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Silage quality as influenced by concentration and type of tannins present in the material ensiled: A meta-analysis

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Abstract

Protein degradation during ensiling is a major problem. Tannins are known to prevent or decelerate protein degradation in the rumen and may be able to do so in silages as well. Therefore, the present evaluation aimed to analyse the influence of tannins on silage quality. This was done by integrating from all suitable experiments found in literature on the topic in a meta-analysis approach. A total of 122 datasets originating from 28 experiments obtained from 16 published articles and one own unpublished experiment were included in the database. Tannins in the silages originated either from the plants ensiled or from supplementations of tanniferous plants or tannins extracted from such plants. Tannin concentrations ranged from 0 to 57.8 g/kg dry matter, and the ensiling period varied from 30 to 130 days. The analysis was based on the linear mixed model methodology in which the different studies were considered as random effects and tannin-related properties (either concentration or type of tannins) were treated as fixed effects. Results revealed that greater concentrations of tannins were associated with a decrease of butyrate concentration in the silages ($p < 0.05$). An increasing tannin concentration was also accompanied with smaller proportions of soluble N, free amino acid N, non-protein nitrogen and NH_3-N in total silage *N* ($p < 0.05$). The relationships between hydrolysable and condensed tannins and the decline in butyrate and NH_3-N concentrations in the silages were of different magnitude ($p < 0.05$). A higher tannin concentration was associated with a decline in in vitro dry matter digestibility. It was concluded that tannins apparently have the ability to limit extensive proteolysis which may occur during ensiling and thus may improve the fermentative quality of silages. A desired side effect seems to be given by the tannins' apparent property to limit the activity of the butyrate-producing microbes.

KEYWORDS

ammonia, butyrate, polyphenol, proteolysis, silage, tannin

1 | INTRODUCTION

Ensiling is a common practice of preserving forages and it is characterized by lower risks of loss of feeding value due to unfavourable weather conditions and harvest loss in comparison with haymaking (Bolsen, Ashbell, & Weinberg, 1996; Coblentz & Akins, 2018). Silage can be made both in hot and cold regions (Bernardes et al., 2018), and it may be fed to livestock either in summer (Woodward, Chaves,

TABLE 1 Studies included in the meta-analysis of the effects of dietary tannin concentrations on silage quality

Exp.	Reference	Substrate	Tannin source	Tannin form	Tannins (g/kg DM)	Ensiling period (days)
1	Albrecht and Muck (1991)	*Sericea lespedeza*, birdsfoot trefoil, red clover, sainfoin, cicer milk vetch	Substrates are sources	Intact material	0–26.5	35
2	Albrecht and Muck (1991)	*Sericea lespedeza*, birdsfoot trefoil, red clover, sainfoin, cicer milk vetch, lotus	Substrates are sources	Intact material	0–31.1	35
3	Salawu, Acamovic, Stewart, Hvelplund, and Weisbjerg (1999)	Ryegrass	Mimosa (CT), myrabolam (CT), quebracho (CT)	Extract	0–50.0	32
4	Salawu et al. (1999)	Ryegrass	Mimosa (CT), quebracho (CT)	Extract	0–50.0	49
5	Salawu, Warren, and Adesogan (2001)	Pea/wheat 3:1	Quebracho (CT)	Extract	0–53.2	112
6	Adesogan and Salawu (2002)	Pea/wheat 3:1	Quebracho (CT)	Extract	0–51.6	112
7	Adesogan and Salawu (2002)	Pea/wheat 1:3	Quebracho (CT)	Extract	0–44.9	112
8	Kondo, Kita, and Yokota (2004a)	Sudangrass	Green tea waste, oolong tea waste, black tea waste	Intact material	2.7–15.1	30
9	Kondo, Naoki, Kazumi, and Yokota (2004)	Oat	Green tea waste	Intact material	0–5.0	30
10	Kondo et al. (2004)	Sudangrass	Green tea waste, green tea polyphenols	Intact material	0–10.2	30
11	Kondo et al. (2004)	Sudangrass	Green tea waste, green tea polyphenols	Intact material	0–34.7	30
12	Kondo et al. (2004)	Sudangrass	Green tea waste	Intact material	0–4.6	45
13	Kondo, Kita, and Yokota (2004b)	Oat	Green tea waste	Intact material	1.5–11.5	50
14	Kondo, Kita, and Yokota (2006)	Tofu cake	Green tea waste	Intact material	2.2–11.1	30
15	Kondo et al. (2006)	Tofu cake/rice bran	Green tea waste	Intact material	2.3–8.7	30
16	Kondo et al. (2006)	Tofu cake/rice straw	Green tea waste	Intact material	2.4–5.9	30
17	Kondo et al. (2006)	Tofu cake/rice bran/rice straw	Green tea waste	Intact material	2.4–5.4	30
18	Tabacco et al. (2006)	Alfalfa	Chestnut (HT)	Extract	0–46.2	70
19	Tabacco et al. (2006)	Alfalfa	Chestnut (HT)	Extract	0–30.8	78
20	Xu, Cai, Moriya, and Ogawa (2007)	Total mixed ration	Green tea	Intact material	0.6–15.9	45
21	Cavallarin, Tabacco, and Borreani (2007)	Lucerne	Chestnut (HT)	Extract	0–30.8	120
22	de Oliveira, Berchielli, Reis, Vechetini, and Pedreira (2009)	Sorghum	Substrate	Intact material	0.2–5.9	120

