

Tannin extract dietary thresholds for preventing unacceptable suppression in intake, digestibility, and growth in sheep and cattle: A meta-analysis

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Abstract

This meta-analysis aimed to identify the optimal inclusion and threshold levels for black wattle, quebracho, and chestnut tannin extracts (TE) in sheep and cattle, in terms of their effects on dry matter intake (DMI), digestibility, weight gain, and nitrogen balance. Data were compiled from 44 scientific publications, representing 580 sheep and 742 cattle. The percentage change approach was used to quantify responses to TE inclusion. Mixed model analysis was performed to evaluate piecewise and linear regressions, with scientific publications treated as random effects, and TE level, TE source, animal species, supplementation period, dietary fibre level, dietary protein level, and animal age as fixed effects. The piecewise model provided the best fit for all responses. The optimal inclusion level was established at a 0% response, while the threshold was established at a 5% reduction in performance. With no differences between TE sources, the optimal inclusion level for DMI was 1.5 g TE/100 g dry matter (DM) and threshold was 3 g TE/100 g DM in both sheep and cattle. Sheep were slightly more able to digest crude protein than cattle ($3.89 \pm 1.91\%$) at the same TE inclusion level, but no other differences were observed between the two species. Black wattle TE reduced organic matter digestibility ($-4.95 \pm 1.82\%$), neutral detergent fibre digestibility ($-8.62\% \pm 4.07$), and acid detergent fibre digestibility (-16.83 ± 6.06) responses, relative to chestnut TE. Moreover, TE inclusion (particularly black wattle TE) raised faecal nitrogen while lowering urinary nitrogen. These differences between TE sources did not significantly influence the weight gain response, with an optimal inclusion level of 1.5 g TE/100 g DM, and a threshold of 2.3 g TE/100 g DM.

Keywords: black wattle, chestnut, mixed model, percentage change, quebracho

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Introduction

Tannins are water-soluble phenolic compounds of high molecular weight found abundantly in plants (Westendarp, 2006; Hassanpour *et al.*, 2011). Based on their molecular structure, tannins are divided into hydrolysable (molecular weight 500–3000 g/mol) and condensed tannins (molecular weight

up to 20000 g/mol) (Denninger *et al.*, 2020; Manzoor *et al.*, 2021). Black wattle (*Acacia mearnsii*), quebracho (*Schinopsis quebracho-colorado*), and chestnut (*Castanea sativa*) are the three most widely used sources of tannin extracts (TE) in animal feed (Duke, 2012). Chestnut TE is considered a source of hydrolysable tannin, while black wattle and quebracho are regarded as condensed tannin sources (Missio *et al.*, 2017).

Tannin extracts are inexpensive, natural, and multifunctional feed additives that can potentially replace a range of synthetic additives used to modulate the production and health of ruminants. For example, TE are increasingly incorporated as supplements in ruminant diets because of their potential ability to modulate rumen fermentation (Makkar, 2003; Grainger *et al.*, 2009; Archimède *et al.*, 2016; Adejoro *et al.*, 2020). Tannins have also shown the potential to increase bypass protein and the availability of amino acids for intestinal absorption by binding to feed proteins in the rumen (Patra & Saxena, 2011; Bunglavan & Dutta, 2013; Besharati *et al.*, 2022). This tannin-protein interaction can reduce excessive protein degradation by rumen microbes and can shift nitrogen excretion away from urinary excretion and towards more stable faecal nitrogen compounds (Adejoro *et al.*, 2020; Ibrahim & Hassen, 2022). This can help improve overall nitrogen utilisation efficiency and reduce potential nitrous oxide emissions associated with higher urine nitrogen excretion (Aguerre *et al.*, 2020; Avila *et al.*, 2020).

Tannin extract supplementation may also mitigate enteric methane emissions from ruminants, which account for approximately half of the greenhouse gases produced by the livestock sector (Gerber *et al.*, 2013; Yanza *et al.*, 2021). This reduction in methane emission is thought to occur through the direct inhibition of methanogens, as well as through indirect effects on protozoa and decreased fibre digestion (Beauchemin *et al.*, 2007; Patra & Saxena, 2011; Adejoro, 2019). Beyond these effects on nutrition and the environment, research also indicates that TE may provide health benefits, exhibiting antioxidant (Liu *et al.*, 2013; Liu *et al.*, 2016), antiparasitic (Athanasiadou *et al.*, 2001; Minho *et al.*, 2008), antibacterial (Olajuyigbe & Afolayan, 2012; Biswas & Roymon, 2013), antiviral (Raheel *et al.*, 2013), and immunity-stimulating properties (Tibe *et al.*, 2012; Lakhani *et al.*, 2019). Hence, there is the potential to develop tannins as phytogetic feed additives suitable for sustainable organic ruminant animal production, as they provide multiple co-benefits, including replacing antibiotics and synthetic feed additives.

