



Effect of dietary tannins on milk yield and composition, nitrogen partitioning and nitrogen use efficiency of lactating dairy cows: A meta-analysis

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Abstract

Tannins are secondary plant compounds which have been extensively studied in order to improve the nitrogen use efficiency (NUE) of ruminants. A meta-analysis was performed of 58 in vivo experiments comparing milk yield, composition and nitrogen metabolism of lactating dairy cows fed diets with or without tannins. The meta-analysis shows that tannins have no impact on corrected milk yield, fat and protein content or NUE ($p > .05$). However, tannins reduce ruminal ammoniacal nitrogen (N) production by 16% (from 10.95 to 8.47 mg/dl on average), milk urea by 9% (from 15.82 to 14.03 mg/dl) and urinary N excretion (-11%; $p < .05$). This is compensated for by a lower apparent N digestibility (61.51% with dietary tannins compared to 66.17% without). The effect of tannin on N metabolism parameters increases with tannin dose ($p < .05$). The shift from urinary to faecal N may be beneficial for environment preservation, as urinary N induces more harmful emissions than faecal N. From a farmer's perspective, tannins seem unable to increase fat- and protein-corrected milk yield or reduce feed protein requirements and thus have no direct economic benefit. Potentially less costly than tannin extracts, forage or by-products naturally rich in tannins could still be useful to reduce the environmental impact of ruminant protein feeding.

KEYWORDS

polyphenols, protein, ruminant, secondary plant metabolites

1 | INTRODUCTION

Ruminants are able to convert non-human edible feed, such as grass, into highly nutritive human food, such as milk and meat. However, conversion efficiency is often low: around 25% of nitrogen intake is converted into milk by dairy cows (Calsamiglia, Ferret, Reynolds, Kristensen, & van Vuuren, 2010). This is because soluble and degradable proteins found in grass and legumes are quickly available to rumen micro-organisms. When taken in excess, a significant part of this nitrogen is excreted through urine and lost in the environment.

Low nitrogen use efficiency (NUE) makes the purchase of high-protein feed necessary, increases feed costs and causes nitrogen pollution. Reducing nitrogen degradability in the rumen using secondary plant metabolites has been identified as a strategy to improve NUE (Broderick, 2018). Among these molecules, tannins have been extensively studied in recent years. Thanks to their ability to form complexes with proteins, tannins can affect nitrogen metabolism in ruminants. Stable between pH values of 3.5 and 7, tannin-protein complexes are thought to protect proteins against lysis in the rumen and dissociate and release proteins at abomasal or duodenal pH

(Jones & Mangan, 1977 cited by Piluzza, Sulas, & Bullitta, 2014). The greater flow of digestible feed protein to the intestine could then potentially improve NUE and milk yield. However, this simplistic assumption is not always confirmed by published studies and what happens to released tannins remains unclear (Piluzza et al., 2014).

The objectives addressed in this article were (a) to evaluate the effect of dietary tannins on the nitrogen balance and milk production of dairy cows and (b) to describe these effects according to tannin dose and tannin type. Through a statistical treatment, the meta-analysis process allows a quantitative and systematic review of the literature in order to reach these objectives (Philibert, Loyce, & Makowski, 2012). The use of meta-analysis in agronomy is recommended to review considerable amounts of experimental data containing heterogeneous information (Doré et al., 2011).

2 | MATERIALS AND METHODS

2.1 | Data collection and selection

We collected peer-reviewed scientific publications through a comprehensive literature search. Three databases were used (Scopus, ScienceDirect and Google Scholar), and the search was extended to all fields. The following Boolean search string (Equation 1), containing most common tannins and tannin-containing plants, was used in the databases:

$$(tannin \text{ OR chestnut OR quebracho OR birdsfoot OR lotus OR sainfoin OR onobrychiss OR sulla OR hedysarum OR proanthocyanidin) \text{ AND } (dairy \text{ OR milk}) \text{ AND } (cow \text{ OR cattle OR ruminant}). \quad (1)$$

The searches were conducted in June 2017 and updated in September 2018 and led to the identification of 604 scientific publications. These publications went through a two-step selection process. A first screening of titles and abstracts excluded *in vitro* and simulation studies, reviews and irrelevant articles. The second step was the analysis of full-text articles in order to select articles meeting several criteria: (a) presence of cows' milk production data; (b) similarity between the control and experimental groups except for the presence of tannin; (c) quantification or possible determination of ingested tannin quantity and iv) only peer-reviewed and non-predatory journal articles (according to the Beall's list, accessed on 10 September 2018).

