<i>In vivo</i> cattle	-	<i>Hedysarium coronarium</i>	Non-extracted	0 and 27.2
8	Woodward <i>et al.</i> (2004)	<i>In vivo</i> cattle	-	<i>Lotus corniculatus</i>	Non-extracted	0 and 26.2
9	Pinares-Patino <i>et al.</i> (2003)	<i>In vivo</i> alpaca	-	<i>Lotus corniculatus</i>	Non-extracted	0.9 and 23.5
10	Pinares-Patino <i>et al.</i> (2003)	<i>In vivo</i> sheep	-	<i>Lotus corniculatus</i>	Non-extracted	0.8 and 43.6
11	Carulla <i>et al.</i> (2005)	<i>In vivo</i> sheep	Ryegrass, red clover, alfalfa	<i>Acacia mearnsii</i>	Extract	0 and 25.0
12	Puchala <i>et al.</i> (2005)	<i>In vivo</i> goat	-	<i>Digitaria ischaemum</i> , <i>Lespedeza cuneata</i>	Non-extracted	5.0 and 177
13	Beauchemin <i>et al.</i> (2007)	<i>In vivo</i> cattle	Barley silage, concentrate	Quebracho	Extract	0 to 18.2
14	De Oliveira <i>et al.</i> (2007)	<i>In vivo</i> cattle	-	Sorghum silage	Non-extracted	0.2 to 1.0
15	Animut <i>et al.</i> (2008a)	<i>In vivo</i> goat	-	<i>Lespedeza cuneata</i> , <i>Lespedeza striata</i>	Non-extracted	140 to 151
16	Animut <i>et al.</i> (2008b)	<i>In vivo</i> goat	Sorghum, Sudan grass	<i>Lespedeza striata</i>	Non-extracted	0.3 to 151
17	Tiemann <i>et al.</i> (2008)	<i>In vivo</i> sheep	Palisade grass	<i>Flemingia macrophylla</i> , <i>Calliandra calothyrsus</i>	Non-extracted	0 to 32.8
18	Grainger <i>et al.</i> (2009)	<i>In vivo</i> cattle	Ryegrass pasture, concentrate	<i>Acacia mearnsii</i>	Extract	0 to 18
19	Ramirez-Restrepo <i>et al.</i> (2010)	<i>In vivo</i> sheep	-	<i>Salix</i> spp.	Non-extracted	2.1 and 34.2

CT, condensed tannins

*) The CT sources were not mixed with any of basal feeds.

Hohenheim gas test, syringe gas generator and glass bottle incubation. For the *in vivo* database, all ruminant species were included, i.e. alpaca, goat, sheep, and cattle.

Statistical Analysis

The analysis of the data assembled in the database was made by a statistical meta-analysis approach (Sauvant *et al.*, 2008). Studies were treated as random effects whereas dietary condensed tannins were treated as fixed effects using the procedure MIXED of SAS version 9.2 (SAS Institute Inc., 2008) and using the following model:

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

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 condensed 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 did not contain any quantitative information. In addition, an unstructured variance-covariance matrix (type = un) was declared as the random part of the model to avoid the positive correlation between the intercepts and slopes (St-Pierre, 2001). Data were unweighted by the number of replicates in each study (Desnoyers *et al.*, 2009). Presence of outliers was identified by examining studentized residuals; values beyond ± 3 SD were considered as outliers and, therefore, were removed from the dataset. For graphical presentation of the meta-analysis results, adjustments were made to the response variables to take into account the random effect of study (Patra, 2010).

For the N retention variable, body size of the ruminants was standardized by relating them to meta-

bolic body weight ($BW^{0.75}$) to counterbalance the variation associated with body weight among and within ruminant species. Data reported in different units of measurements were transformed into the same units. Microbial counts variables (both bacterial and protozoal counts) 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). Since the data were unbalance across all variables, meta-analysis was performed based on the available data for each variable.

RESULTS AND DISCUSSION

Effects on Digestion and Ruminal Fermentation

In the *in vitro* studies, organic matter digestibility (OMD) decreased linearly ($P=0.002$) as CT concentration in feed increased (Figure 1). Likewise, such linear decrease of OMD at increasing CT was observed as well in the *in vivo* studies ($P<0.001$) (Figure 2). The results indicated that the nutrient digestibility was clearly hampered by increasing level of CT as confirmed by both *in vitro* and *in vivo* studies. This might be connected to a theory that tannins form complexes with natural polymers such as proteins and carbohydrates (McSweeney *et al.*, 2001; Makkar, 2003; Mueller-Harvey, 2006; Jayanegara *et al.*, 2009) and, therefore, may reduce their digestibility in the digestive tract of ruminants. The binding property of tannins is resulted from a large number of free phenolic groups that form strong hydrogen bonds at multiple sites with proteins (Silanikove *et al.*, 2001). Tannins may also form complexes with proteins through hydrophobic binding between the aromatic ring structure of tannins and hydrophobic regions of the proteins (Smith *et al.*, 2005). Additionally, covalent bonds may also be formed between protein and tannins through oxidative polymerization reactions as a result of heating, exposure to UV radiation and the action of polyphenol oxidase (Reed,