However, many of these opportunities and applications are challenged by reports of the negative impacts of TE on feed intake and digestibility, and thus animal performance (Dschaak *et al.*, 2011; Patra & Saxena, 2011; Adejoro *et al.*, 2019). Previous studies have reported that the risks of negative consequences increase as the TE inclusion level increases (Dschaak *et al.*, 2011; Patra & Saxena, 2011; Adejoro *et al.*, 2019). Thus, identifying the optimum inclusion and threshold levels for TE supplementation in animal feed may allow the exploitation of various applications and co-benefits associated with tannin-based feed additives, without compromising animal performance. Establishing optimum inclusion and threshold levels will also benefit research stakeholders who need quantitative data on the responses of animals to TE dietary additives.

Surprisingly, considering the volume of data available from the many *in vivo* experiments done on TE inclusion in animal feeds (especially for black wattle, quebracho, and chestnut TE), no meta-analysis has been done to specifically identify optimum inclusion and threshold levels. A recently published meta-analysis (Yanza *et al.*, 2021) examined the effects of TE supplementation at various levels on ruminant (cattle, sheep, and goats) performance and digestibility parameters, and, in addition to the TE inclusion level, also examined the effects of tannin type (hydrolysable versus condensed tannins). However, there are several other factors that may influence the animal's response to TE inclusion. These include the TE source, animal species, supplementation period, dietary fibre level, dietary protein level, and animal age, and need to be taken into account during meta-analyses.

In addition, a suitable approach to identify the TE inclusion threshold level is needed. Different approaches have been used to study the relationship between TE inclusion and animal performance (sheep and cattle) in several meta-analyses (Jayanegara & Palupi, 2010; Jayanegara *et al.*, 2012; Méndez-Ortiz *et al.*, 2018; Jayanegara *et al.*, 2019; Herremans *et al.*, 2020; Purba *et al.*, 2020; Orzuna-Orzuna *et al.*, 2021a; Orzuna-Orzuna *et al.*, 2021b; Yanza *et al.*, 2021). One approach was to link the dependent variable, such as the dry matter (DM) intake (DMI, in kg/day), to the tannin inclusion level (in g/kg DM), so that the effect of TE inclusion could be seen as a decrease or increase in the DMI regression line, depending on the TE inclusion level used. However, the DMI of each animal depends on its weight, with cattle typically consuming more than sheep. The variability in animal live weight is unrelated to the TE inclusion level, and the effect thereof can be reduced by dividing the DMI by the

body weight or metabolic body weight (MBW) (Jayanegara *et al.*, 2012; Méndez-Ortiz *et al.*, 2018). Nevertheless, using the percentage change approach might better suit the purpose of this meta-analysis (Congio *et al.*, 2022). This approach involves subtracting the DMI (dependent variable) of the control group from that of the treated group, dividing the result by the control's DMI, and multiplying by 100, with the outcome indicating either a positive or negative direction of change. A regression analysis can then be performed based on the generated observations, while considering the factors that may affect the relationship between the TE inclusion level and the DMI response (for example, the TE source or animal species). The same principle can be applied to any dependent variable, including the nutrient digestibility and animal weight gain.

Nonetheless, observations within one study are more correlated than observations across studies, and there are unignorable differences in measurement accuracy within and across studies (St-Pierre, 2001). The consequence of discounting these two facts is that the regression equation under consideration may create biased estimated parameters (St-Pierre, 2001). Mixed models allow for the study effect to be accounted for as a random effect and the measurement accuracy (variation) to be accounted for as different weights (St-Pierre, 2001; St-Pierre, 2007). We hypothesised that nutrient digestibility, intake, nitrogen balance, and animal performance responses to TE inclusion are not affected by the TE inclusion level, TE source, animal species, supplementation period, dietary fibre level, dietary protein level, or animal age. The present meta-analysis thus aimed to: (i) determine the effects of TE inclusion on animal performance, with the specific objectives of establishing optimum and threshold inclusion levels for black wattle, quebracho, and chestnut TE that prevent the suppression of animal performance in sheep and cattle, and to (ii) determine the effects of TE inclusion on the nitrogen balance, in order to quantify the co-benefits of shifting urine nitrogen to faecal nitrogen excretion.