(*Continues*)

TABLE 1 (Continued)

Exp.	Reference	Substrate	Tannin source	Tannin form	Tannins (g/kg DM)	Ensiling period (days)
23	Colombini, Colombari, Crovetto, Galassi, and Rapetti (2009)	Lucerne	Chestnut (HT)	Extract	0–46	130
24	Deaville et al. (2010)	Ryegrass	Mimosa (CT), chestnut (HT)	Extract	0–55.6	90
25	Lorenz, Eriksson, and Uden (2010)	Direct-cut sainfoin	Substrate is source	Intact material	32.2–42.4	60
26	Lorenz et al. (2010)	Wilted sainfoin	Substrate is source	Intact material	41.2–57.8	60
27	Santoso, Hariadi, Manik, and Abubakar (2011)	King grass	Acacia (CT)	Extract	0–10.3	30
28	Own unpublished data	Total mixed ration	Chestnut (HT), quebracho (CT)	Extract	0–46.7	30

Note. CT, condensed tannin; DM, dry matter; HT, hydrolysable tannin.

Waghorn, Brookes, & Burke, 2006) or winter (Keogh, French, McGrath, Storey, & Mulligan, 2009). Another main benefit of preparing silage is the bale technology where storage can be accomplished without buildings. It thus plays an important role especially in intensive livestock production systems (Weinberg et al., 2011). Almost all forages including maize, sorghum, grasses and legumes can be preserved as silages. However, ensiling of forages that are rich in protein, especially the legumes such as alfalfa, has some drawbacks, whereof the most important is the potentially rapid and extensive degradation of protein before and during fermentation (Lynch, Jin, Lara, Baah, & Beauchemin, 2014; Nguyen, Kawai, Takahashi, & Matsuoka, 2004; Owens, Albrecht, Muck, & Duke, 1999). This degradation is the result of the action of plant and microbial enzymes, particularly proteases, during the ensiling process. It mostly consists in a conversion of plant protein to non-protein nitrogen (NPN), a change which may negatively influence intake and utilization of forage N by ruminant livestock (Givens & Rulquin, 2004; Huhtanen, Rinne, & Nousiainen, 2008). Further, the formation of the basic ammonium ions counteracts a rapid pH decline in the ensiling process which is essential for producing silage resistant against deterioration before (butyrate) and after opening (resumed fermentation). Therefore, effective, economic and save methods to protect or at least decelerate protein degradation during ensiling of high protein forages are required to preserve or even increase the proportion of rumen undegradable (by-pass) protein as a component of the metabolizable protein, that is the protein that is absorbed in the small intestine (Burroughs, Nelson, & Mertens, 1975; Rinne, Nousiainen, & Huhtanen, 2009).