The selection process is illustrated in Figure 1. When several selected articles presented data from the same experiment, they were pooled. When referenced in selected articles, data from the same experiment published in other articles were also extracted. All articles containing data used in the meta-analysis are cited in Appendix 1.

2.2 | Data extraction

Mean milk yield was extracted for control and treatment groups from all studies, which sometimes included several trials. When available, 15

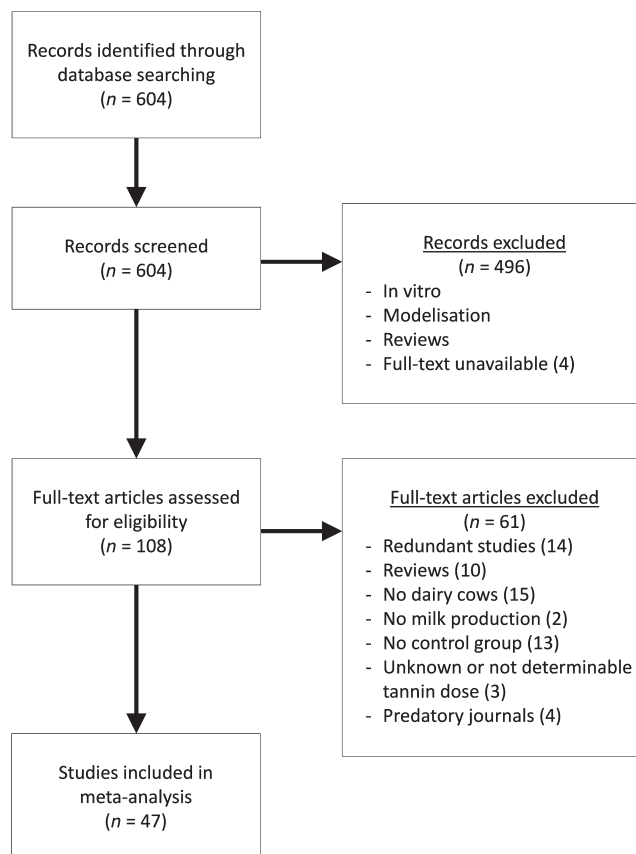


FIGURE 1 Study selection flow diagram

other response parameters were also extracted (listed in the extracted response parameters section of Table 1). For each parameter and each study, a ratio (R) of mean value with tannin in the diet (Y_{tannin}) to mean value without tannin (Y_{control}) was calculated ($R = Y_{\text{tannin}}/Y_{\text{control}}$). A ratio of 1 indicated the same value for tannin and control diets. Most studies presented standard errors of the mean (SEM), so that standard deviations (SD) were evaluated using the following equation: $SEM = SD/\sqrt{n}$, with n being the number of observations. Some articles did not provide SD , SEM or any other value allowing computing SD so that missing SD were replaced by mean or maximum SD of the other studies according to the model (see Section 2.3). Information on type of animals, diet, type and dose of tannins was also extracted. For four studies which did not determine the tannin content of the diet, the dose of tannins was estimated according to Feedipedia (<http://feedipedia.org>) values or from two publications of Jackson, McNabb, Barry, Foo, and Peters (1996) and Zimmer and Cordesse (1996).

2.3 | Data analysis

Statistical analyses were performed with R software and the "metafor" package (Viechtbauer, 2010). All statistical analyses were performed on the logarithm of the ratios previously calculated ($L = \ln(R)$). The homogeneity of effect sizes was assessed by the Q test (Hedges, Gurevitch, & Curtis, 1999) and supported the choice of model with or

TABLE 1 Data description (means and *SD* between studies)