Materials and methods

Peer-reviewed articles, theses, and dissertations for the subject under investigation were searched for in the Web of Science and Google Scholar databases. There was no time range or limitation on the publication date, and the last search was done on 20 November, 2021. The following keywords were used: "condensed tannin OR condensed tannins OR hydrolysable tannin OR hydrolysable tannins OR mimosa tannin OR mimosa tannins OR quebracho tannin OR quebracho tannins OR chestnut tannin OR chestnut tannins OR *Castanea sativa* tannin OR *Castanea sativa* tannins OR *Acacia mearnsii* tannin OR *Acacia mearnsii* tannins OR black wattle tannin OR black wattle tannins AND ram OR rams OR ewe OR ewes OR lamb OR lambs OR cattle OR heifer OR heifers OR cow OR cows OR bull OR bulls OR calf OR calves OR steer OR steers OR wether OR wethers".

The online search resulted in 153 published documents, and a number of criteria were adapted to include or exclude these studies from this meta-analysis. (1) The study had to be an *in vivo* experiment done either on sheep or cattle. (2) The source of tannin used in the experiment had to be a TE, not a plant or part of a plant. (3) Tannin extract sources had to be limited to the top three sources used, namely black wattle, quebracho, and chestnut, and studies on other sources of TE were excluded. (4) The TE used had to be from one source, and studies where the animal was provided with a mixture of TE sources were excluded. (5) The animals used had to be healthy (i.e. studies in which a TE was used as an anthelmintic agent were excluded). (6) The concentration of the TE in the feed had to be reported or calculable. (7) The TE-containing feed had to be consumed by the animal voluntarily; thus, studies where the TE was infused into the animal rumen were excluded. (8) The TE-containing feed had to be provided to the animals *ad libitum*, or a refusal rate of $\geq 10\%$ had to be reported. However, it should be noted that the last two conditions were not taken into account for the data sets for the digestibility and nitrogen balance parameters, when applied to the DMI and weight gain data sets.

Of the 153 published documents screened, 43 peer-reviewed articles and one PhD thesis met the selection criteria for inclusion in the analysis (Table 1).

Table 1 Summary of the studies included in the meta-analysis of tannin extract threshold levels in sheep and cattle feed

No	Study name	Country	Diet used	Animal species	Animal status	Tannin extract source	Inclusion method
1	Aboagye <i>et al.</i> (2018)	Canada	Total mixed ration	Cattle	Steers	Chestnut	In feed
2	Aboagye <i>et al.</i> (2019)	Canada	Total mixed ration	Cattle	Steers	Chestnut	In feed
3	Adejoro <i>et al.</i> (2019)	South Africa	Total mixed ration	Sheep	Wethers	Black wattle	In feed
4	Adejoro <i>et al.</i> (2020)	South Africa	Total mixed ration	Sheep	Lambs	Black wattle	In feed
5	Ahnert <i>et al.</i> (2015)	Germany	Forage + concentrates	Cattle	Heifers	Quebracho	Intra-ruminally infused
6	Al-Dobaib (2009)	Saudi Arabia	Forage	Sheep	Rams	Quebracho	In feed
7	Avila <i>et al.</i> (2020)	Brazil	Total mixed ration	Cattle	Steers	Black wattle	In feed
8	Beauchemin <i>et al.</i> (2007)	Canada	Total mixed ration	Cattle	Heifers	Quebracho	In feed
9	Benchaar <i>et al.</i> (2008)	Canada	Total mixed ration	Cattle	Lactating cows	Quebracho	In feed
10	Carulla <i>et al.</i> (2005)	Colombia	Forage	Sheep	Lambs	Black wattle	In feed
11	Costa <i>et al.</i> (2021)	Brazil	Total mixed ration	Sheep	Lambs	Black wattle	In feed
12	Dallastra <i>et al.</i> (2018)	Brazil	Total mixed ration	Sheep	Lactating ewes	Black wattle	In feed
13	Dawson <i>et al.</i> (1999)	UK	Forage (pelleted dried grass)	Sheep	Lambs	Quebracho	In feed
14	Deaville <i>et al.</i> (2010)	UK	Silage	Sheep	Wethers	Black wattle	In feed
15	Dschaak <i>et al.</i> (2011)	USA	Total mixed ration	Cattle	Lactating cows	Quebracho	In feed
16	Ebert <i>et al.</i> (2017)	USA	Finishing diet	Sheep	Steers	Quebracho	In feed
17	Frutos <i>et al.</i> (2004)	Spain	Forage	Sheep	Lambs	Chestnut	In feed
18	Gerlach <i>et al.</i> (2018)	Germany	Total mixed ration	Cattle	Lactating cows	Black wattle	In feed
19	Henke <i>et al.</i> (2017)	Germany	Total mixed ration	Cattle	Lactating cows	Quebracho	In feed
20	Kamel <i>et al.</i> (2018)	Saudi Arabia	Total mixed ration	Sheep	Lambs	Quebracho	In feed
21	Kapp-Bitter <i>et al.</i> (2020)	Switzerland	Forage	Cattle	Lactating cows	Chestnut	In feed
22	Koenig <i>et al.</i> (2018)	Canada	Total mixed ration	Cattle	Steers	Black wattle	In feed
23	Kozloski <i>et al.</i> (2012)	Brazil	Forage	Sheep	Wethers	Black wattle	Intra-ruminally infused
24	Krueger <i>et al.</i> (2010)	USA	Total mixed ration	Sheep	Steers	Chestnut	In feed