A number of either direct or indirect treatments aiming to protect protein degradation during ensiling are or have been applied. These include the use of formaldehyde and of formic acid (Guo, Ding, Han, & Zhou, 2008). Despite their successful application, these substances are considered as toxic chemicals and may cause irritation on skin, eye and respiratory organs (Golden, 2011; Liesivuori & Savolainen, 1991). A group of natural substances present in various plants that has the property of protecting protein from microbial degradation is given by the tannins (Mueller-Harvey, 2006). This interaction between tannins and proteins is possible due to the presence of multiple phenolic hydroxyl groups in tannins which facilitate the establishment of bonds with proteins. The nature and stability of the bonds depend on the characteristics of both tannins and proteins in terms of molecular weight, tertiary structure, isoelectric point and compatibility of binding sites (Reed, 1995). Apart from their ability to protect protein degradation, or as a result of it, tannins have quite often been reported to provide other beneficial effects such as increasing the productivity of ruminants (Mueller-Harvey, 2006), decreasing gastro-intestinal parasitic nematodes (Azuhnwi et al., 2013; Hoste, Jackson, Athanasiadou, Thamsborg, & Hoskin, 2006), mitigating ruminal methane emission (Jayanegara, Leiber, & Kreuzer, 2012; Jayanegara, Marquardt, Wina, Kreuzer, & Leiber, 2013), inhibiting biohydrogenation of polyunsaturated fatty acids in the rumen (Jayanegara, Kreuzer, & Leiber, 2012; Jayanegara, Kreuzer, Wina, & Leiber, 2011) and elevating proportions of beneficial fatty acid in animal-source foods (Vasta, Nudda, Cannas, Lanza, & Priolo, 2008). This indicates that silages containing tannins may not only ferment in a more favourable way but may also have an inherently higher feeding value as such in addition.

Although there have been some studies on the influence of tannins on silage fermentation quality, no study has yet summarized the results obtained across different studies. Furthermore, it appears that there are contrasting results on whether or not tannins can improve the quality of silage or if they may even decrease its quality. This warrants data analysis with respect to the nature of the tannins present in the material ensiled in the experiments carried out so far. Therefore, the present meta-analysis aimed to relate the nature of influence of tannins on silage quality with both concentration and type of tannins, namely hydrolysable tannins (HT) and condensed tannins (CT). It was hypothesized that tannins would inhibit proteolysis in silage, and different type of tannins would elicit different responses in inhibiting the proteolysis.

2 | MATERIALS AND METHODS

2.1 | Database development

A database was constructed from published literature reporting the influence of dietary tannin concentration on silage quality. The literature included was obtained from the Scopus database, searched with "tannin," "silage" and "quality" as keywords. From that, initially 32 articles were identified. Criteria for studies to be included in the database were as follows: (a) the article was published in English, (b) tannin concentration of the materials ensiled was reported, and (c) silage fermentation parameters were reported. After subsequent abstract and full-text evaluation, 16 articles (describing of 27 experiments in total) met the respective criteria (Table 1). Additionally, data a 28th experiment (own and unpublished) were also integrated in the database.

There was a distinct variation among experimental conditions in the studies considered. Tannins were either added from an external source or were inherently present in the plant material ensiled. In addition, tannin presentation differed by either tannins being present in non-extracted (plant containing tannins) or in a concentrated form after extraction. Data from all types of tannins were included in the database and were classified into HT, CT or "not specified." Tannin from chestnut extract was categorized as HT whereas tannin extracts from mimosa, myrabolam, quebracho and acacia were categorized as CT (Jayanegara, Goel, Makkar, & Becker, 2015). The "non-specified tannins" referred to the plant containing tannins, that is *Sericea lespedeza*, birdsfoot trefoil, red clover, sainfoin, cicer milk vetch, lotus, tea waste and sorghum; these plants contain both HT and CT at varying proportions. In the experiments, the concentrations of tannins ranged from 0 (typically in control substrate) to 57.8 g/kg DM. Ensiling periods varied from 30 to 130 days. Data from variants where the material had been treated with polyethylene glycol were not included in the database since this compound is known to be a tannin-deactivating agent (Kondo et al., 2014). Silage fermentation quality criteria included in the dataset were pH, lactate, acetate, propionate, butyrate, ethanol, total N, soluble N, free amino acid N (free AA-N), non-protein nitrogen (NPN), ammonia N (NH_3-N) and in vitro dry matter digestibility (IVDMD). When an article reported more than one experiment, each individual entity was encoded separately. An experiment reporting the effects obtained with more than one ensiling material was also encoded separately. Prior to being transferred to the database, all data were recalculated to the same units of measurements in order to allow comparison within variables.

