**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 contents 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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**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.

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Key words: condensed tannin, meta-analysis, nitrogen digestion, ruminant
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 (isoSCFA) 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 \((\text{in vitro and in vivo})\), 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. \(\text{in vitro}\) (6 studies, 65 treatments) and \(\text{in vivo}\) experiments (13 studies, 35 treatments). \(\text{In vitro}\) studies were conducted using

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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 et al. (2005) | \textit{In vitro} | Medicago sativa, Lotus pedunculatus | Non-extracted | 0.2 and 99.0 |
| 2         | Jayanegara et al. (2009) | \textit{In vitro} | Rhus typhina, Salix alba              | Non-extracted | 0 to 14.5   |
| 3         | Bhatta et al. (2009)       | \textit{In vitro} | Mimosa, quebracho                    | Extracts                  | 13.3 to 36.7|
| 4         | Own study 1                | \textit{In vitro} | Various tropical plants (n=27)       | Non-extracted             | 0 to 86.0  |
| 5         | Own study 2                | \textit{In vitro} | Various temperate plants (n=18)      | Non-extracted             | 0 to 9.0   |
| 6         | Own study 3                | \textit{In vitro} | Grass hay and concentrate            | Purified extract          | 0 to 87.7  |
| 7         | Woodward et al. (2002)     | \textit{In vivo} | Hedysarium coronarium                | Non-extracted             | 0 and 27.2 |
| 8         | Woodward et al. (2004)     | \textit{In vivo} | Lotus corniculatus                   | Non-extracted             | 0 and 26.2 |
| 9         | Pinares-Patino et al. (2003) | \textit{In vivo} | Lotus corniculatus                   | Non-extracted             | 0.9 and 23.5|
| 10        | Pinares-Patino et al. (2003) | \textit{In vivo} | Lotus corniculatus                   | Non-extracted             | 0.8 and 43.6|
| 11        | Carulla et al. (2005)      | \textit{In vivo} | Acacia mearnsii                      | Extract                   | 0 and 25.0 |
| 12        | Puchala et al. (2005)      | \textit{In vivo} | Digitaria ischaemum, Lespedeza cuneata | Non-extracted             | 5.0 and 177|
| 13        | Beauchemin et al. (2007)   | \textit{In vivo} | Barley silage, concentrate           | Quebracho                 | 0 to 18.2  |
| 14        | De Oliveira et al. (2007)  | \textit{In vivo} | Sorghum silage                       | Non-extracted             | 0.2 to 1.0 |
| 15        | Anmut et al. (2008a)       | \textit{In vivo} | Lespedeza cuneata, Lespedeza striata | Non-extracted             | 140 to 151 |
| 16        | Anmut et al. (2008b)       | \textit{In vivo} | Lespedeza striata                    | Non-extracted             | 0.3 to 151 |
| 17        | Tiemann et al. (2008)      | \textit{In vivo} | Flemingia macrophylla, Calliandra caleothyes | Non-extracted             | 0 to 32.8  |
| 18        | Grainger et al. (2009)     | \textit{In vivo} | Acacia mearnsii                      | Extract                   | 0 to 18    |
| 19        | Ramirez-Restrepo et al. (2010) | \textit{In vivo} | Salix 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 vitro 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:

\[ Y_{ij} = B_0 + B_1 X_{ij} + s_i + b_i + e_{ij} \]

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), \( s_i \) = value of the continuous predictor variable (dietary condensed tannins), \( b_i \) = random effect of study \( i \), \( e_{ij} \) = 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 metabolic 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,
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 iso butyrate and iso valerate, 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 ($P=0.051$) decreased linearly as increasing level of CT (Figure 4). 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 | Intercept | SE intercept | $P$ intercept | Slope | SE slope | $P$ slope |
|-------------------|---|-----------|--------------|--------------|-------|----------|----------|
| **In vitro 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       |
| **In vivo 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.

Figure 3. Relationships between dietary condensed tannin concentration and ruminal NH$_3$ ($\circ-$, full regression line; $\text{NH}_3 = 10.3 + 7.66 e^{-0.073CT}, P<0.001, R^2=0.443$) and isoSCFA ($\Delta-$, dashed regression line; $\text{isoSCFA} = 0.85 + 0.70 e^{-0.030CT}, P<0.001, R^2=0.48$) concentrations in the in vitro studies.

Figure 4. Relationships between dietary condensed tannin concentration and ruminal NH$_3$ ($\circ-$, full regression line; $\text{NH}_3 = 10.6 – 0.024 CT, P = 0.002, R^2=0.40$) and isoSCFA ($\Delta-$, dashed regression line; $\text{isoSCFA} = 2.79 – 0.006 CT, P = 0.051, R^2=0.281$) concentrations in the in vivo studies.
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 NH3 and isoSCFA variables in the both types of studies; ruminal NH3 and isoSCFA 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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