Table 1 Summary of the studies included in the meta-analysis of tannin extract threshold levels in sheep and cattle feed (continued)

No	Study name	Country	Diet used	Animal species	Animal status	Tannin extract source	Inclusion method
25	Liu <i>et al.</i> (2013)	China	Total mixed ration	Cattle	Lactating cows	Chestnut	In feed
26	Liu <i>et al.</i> (2016)	China	Total mixed ration	Sheep	Lambs	Chestnut	In feed
27	Mezzomo <i>et al.</i> (2011)	Brazil	Concentrates	Cattle	Bulls	Quebracho	In feed
28	Norris <i>et al.</i> (2020)	USA	Total mixed ration	Cattle	Steers	Quebracho	In feed
29	Orlandi <i>et al.</i> (2015)	Brazil	Forage + concentrates	Cattle	Steers	Black wattle	In feed
30	Piñeiro-Vázquez <i>et al.</i> (2017)	Mexico	Forage	Cattle	Heifers	Quebracho	In feed
31	Piñeiro-Vázquez <i>et al.</i> (2018)	Mexico	Forage	Cattle	Heifers	Quebracho	In feed
32	Rivera-Méndez <i>et al.</i> (2017)	USA	Finishing diet	Cattle	Steers	Quebracho & chestnut	In feed
33	Salawu <i>et al.</i> (1997)	UK	Forage	Sheep	Rams	Quebracho	In feed
34	Sinz <i>et al.</i> (2019)	Switzerland	Forage + concentrates	Sheep	Lambs	Black wattle	In feed
35	Śliwiński <i>et al.</i> (2002)	Switzerland	Forage + concentrates	Sheep	Lambs	Chestnut	In feed
36	de Souza <i>et al.</i> (2021)	Brazil	Silage + concentrates	Sheep	Lambs	Black wattle	In feed
37	Staerfl <i>et al.</i> (2012)	Switzerland	Finishing diet	Cattle	Bulls	Black wattle	In feed
38	Taha (2015)	UK	Silage	Sheep	Cows	Chestnut	In feed
39	Toral <i>et al.</i> (2013)	Spain	Total mixed ration	Sheep	Lactating ewes	Quebracho	In feed
40	Tseu <i>et al.</i> (2020)	Brazil	Total mixed ration	Cattle	Cows	Black wattle	In feed
41	Valenti <i>et al.</i> (2019)	Italy	Concentrates	Sheep	Lambs	Black wattle & chestnut	In feed
42	Vasta <i>et al.</i> (2009)	Italy	Forage + concentrates	Sheep	Lambs	Quebracho	In feed
43	Wischer <i>et al.</i> (2014)	Germany	Forage + concentrates	Sheep	Wethers	Chestnut	In feed
44	Zimmer & Cordesse (1996)	France	Forage	Sheep	Wethers	Chestnut	In feed