2.2 | Statistical analysis

Data analysis was performed according to a meta-analysis technique based on the linear mixed model methodology (Sauvant, Schmidely, Daudin, & St-Pierre, 2008), in which different experiments were considered as random effects and tannin-related factors (either concentration or form or type of tannins) were treated as fixed effects. The assessment of the influence of tannin concentration and tannin form (non-extracted or extracted) or tannin type (CT or HT; considering only treatments where tannin extracts were added) on silage quality was accomplished by employing the following statistical model:

$$Y_{ijk} = \mu + s_i + \tau_j + s\tau_{ij} + B_1 X_{ij} + b_i X_{ij} + B_j X_{ij} + e_{ijk}$$

where Y_{ijk} = dependent variable, μ = overall mean, s_i = random effect of the ith experiment, τ_j = fixed effect of the jth level of factor τ, $s\tau_{ij}$ = random interaction between the ith experiment and the jth level of factor τ, B_1 = linear regression coefficient of Y on X (fixed effect), X_{ij} = value of the continuous predictor variable (tannin concentration), b_i = random effect of experiment i on the regression coefficient of Y on X in experiment i, B_j = effect of jth level of the discrete factor τ on the regression coefficient (fixed effect), and e_{ijk} = the unexplained residual error. The model used was weighting the observations with their number of replicates. The variables "experiment," "tannin form" and "tannin type" were stated in the class statement since these variables did not contain any quantitative information. p-Values and root mean square errors (RMSE) were used as model statistics. Significance of an effect was stated at $p < 0.05$. All statistical analyses were performed with SAS Software version 9.1 (SAS Institute, 2008).

3 | RESULTS

According to the calculations, increasing concentrations of tannins were not associated with changes in silage pH (Table 2). Similarly, concentrations of lactate, acetate, propionate and ethanol were not related by tannin concentration in the ensiled material. However, a higher concentration of tannins was associated to a decrease in butyrate concentration ($p < 0.05$; Figure 1). The tannin concentration was accompanied with changes in the

TABLE 2 Regression equations on the influence of tannin concentration (T, in g/kg DM; independent factor) on silage quality

			Parameter estimates				Model statistics		
Response variable	Unit	*n*	Intercept	SE intercept	Slope	SE Slope	*p*-Value	RMSE	NE vs E
pH	(no unit)	122	4.42	0.084	0.0004	0.0016	0.827	0.277	0.225
Lactate	g/kg DM	89	46.7	4.96	−0.063	0.0958	0.516	15.0	0.418
Acetate	g/kg DM	91	19.6	2.97	−0.084	0.0515	0.107	8.06	0.145
Lactate/acetate	(no unit)	89	4.20	0.798	−0.0081	0.0151	0.592	2.37	0.015
Propionate	g/kg DM	42	2.50	0.959	−0.0092	0.0266	0.733	2.93	0.368
Ethanol	g/kg DM	20	14.7	5.44	−0.038	0.0604	0.544	6.79	Na
Total N	g/kg DM	24	30.2	1.47	−0.015	0.0076	0.061	0.892	Na
Soluble N	g/kg total N	60	526	24.1	−3.78	0.536	<0.001	75.3	<0.001
Free AA-N	g/kg total N	42	379	32.8	−2.59	0.868	0.005	71.9	Na
NPN	g/kg total N	16	541	871.0	−2.47	0.322	<0.001	21.7	0.803
IVDMD	g/kg DM	17	647	25.8	−0.533	0.221	0.037	21.7	0.364

Note. AA-N, amino acid nitrogen; DM, dry matter; E, extracted tannin form; IVDMD, in vitro dry matter digestibility; *n*, number of observation; N, nitrogen; na, not available; NE, non-extracted tannin form; NPN, non-protein nitrogen; RMSE, root mean square error; SE, standard error.