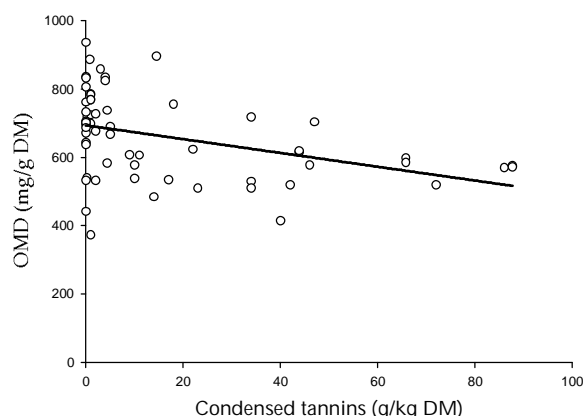


Figure 1. Relationship between dietary condensed tannin concentration and organic matter digestibility in the *in vitro* studies (OMD= $693.8 - 2.02 \text{ CT}$, $P=0.002$, $R^2=0.160$).

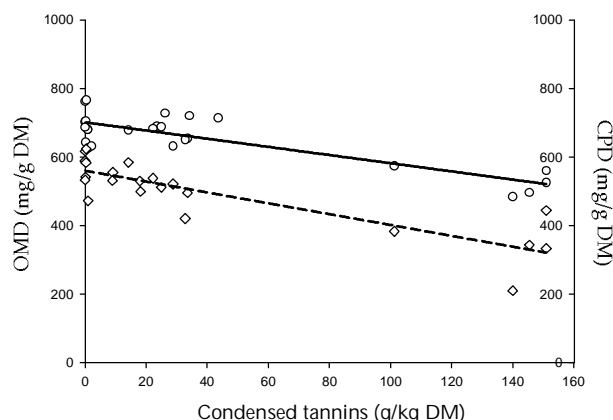


Figure 2. Relationships between dietary condensed tannin concentration and organic matter digestibility (\circ -, full regression line; OMD= $701.2 - 1.19 \text{ CT}$, $P<0.001$, $R^2=0.701$) and crude protein digestibility (\diamond -, dashed regression line; CPD= $559.7 - 1.59 \text{ CT}$, $P<0.001$, $R^2=0.730$) in the *in vivo* studies.

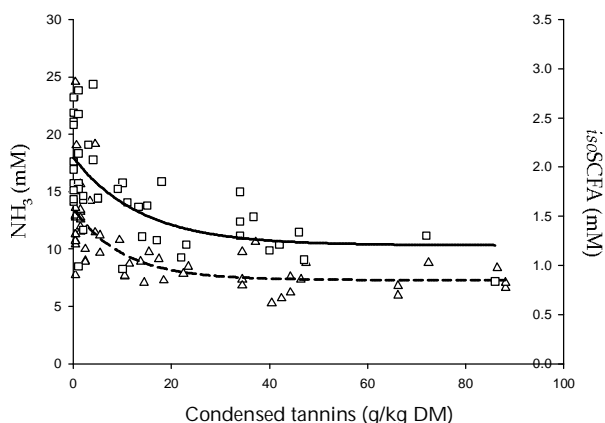


Figure 3. Relationships between dietary condensed tannin concentration and ruminal NH_3 (-□-, full regression line; $\text{NH}_3 = 10.3 + 7.66 e^{-0.073 \text{ CT}}$, $P < 0.001$, $R^2 = 0.443$) and isoSCFA (-Δ-, dashed regression line; $\text{isoSCFA} = 0.85 + 0.70 e^{-0.099 \text{ CT}}$, $P < 0.001$, $R^2 = 0.48$) concentrations in the *in vitro* studies.

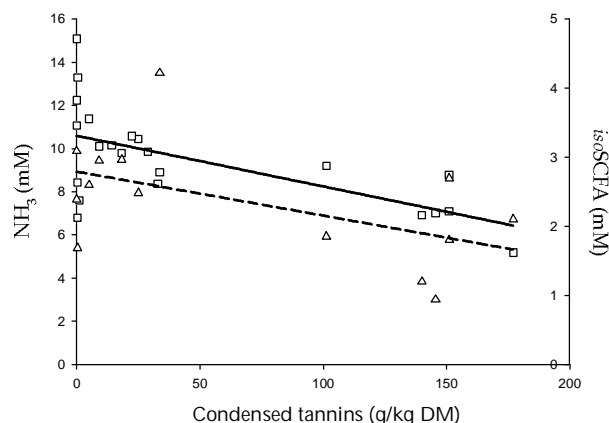


Figure 4. Relationships between dietary condensed tannin concentration and ruminal NH_3 (-□-, full regression line; $\text{NH}_3 = 10.6 - 0.024 \text{ CT}$, $P = 0.002$, $R^2 = 0.40$) and isoSCFA (-Δ-, dashed regression line; $\text{isoSCFA} = 2.79 - 0.006 \text{ CT}$, $P = 0.051$, $R^2 = 0.281$) concentrations in the *in vivo* studies.