Parameter	n_{Control}	Mean _{Control}	n_{Tannin}	Mean _{Tannin}	<i>SD</i>
Diet features					
Forage in diet ^a (g/100 g DM)	–	55.6	–	56.7	28.19
CP (g/100 g DM)	–	16.6	–	16.8	3.47
NDF (g/100 g DM)	–	40.3	–	39.2	10.50
ADF (g/100 g DM)	–	24.9	–	25.2	7.08
Tannin dose (g/day)	–	–	–	183	–
Tannin relative dose (g/100 g DMI)	–	–	–	0.95	–
Extracted response parameters					
DMI (kg DM/day)	46	19.47	84	19.57	5.639
Milk yield (kg/day)	58	23.88	102	24.00	10.989
FPCM yield (kg/day)	55	23.76	94	24.20	9.792
Fat (g/100 g)	55	3.96	94	3.98	0.499
Protein (g/100 g)	55	3.32	94	3.30	0.379
Rumen NH ₃ -N (mg/dl)	18	10.95	35	8.47	2.079
BUN (mg/dl)	14	17.58	27	15.20	5.419
MUN (mg/dl)	27	15.82	48	14.03	4.837
UN (%N intake)	17	35.75	34	30.88	10.558
FN (%N intake)	22	34.39	42	38.39	6.174
DM digestibility (%)	18	65.64	37	64.87	5.523
OM digestibility (%)	27	66.17	40	66.03	7.032
NDF digestibility (%)	18	54.88	40	52.60	7.939
ADF digestibility (%)	15	50.06	33	50.13	8.984
N digestibility (%)	22	66.17	42	61.51	6.657
NUE (%)	26	27.52	47	27.47	4.178

Abbreviations: ADF, acid detergent fibre; BUN, blood urea nitrogen; CP, crude protein; DM, dry matter; DMI, dry matter intake; FN, faecal nitrogen; FPCM, fat- and protein-corrected milk; MUN, milk urea nitrogen; *n*, number of treatments included in the meta-analysis; NDF, neutral detergent fibre; NUE, nitrogen use efficiency; OM, organic matter; *SD*, standard deviation; UN, urinary nitrogen.

^aForage excluding corn silage.

without random effects. As between-study heterogeneity was found, the study was used as a random factor. For each response parameter, two models were compared: missing *SD* were replaced by either the mean or the maximum *SD* of other studies. As our models used study variance as a weighting factor, the use of SD_{max} minimised the influence of data with missing *SD* compared with the use of SD_{mean} . The sensitivity of our results to the model choice was assessed by comparing these two models, based on the Akaike information criteria. For each response parameter individually, the two models were fitted using the “rma” function (linear mixed-effects model) of the “metafor” package, with the restricted maximum likelihood method. Transformed ratios (*L*) were used as the only independent variable, study as random factor and study variance as weight. If *L* significantly differs from 0 (if *R* differs from 1), the meta-analysis indicates that tannins have a significant effect on the tested response parameter. This analysis is a first approach to determine if tannins globally affect each response parameters.

Then, the effects of dose (g/day), relative dose (g/kg DMI), tannin type (hydrolysable versus. condensed), tannin source

(extracted or not) and diet composition on the mean effect size were individually tested with the “lme” function (linear mixed-effects model) of the “nlme” package. Study was still used as a random factor and variance as weight. Dose, tannin type or source or diet composition were set as a fixed factor, one at a time with *L* as independent variable and this for each response parameter individually. It should be noted that the effect of cow's breed could not be analysed because of insufficient data. This second and independent approach aimed at determining which factors generally impact the effect of tannins on the response parameters and in what ways. The level of significance was set at $\alpha = 0.05$ for all analyses.

2.4 | Publication bias analysis

Funnel plots were created for each parameter, excluding studies with missing *SD*s. Publication bias was assessed with Egger's method using a linear regression between normalised effect size and

precision (Makowski, Piraux, & Brun, 2018) defined as $\frac{L}{SD_L} - \frac{1}{SD_L}$ with

SD_L being the standard deviation of $L = \ln(R)$.

If the intercept of the regression line differed from 0, a publication bias was detected.

The trimfill function (Duval & Tweedie, 2000) with the default arguments was used on parameters subject to publication bias in order to evaluate the effect of this bias on the outcomes.

3 | RESULTS

Through the database search, 47 studies (presented in Appendix 1) were included in the meta-analysis totalling 58 experiments, 160 treatments and 1,347 animals. Of these experiments, 42% were performed with pure Holstein dairy cows and 27% with crossbred Holstein. Around 30% of the experiments took place in Europe, 20% in Asia, 17% in North America and 17% in Australia or New Zealand. Mean diet characteristics are presented in Table 1; more than 50% of the average diet consisted of grass or legume forages and almost 17% of crude protein (CP). Experimental tannin doses ranged from 10 to more than 800 g per animal per day, representing a range of between 1 and more than 40 g/kg DM. Two tannin types were studied: 12% of the treatments used hydrolysable tannins and the other 88% used condensed tannins. Around 65% of treatments used forage or by-products naturally containing tannins whereas 35% added tannin extracts.