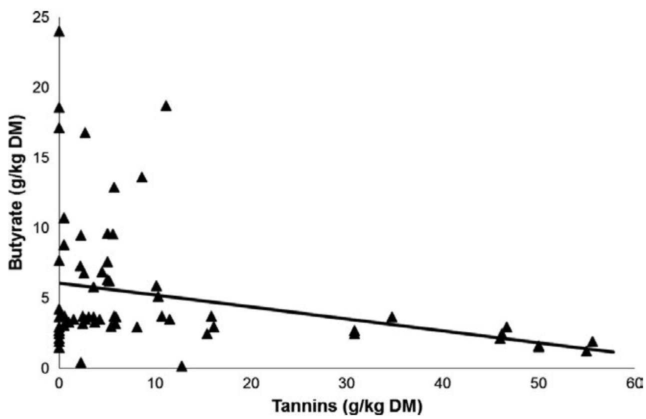

FIGURE 1 Relationship between tannins and butyrate concentration in silage based on the results of the meta-analysis. Equation: Butyrate (g/kg dry matter) = 6.07 − 0.085 × tannins (g/kg dry matter) ($n = 75$; $p < 0.05$; root mean square error = 4.44)

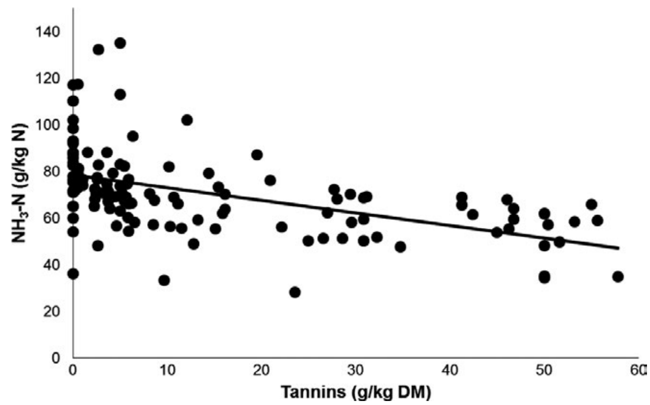

FIGURE 2 Relationship between tannins and ammonia (NH_3-N) concentration in silage based on the results of the meta-analysis. Equation: NH_3-N (g/kg total N) = 78.2 − 0.50 × tannins (g/kg dry matter) ($n = 128$; $p < 0.001$; root mean square error =17.96)

proportions of all N fraction analysed in the silages. Accordingly, with tannins concentrations of soluble N, free AA-N, non-protein nitrogen and NH_3-N were lower ($p < 0.05$; Figure 2). A higher concentration of tannins was also associated with a linear decline ($p < 0.05$) in IVDMD (Table 2). Generally, there was no significant differentiation between different forms of tannins (non-extracted vs extracted) on silage quality parameters except for lactate-to-acetate ratio and soluble N, in which the decline of these parameters was greater with non-extracted tannins than those of extracted tannins.

A higher concentration of tannin extracts was associated with lower concentrations of lactate, butyrate, soluble N, NPN and NH_3-N in the silages ($p < 0.05$), and with a trend towards lower total N and free AA-N ($p < 0.10$; Table 3). Some other parameters such as pH, acetate, propionate, ethanol and IVDMD were not altered when tannins extracts were added. There were different magnitudes of the relationships between CT and HT in silages on butyrate and NH_3-N concentrations ($p < 0.05$). Low concentrations of HT were already effective in lowering butyrate (Figure 3) and NH_3-N concentrations (Figure 4), whereas with high HT there was apparently no further decrease these silage parameters. Conversely, the changes found at low concentrations of CT were not as high as that in HT concerning the decline in butyrate and NH_3-N concentrations; the changes were only equivalent at higher CT concentrations compared to those associated with HT.