1995). It is of interest to note that the variation on *in vitro* OMD was higher at lower level of CT. This may be addressed as an explanation on the inconsistency effect of CT on nitrogen digestion at low level of application.

From the *in vivo* studies, crude protein digestibility (CPD) decreased as CT level increase, and the relationship was stronger than that of OMD. This suggests that CT may have a stronger interaction with protein than those of other organic components in the diet, particularly fiber fractions. In agreement with McSweeney *et al.* (2001), higher negative effect of dietary tannins on CP digestibility than that of fiber suggests that the effect of tannins on fiber digestion is a secondary effect as compared to its effect on protein digestion. Protein appears to have more possible binding sites with tannins than that of fiber since fiber appears to interact with tannins through only hydrogen bonds (Silanikove *et al.*, 2001); protein may also complex with tannins through hydrophobic binding and covalent bonds as discussed above. It may be possibly also that proteolytic bacteria are more tannin sensitive than those of fiber degrading bacteria. This is perhaps supported by the work of

Min *et al.* (2002) who observed that condensed tannins in *Lotus corniculatus* reduced the populations of some proteolytic bacteria, but total ruminal microbial protein were remain unchanged.

The negative effect of CT on CP digestibility was supported by the results from NH_3 and isoSCFA variables. During ruminal digestion and fermentation, protein is degraded by certain microbes to result NH_3 . IsoSCFA, mainly comprised of isobutyrate and isovalerate, are also connected to protein degradation in the rumen. They are the specific products from deamination of branched chain amino acids (Hoffmann *et al.*, 2008). Therefore, these variables could be used as indicators for the extent of protein degradation in the rumen. Ruminal NH_3 ($P < 0.001$) and isoSCFA production ($P < 0.001$) decreased with the increasing level of CT in the *in vitro* studies (Figure 3). However, the decrease was not linear. Instead, both curves followed exponential decay models and stabilized after around 40 g/kg CT. Different pattern was observed from the *in vivo* studies. Within this type of studies, ruminal NH_3 ($P = 0.002$) and isoSCFA ($P = 0.051$) decreased linearly as increasing level of CT (Figure 4).

Table 2. Equations for linear regression of dietary condensed tannin (in g/kg DM) on microbial counts and nitrogen retention

Response variable	n	Variable estimates					
		Intercept	SE intercept	P intercept	Slope	SE slope	P slope
<i>In vitro</i> studies							
Log bacteria	45	9.49	0.024	0.002	0.002	0.0006	<0.001
Log protozoa	48	4.36	0.080	<0.001	0.001	0.0008	ns
<i>In vivo</i> studies							
Log bacteria	9	10.34	0.374	0.001	−0.001	0.0022	ns
Log protozoa	13	5.87	0.160	<0.001	−0.001	0.0014	ns
Nitrogen retention (g/d)	13	2.18	1.346	ns	0.013	0.0099	ns

n, number of observation; ns, not significant at $P < 0.05$; SE, standard error.

Effects on Microbial Counts and Nitrogen Retention

Increasing CT level in feed led to a linear increase in log bacterial counts from the *in vitro* studies ($P < 0.001$), although the increase was quite small (Table 2). However, the significance could not be observed in the *in vivo* studies. Log protozoal counts were not significantly affected by CT concentrations in both *in vitro* and *in vivo* studies. This is in agreement with Makkar (2003) who stated that the effects of tannins on protozoal counts are variable. Such result might be related to a view that holotrichs seem to be more susceptible to tannins than those of entodiniomorphs (Makkar *et al.*, 1995; Carulla *et al.*, 2005) although the population of holotrichs is much lower. So, the large pool of protozoa appears to be not that sensitive to the presence of tannins in the rumen. In addition to such inconsistent effect of CT on ruminal protozoa, Patra & Saxena (2009) suggested that tannins present in all types of plants are not equally effective on protozoa.

Interestingly, although CT was shown to have a negative effect on ruminal nitrogen digestion, it did not significantly affect nitrogen retention in the body of ruminants. This could be possibly due to the high variability of N retention over different CT levels. Tannins may reduce the amount of protein that is degraded in the rumen and increase the amount of available protein in the small intestine or known as ruminal by-pass protein (Min *et al.*, 2003). Therefore, lower N absorption in the rumen may occur and shifted towards higher N flow to the small intestine. As the consequence, lower urinary N and higher faecal N could be expected. However, tannins have complicated and great structural diversity. This implies to the diverse activity of tannins and even slight changes in the structures can produce measurable effects (Mueller-Harvey, 2006). It is then unsurprising that the N retention is quite variable in the presence of CT.

CONCLUSION

Dietary condensed tannins reduced organic matter digestibility linearly in both *in vitro* and *in vivo* studies. At low level of CT, the variation on *in vitro* OMD was higher. This was supported by the results from NH_3 and *iso*SCFA variables in the both types of studies; ruminal NH_3 and *iso*SCFA decreased with increasing level of dietary CT. No clear effects of CT on microbial (bacterial and protozoal) counts were observed. Although CT was shown to have a negative effect on ruminal nitrogen digestion, it did not significantly affect nitrogen retention in the body of ruminants.

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