The Q tests were significant ($p < .05$) for all parameters, suggesting between-study heterogeneity. For this reason, models with random effects were chosen in this meta-analysis. Differences between models using SD_{max} or SD_{mean} were small. Three parameters (DMI, milk yield and DM digestibility) were significantly influenced by tannins ($p < .05$) in only one of the two models, but they tended to be affected by tannins in the second model ($p < .10$). Thus, estimated mean effect sizes and confidence intervals were not very sensitive to model assumption on SD . According to the best-fitted models (Table 2), tannins significantly affected milk yield, ruminal ammonia nitrogen ($N-NH_3$), blood urea nitrogen (BUN), milk urea nitrogen (MUN), urine and faecal nitrogen and nitrogen digestibility ($p < .05$). While milk yield was slightly improved by tannins (+1.7%), ruminal $N-NH_3$, MUN and BUN decreased by 16%, 8% and 9% respectively. Urine N excretion fell by 11% whereas faecal N excretion rose by 10%, also reducing N digestibility by 7%. The digestibility of DM and OM tended to decrease in the presence of tannins ($p < .10$; -1.5% and -1.2% respectively). Depending on the model, tannins had little or no impact on DMI. Corrected milk yield, milk fat and protein, NUE and fibre digestibility were not influenced by the presence of tannins in the diet.

All significant parameters except milk yield and urinary N were influenced by tannin dose in both absolute and relative values (Table 3). Increasing tannin doses significantly decreased ruminal $N-NH_3$, BUN, MUN and N digestibility ($p < .05$), whereas they increased FN ($p < .001$). The tannin type (condensed versus

hydrolysable) only influenced faecal N excretion ($p < .05$), with condensed tannins causing more faecal excretion than hydrolysable tannins (+15%). The source of tannin (naturally contained in feed or extracts added to diet) significantly affected milk yield ($p < .01$). Feed naturally containing tannins improved milk production compared with tannin extracts added to the diet (+4%). Diet characteristics mostly interacted with tannin's effect on ruminal $N-NH_3$ and BUN. A rise in diet CP content enhanced the reduction effect of tannins on these parameters, whereas a high forage proportion in the diet lowered the impact of tannins. Diet CP and forage content did not affect tannin impact on milk yield or NUE ($p > .05$; data not shown).

Figure 2 shows that BUN, FN and DM digestibility could present a publication bias, with the intercept of Egger's regression line being significantly different from 0 ($p < .05$). The use of the trimfill function only affected FN outcomes, increasing the mean ratio from 1.1031 to 1.1373, which remains significantly different from 1 ($p < .001$). The function suggests that eight treatments were missing to avoid the publication bias in addition to the 42 treatments identified in the meta-analysis.

4 | DISCUSSION

The present study showed that tannins had several impacts on dairy cows' milk production and nitrogen partitioning. The major findings are an absence of effect of tannins on corrected milk yield and NUE of dairy cows. Ruminal $N-NH_3$, MUN and urinary N excretion indicated that ruminal N degradability would be reduced because of tannins. However, the fall observed in N digestibility can explain why tannins did not influence NUE. Dairy cows seem thus unable to make better use of dietary proteins thanks to tannins. Consistently with ruminal ammonia and MUN decreases, lower BUN was observed with tannins in the diet but this response parameter was subject to publication bias. The same bias applied to DM digestibility and FN. Tannins effects on ruminal $N-NH_3$, MUN and N digestibility were magnified by increasing tannin doses. Tannin type influenced none of the assessed response parameters, except for FN which was subject to publication bias and thus difficult to interpret.

According to the best-fitted model, a significant increase in milk yield was observed in the presence of tannins, but the mean effect size ratio suggested that the increase was less than 2%. Our results suggest that this increase is greater when cows are fed forage or by-products naturally containing tannins than when they are given tannin extracts, but tannin dose or type (hydrolysable vs. condensed) had no effect. The source of tannin (naturally present or extracted) did not influence parameters other than milk yield, which makes this effect difficult to interpret. Milk fat and protein were not affected by tannins, and corrected milk yield was not influenced either. It is possible that protein was not a limiting factor in the diet in most studies, so that an improved digestible protein supply linked to tannins would not result in an increased milk yield. However, the absence of effect of dietary CP content on mean effect size of tannins on milk yield suggests that even in low-protein diets, milk production would not be substantially