4 | DISCUSSION

4.1 | Effects of tannins on carbohydrate degradation during ensiling

A decreasing concentration of butyrate at an increasing concentration of tannins indicates that the compounds negatively affect

TABLE 3 Regression equations on the influence of tannin extract concentration (T, in g/kg DM; independent factor) on silage quality

			Parameter estimates				Model statistics		
Response variable	Unit	*n*	Intercept	SE intercept	Slope	SE Slope	*p*-Value	RMSE	CT vs HT
pH	(no unit)	44	4.57	0.145	0.0004	0.0010	0.670	0.150	0.204
Lactate	g/kg DM	44	53.5	7.84	−0.136	0.0566	0.023	8.47	0.100
Acetate	g/kg DM	44	25.8	4.92	−0.109	0.0697	0.129	10.5	0.086
Lactate/acetate	(no unit)	44	3.14	0.591	−0.0023	0.0050	0.654	0.754	0.582
Propionate	g/kg DM	24	3.06	0.782	−0.0197	0.0154	0.221	1.66	0.724
Butyrate	g/kg DM	38	4.30	1.79	−0.092	0.0381	0.023	5.09	0.012
Ethanol	g/kg DM	20	14.7	5.44	−0.038	0.0604	0.544	6.79	Na
Total N	g/kg DM	24	30.2	1.47	−0.015	0.0076	0.061	0.892	0.585
Soluble N	g/kg total N	24	505	25.3	−2.23	0.222	<0.001	26.4	0.578
Free AA-N	g/kg total N	6	307	27.5	0.303	0.0758	0.057	2.92	Na
NPN	g/kg total N	10	613	493.4	−2.38	0.200	<0.001	13.2	na
NH_3-N	g/kg total N	44	106	8.97	−0.465	0.137	0.002	20.7	0.013
IVDMD	g/kg DM	10	620	30.8	−0.503	0.306	0.162	29.7	0.905

Note. AA-N, amino acid nitrogen; CT, condensed tannins; DM, dry matter; HT, hydrolysable tannins; IVDMD, in vitro dry matter digestibility; *n*, number of observation; N, nitrogen; na, not available; NPN, non-protein nitrogen; RMSE, root mean square error; SE, standard error.

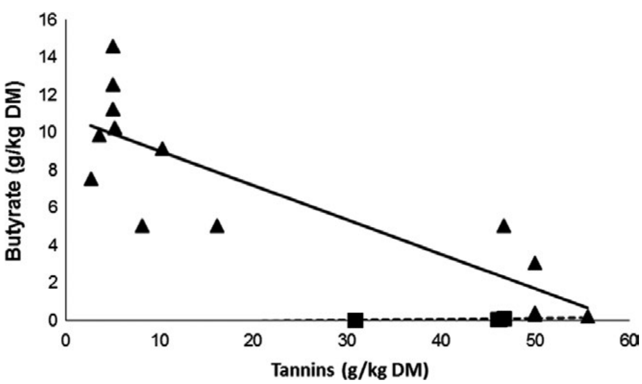

FIGURE 3 Relationship between levels of different tannin types, either condensed tannins (CT, ▲) or hydrolysable tannins (HT, ■) and butyrate concentration in silage based on the results of the meta-analysis. Equation for CT: Butyrate (g/kg dry matter) = 10.9 − 0.183 × tannins (g/kg dry matter) ($n = 14$; $p < 0.001$; root mean square error = 2.56). Equation for HT: Butyrate (g/kg dry matter) = −0.102 + 0.005 × tannins (g/kg dry matter) ($n = 7$; $p = 0.008$; root mean square error = 0.032)

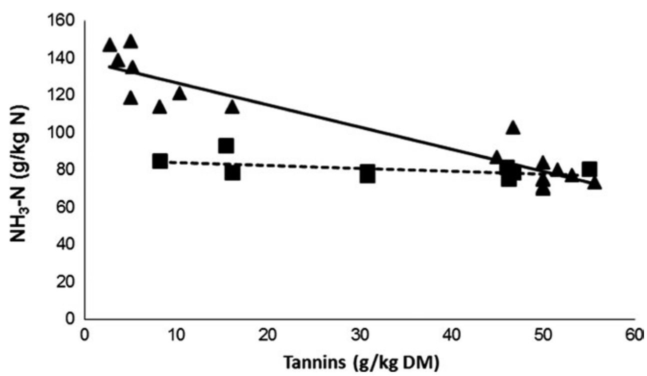

FIGURE 4 Relationship between levels of different tannin types, either condensed tannins (CT, ▲) or hydrolysable tannins (HT, ■) and ammonia (NH_3-N) concentration in silage based on the results of the meta-analysis. Equation for CT: NH_3-N (g/kg total N) = 139 − 1.19 × tannins (g/kg dry matter) ($n = 18$; $p < 0.001$; root mean square error = 9.80). Equation for HT: NH_3-N (g/kg total N) = 85.7 − 0.16 × tannins (g/kg dry matter) ($n = 11$; $p = 0.127$; root mean square error = 4.54)

clostridia. This is possible since tannins are known to possess some antimicrobial effects on a wide range of microorganisms (Daglia, 2012; Mueller-Harvey, 2006). This would make tannins a valuable silage conserving additive. Clostridia have been widely known to cause a lower silage quality since these bacteria convert sugars and organic acids, among them lactate, to butyrate thus counteracting trends to stable silage (Bolsen et al., 1996; Charmley, 2001). This typically massively reduces forage quality. However, tannins apparently have only a minor influence on the aerobic or microbial fermentation of carbohydrates to lactate, acetate and propionate. This is also supported by the lack of a significant relationship with pH at increasing concentrations of tannins in silage. Obviously LAB are not very sensitive to the presence of tannins. Certain LAB species were found to be able to adapt to tannins or phenolic compounds in general. For instance, *Lactobacillus plantarum* was shown to possess tannase activity that enables the microbes to degrade tannins, especially the

HT (Vaquero, Marcobal, & Munoz, 2004). In another study, it was reported that *L. plantarum* degraded gallotannins through the depolymerization of high molecular weight tannins and a concomitant degradation of low molecular weight tannins, producing gallic acid and pyrogallol as final metabolic products (Rodríguez, de las Rivas, Gómez-Cordovés, & Muñoz, 2008).

There is, however, a drawback in using tannins during ensiling which is not related to the process of ensiling but consists in reducing its feeding value in the ruminant. A depression in ruminal digestibility occurs at higher concentration of tannin as shown by the negative slope of IVDMD parameter. Part of this effect is desired, when it concerns the proteins (see discussion below), but the constraints comprise other nutrients as well. For that reason, tannins are even considered as an anti-nutritional factor especially when present at high concentration, that is above 50 g/kg DM (Makkar, 2003). The formation of hydrogen bonds between free phenolic groups in tannins and carbohydrate macromolecules, especially those present in fibre (Silanikove, Perevolotsky, & Provenza, 2001), provide an explanation for the decline in IVDMD observed together with an increase in tannin concentration. This means that in diets limited in energy content the use of tannins in ensiling might result in silages which do not fully support performance of these animals.

4.2 | Effects of tannins on protein degradation during ensiling

The main action of tannins in silage apparently consists in the reduction of the extent and rate of proteolysis as shown by the negative slopes of the proportions of soluble N, free AA-N, NPN and NH_3-N of total silage N on tannin concentration. All these N fractions are contributing to the often already high concentration of rumen-degradable protein. As a consequence of this, tannins present in silage were indeed shown to have a negative correlation with rumen-degradable protein (Coblentz & Grabber, 2013) and thus may potentially improve digestive and metabolic protein utilization as well as animal performance. Proteolysis in silage is influenced by a number of factors such as forage species, maturity stage of the forage at harvest, degree of wilting and the rate of pH decline (Tabacco et al., 2006). Further, proteolytic clostridia may cause secondary protein fermentation by degrading amino acids to ammonia, amines and volatile organic acids (Albrecht & Muck, 1991; Givens & Rulquin, 2004), thus may reduce forage true protein by up to 80% (Winters, Cockburn, Dhanoa, & Merry, 2000). In case the silages provide an excessive supply of rumen-degradable protein, the ammonia produced during ensiling is not utilized by rumen microbes. This enhances nitrogen losses by the animal via urine and contributes to environmental pollution (Dijkstra et al., 2013). It is therefore very important to find ways to prevent or at least decelerate such a degradation of protein during ensiling in order to maintain the quality of silage as well as to minimize N emissions to the environment.