TABLE 2 Mean effect size and model confidence intervals, Akaike's information criterion (AIC) and *p*-values calculated from 58 experiments using random-effect models with SD_{\max} or SD_{mean} replacing missing *SD*

Parameter	<i>n</i>	Best-fitted model	Mean ratio	CI 95%	<i>p</i> -value
DMI	84	SD_{mean}	1.0118	0.9971–1.0266	.1150
Milk yield	102	SD_{\max}	1.0170	1.0021–1.0322	.0255
FPCM yield	94	SD_{\max}	1.0117	0.9950–1.0287	.1699
Fat	94	SD_{mean}	1.0037	0.9934–1.0142	.4776
Protein	94	SD_{mean}	0.9913	0.9821–1.0006	.0665
Rumen NH_3 -N	35	SD_{mean}	0.8389	0.7785–0.9039	<.001
BUN	27	SD_{\max}	0.9085	0.8534–0.9671	.0026
MUN	48	SD_{\max}	0.9242	0.8974–0.9521	<.001
UN	34	SD_{\max}	0.8906	0.8437–0.9400	<.001
FN	42	SD_{\max}	1.1031	1.0666–1.1408	<.001
DM digestibility	37	SD_{\max}	0.9857	0.9715–1.0000	.0501
OM digestibility	40	SD_{mean}	0.9878	0.9754–1.0003	.0563
NDF digestibility	40	SD_{mean}	0.9807	0.9561–1.0058	.1304
ADF digestibility	33	SD_{mean}	0.9878	0.9600–1.0165	.4019
N digestibility	42	SD_{\max}	0.9304	0.9083–0.9530	<.001
NUE	47	SD_{mean}	1.0066	0.9770–1.0371	.6668

Abbreviations: ADF, acid detergent fibre; BUN, blood urea nitrogen; CI, confidence interval; DMI, dry matter intake; FN, faeces/intake nitrogen; FPCM, fat- and protein-corrected milk; MUN, milk urea nitrogen; *n*, number of treatments included in the analysis; NDF, neutral detergent fibre; NUE, nitrogen use efficiency; OM, organic matter; UN, urine/intake nitrogen.

TABLE 3 Significance (*p*-values) of dose, tannin characteristics and diet effects on mean effect size of significant parameters, based on the SD_{\max} model

Parameter	Dose	Relative dose	Tannin type	Tannin source	Diet CP content	Diet forage proportion
Milk yield	0.7302	0.4726	0.2774	0.0045	0.7482	0.4064
Rumen NH_3 -N	<0.0001	<0.0001	0.9077	0.1957	0.0021	0.0085
BUN	0.0002	0.0004	0.4998	0.9691	0.0240	0.0029
MUN	0.0022	0.0011	0.2651	0.5160	0.8898	0.3361
UN	0.4757	0.6723	0.5811	0.5862	0.3538	0.6920
FN	0.0001	0.0002	0.0227	0.6452	0.3808	0.0185
N digestibility	0.0223	0.0223	0.0712	0.9134	0.2793	0.1921

Abbreviations: BUN, blood urea nitrogen; CP, crude protein; FN, faeces/intake nitrogen; MUN, milk urea nitrogen; UN, urine/intake nitrogen.

increased by tannins. According to these results, tannins would probably not represent a suitable option to improve milk yield.

Tannins affected nitrogen metabolism at several levels. Dietary tannins decreased ruminal N-NH_3 and the urea N content of milk. Total N found in urine compared with N intake fell when cows were fed tannins, which was probably compensated for by a rise in faecal N. As a result, N digestibility was reduced. Ruminal ammonia N reduction has already been widely described in the literature: tannins are known for their capacity to bind with proteins at ruminal pH and reduce their degradation by micro-organisms (McNabb, Waghorn, Peters, & Barry, 1996). A lower N-NH_3 concentration in the rumen, reflecting a decrease in ruminal CP degradability, could thus be linked to a greater protein flow to the intestine. Besides, as urea is formed by

the liver from excess ammonia, the decrease in urea N in milk may be directly caused by the lower protein degradation in the rumen and the lower ammonia production. Because less urea is generated, the lower ruminal CP degradation may also explain the fall in urinary N excretion relative to N intake. Consistently with ruminal N-NH_3 and MUN, a decrease in BUN is also observed with tannins but BUN results could be subject to publication bias as shown in Figure 2. Publication bias tends to overestimate the treatment effect (Makowski et al., 2018), so BUN reduction (–9%) with tannins could be lower in reality.

Tannin–protein complexes are formed by means of hydrophobic interactions, hydrogen, and ionic and covalent bonds (Kumar & Singh, 1984 cited by Frutos, Hervas, Giráldez, & Mantecón, 2004). As some of these bonds are pH-dependent, complexes are unstable and dissociate

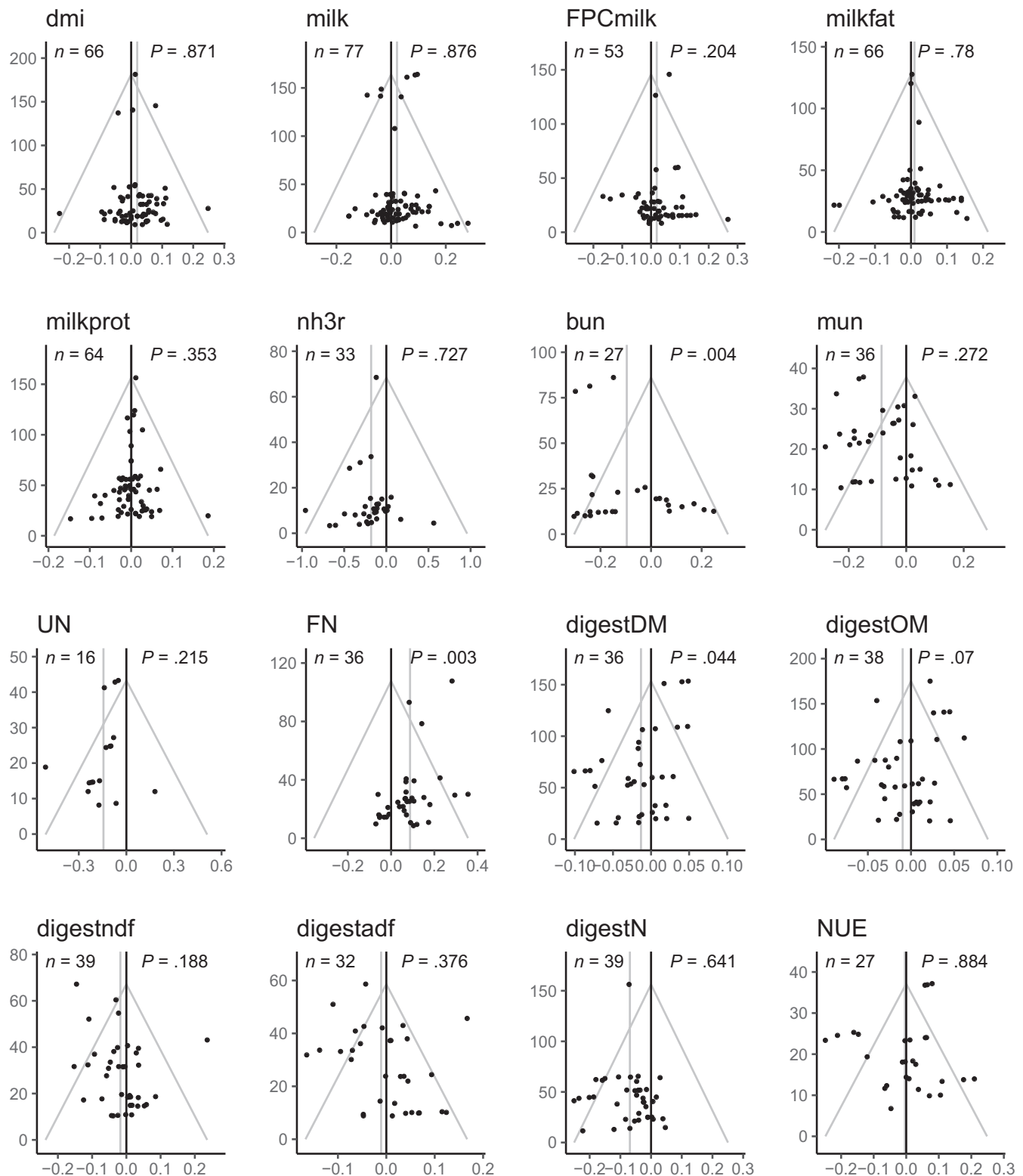


FIGURE 2 Funnel plots and significance (p -value) of Egger's criterion per parameter without missing SD studies (DMI, dry matter intake; FPCM, fat- and protein-corrected milk; nh₃r, ruminal ammonia; BUN, blood urea nitrogen; MUN, milk urea nitrogen; UN, urine/intake nitrogen; FN, faeces/intake nitrogen; DM, dry matter; OM, organic matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; NUE, nitrogen use efficiency)