Tannins may influence proteolysis in silage by inhibiting both plant and microbial proteinases as well as by forming complexes with forage protein (Albrecht & Muck, 1991; Deaville, Givens, & Mueller-Harvey, 2010), making the protein become less susceptible to hydrolysis. Such formation of tannin–protein complexes under acidic environment in silages is possible since it may occur at a broad pH range between 3.5 and 7.5 (Barry & McNabb, 1999). This was supported by a study of Ding, Guo, and Ataku (2013) who observed a reduction in NPN, ammonia N and amino acid N proportions to total N in alfalfa silage when adding tannic acid. Further, supplementation of tannic acid resulted in a decline of the activities of carboxypeptidase and aminopeptidase, which are among the key proteolytic enzymes present in silages (Guo, Zhou, Yu, & Zhang, 2007).

The property of tannins in silage to reduce IVDMD described above does not mean that its use will always decrease animal performance as it may even be positive in cases where metabolizable protein is limiting performance. Part or all of the protein previously protected in the rumen from degradation may be released from its bond with tannins in abomasum at low pH, making it available in the small intestine for subsequent enzymatic degradation and absorption (Cortés et al., 2009; Makkar, 2003). Accordingly, a number of in vivo studies have demonstrated the positive effect of tannins on animal performance either in dairy or meat producing animals. For instance, replacing alfalfa or red clover silage by birdsfoot trefoil (containing CT) silage in total mixed rations increased yields of milk and milk constituents of lactating dairy cows by improving the efficiency of dietary protein utilization (Hymes-Fecht, Broderick, Muck, & Grabber, 2013). A recent study by Jolazadeh, Dehghan-banadaky, and Rezayazdi (2015) showed that the treatment of soybean meal with tannins extracted from pistachio hull increased average daily gains and feed efficiency of Holstein bulls. Further, the tannin treatment also decreased NH_3-N concentration and protozoa population in the rumen thus reducing urine N excretion and the likelihood of excessive ammonia emission from the manure (Carulla, Kreuzer, Machmüller, & Hess, 2005).

4.3 | Importance of the type of tannins for the effects on nutrient degradation during ensiling

In the present evaluation, experiments carried out with sources of HT and with CT were distinguished as these two groups of tannins clearly differ in their properties. Accordingly, HT contain carbohydrate as a central core with hydroxyl groups esterified to phenolic groups (Goel, Puniya, Aguilar, & Singh, 2005). Different from that, CT have no central carbohydrate core and are composed of oligomers and polymers of flavanoid units linked by carbon–carbon bonds with a molecular weight of 2,000–4,000 kDa. Jayanegara et al. (2015) demonstrated that HT (from chestnut and sumach) had a higher protein precipitation capacity and, consequently, biological activity than those of CT (from mimosa and quebracho). It, therefore, seems that higher ability of the HT to limit proteolysis in silages at lower concentrations in comparison to the CT is related to their higher protein precipitation capacity. Higher HT concentrations did not further limit proteolysis possibly due to the partial degradation of HT by LAB as discussed above. This can be explained chemically because, with the presence of the carbohydrate core, HT are more susceptible to enzymatic and non-enzymatic hydrolysis than CT (Mueller-Harvey, 2006). The HT can be absorbed from the digestive tract to some extent and thus may even be toxic to ruminants when consumed in excessive amounts, but they may provide some beneficial effects if consumed at low to moderate concentrations (Reed, 1995). By contrast, the CT are resistant to microbial degradation under anaerobic conditions like in silages and in the rumen and are not absorbed from the digestive tract (Reed, 1995). However, they may bind nutrients quite strongly and make them unavailable even in the lower gut (Makkar, Francis, & Becker, 2007).

5 | CONCLUSION

The present meta-analysis compiled experimental evidence for the observation that tannins have the ability to limit extensive proteolysis when forage is ensiled. Various parameters like the lower proportions of soluble N, free amino acid N, non-protein nitrogen and NH_3-N in total silage N pointed into this direction. Another favourable effect evaluated was the limitation of the production of butyrate by the tannins, another important constraint when producing high quality silage. At the same time, tannins apparently have no negative effect on overall fermentation of silage as shown by the unchanged pH and lactate concentration. This suggests that tannins have a clear potential as a silage additive especially when the silage material is high in degradable protein. Apart from these properties, tannins may potentially provide additional benefits to the animal after being ingested through improving the protein utilization. The evaluation also suggested that hydrolysable tannins are effective at low proportions, but great effects may only be achieved by higher silage proportions of condensed tannins.

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