outside the pH range of 3.5 to 7 (Jones & Mangan, 1977). Protein will then be available at gastric and pancreatic digestion sites. However, the fall in N digestibility suggests either that dissociation is only partial

or that some complexes could reassemble with feed or endogenous proteins in the digestive tract, as proposed by Waghorn (2008). Beauchemin, McGinn, Martinez, and McAllister (2007) observed a

decrease in ADF-bound N (ADIN) digestibility by 43%–93% due to condensed tannin extracts, which represents 31% to 67% more ADIN in tannin-fed cows' faeces compared with control cows' faeces. Powell, Broderick, Grabber, and Hymes-Fecht (2009) also found an increase in ADIN faecal concentrations when high-tannin birdsfoot trefoil was fed to cows. As ADIN intakes were similar in all treatments in the study of Beauchemin et al. (2007), we can hypothesise that ADIN was formed in the digestive tract because of non-reversible complexation between protein and tannins. This trend was also observed by Herremans, Decruyenaere, Cantalapiedra-Hijar, Beckers, and Froidmont (2019) when hydrolysable tannin-treated grass silage was fed to dairy cows. The undigested irreversible tannin–protein complexes therefore appear to lower the N digestibility of tannin-rich diets.

Hagerman, Robbins, Weerasuriya, Wilson, and McArthur (1992) showed that hydrolysable tannins did not affect N digestibility, contrary to condensed tannins. In faeces, they found condensed tannins but no hydrolysable tannins, leading them to suppose the rapid hydrolysis of hydrolysable tannins and the formation of gallic acid after ingestion. The present meta-analysis did not find any effect of tannin type on N digestibility, but higher faecal N was observed for condensed tannins relative to hydrolysable tannins. However, faecal N values were subject to a publication bias that may suggest an over-estimation of the reduction observed with tannins.

While tannins significantly reduced N digestibility by 7%, OM digestibility tended to decrease too, but to a lower extent. With a reduction in less than 2%, the impact of tannins on this parameters is weak. Furthermore, the digestibility of NDF and ADF was similar between treatments. This qualifies the reports in some literature reviews of a reduction in DM, OM and fibre digestibility (Frutos et al., 2004; McMahan et al., 2000; Mueller-Harvey, 2006) caused by cellulolytic enzymes or bacteria inhibition or complexation between tannins and feed components such as starch, carbohydrates or cell walls.

The absence of any effect of tannins on NUE, regardless of the diet's CP or forage content, implies that milk nitrogen yield could not be improved overall despite the likely enhanced protein flow to the intestine. The fall in urinary N is almost exactly compensated for by the rise in faecal N, without any efficiency gain. From a farmer's perspective, tannins are thus of little interest given the lack of economic return. However, tannins could be useful for environmental preservation. By reducing urinary N excretion, tannins can limit N pollution. Śliwiński, Kreuzer, Sutter, Machmüller, and Wettstein (2004) showed a 50% reduction in manure nitrogen loss during 8 weeks of storage resulting from the use of chestnut tannin extract ($p < .05$). Relative to faecal excretion, urinary N is highly susceptible to NH_3 volatilisation (Bussink & Oenema, 1998) and to nitrification, and hence responsible for a high proportion of N_2O emissions (Eckard, Grainger, & de Klein, 2010). Reducing urinary N would therefore help protect the environment.

5 | CONCLUSION

Through the 58 experiments included in the present meta-analysis, we found that tannins have no effects on fat- and protein-corrected

milk production or N use efficiency. However, they do act on dairy cows' nitrogen metabolism by reducing N degradation in the rumen. Their effect on nitrogen metabolism linearly increased with tannin dose. Although tannins have no direct economic benefit for dairy producers, they could be of environmental interest by reducing urinary N losses in favour of faecal N. As a result, N emission from manure is lower in a tannin-rich diet.

CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

ANIMAL WELFARE STATEMENT

No ethical approval was required as this is a review article with no original research data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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APPENDIX 1

LIST OF PUBLICATIONS USED IN THE META-ANALYSIS

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