Our data set included six categorical predictors: (1) TE source, (2) animal species, (3) supplementation period, (4) dietary crude protein (CP) content, (5) dietary neutral detergent fibre (NDF) content, and (6) animal age. The TE source predictor consisted of three categories: chestnut TE, black wattle TE, and quebracho TE. The animal species predictor included sheep and cattle, with data from these two species being included in the analysis to assess the significance and extent of differences between these species in their responses to TE inclusion. Tannin extract supplementation periods were divided by length into two categories: short-term (≤ 1 month) and long-term (> 1 month). The dietary CP content was classified as low ($< 18\%$ CP) or high ($\geq 18\%$ CP), and the dietary NDF content was categorised as low ($\leq 38\%$ NDF) or high ($> 38\%$ NDF). The animals used in the study were grouped by age as young (< 1 year) or old (> 1 year) animals. It is crucial to note that lactating cows and ewes were only included in the DMI and digestibility datasets, and were excluded from the nitrogen balance and weight gain datasets.

To make the comparison between different studies more applicable, we used the change percentage to calculate the dependent variables' responses to TE inclusion (Hou *et al.*, 2015; Congio *et al.*, 2022). The following equation was applied to calculate the DMI response to TE inclusion:

$$DMI \text{ response} = ((DMI_t - DMI_c) \div DMI_c) \times 100$$

where: DMI_t = DMI of the treated group, and DMI_c = DMI of the control group.

The same method was used to calculate the responses of weight gain, digestibility parameters – organic matter digestibility (OMD), CP digestibility (CPD), neutral detergent fibre digestibility (NDFD), and acid detergent fibre digestibility (ADFD) – and nitrogen balance parameters (faecal nitrogen, urinary nitrogen, and retained nitrogen) to TE inclusion. However, for the nitrogen balance, the nitrogen (faecal nitrogen, urinary nitrogen, and retained nitrogen) in grams per 100 g of intake was calculated for both the control and the treated group first, before calculating the response.

The level of TE inclusion can be reported as grams per kilogram DM of feed (g/kg DM) or grams per kilogram of the animal's MBW (g/kg MBW). However, the scales differ between these two methods of expression (e.g. for DMI data, the minimum and maximum values were 2.0 and 89.3 g/kg DM, versus 0.2 and 6.35 g/kg MBW, respectively). To standardise this, the TE inclusion level was expressed as g/100 g of DM (resulting in DMI minimum and maximum values of 0.2 and 8.9 g/100 g DM) in this analysis. Both units for expressing the TE inclusion level (g/100 g DM and g/kg MBW) were calculated, and their relationships with the dependent variables were examined.

Statistical analyses were carried out using R (version 4.2.1, 2022-06-23) and RStudio (version 2022.07.1+554). Studies were treated as random effects, whereas the TE level, TE source, animal species, supplementation period, dietary NDF level, dietary CP level, and animal age were treated as fixed effects, using the following mixed models:

Model 1: Linear model:

$$Y_{irsfvna} = \mu + S_i + \beta_1 X_{irsfvna} + C_r + A_s + P_f + F_v + T_n + G_a + e_{irsfvna}$$

Model 2: Piecewise model:

$$Y_{irsfvna} = \mu + S_i + \beta_2 X_{irsfvna} I(X_{irsfvna} < \lambda) + \beta_3 X_{irsfvna} I(X_{irsfvna} \geq \lambda) + C_r + A_s + P_f + F_v + T_n + G_a + e_{irsfvna}$$

where: $Y_{irsfvna}$ = response variable; μ = overall mean; S_i = random effect of the i th study; β_1 = linear regression coefficient; β_2 = linear regression coefficient ($X_{irsfvna}$) when $X_{irsfvna} < \lambda$; $X_{irsfvna}$ = value of the continuous predictor variable (TE level); β_3 = linear regression coefficient when $X_{irsfvna} \geq \lambda$; λ = the breakpoint/knot for the piecewise model; $I()$ = an indicator function that returns 1 if the condition inside is true, 0 otherwise; C_r = TE source ($r = 1-3$); A_s = animal species ($s = 1-2$); P_f = supplementation period ($f = 1-2$); F_v = dietary fibre level ($v = 1-2$); T_n = dietary protein level ($n = 1-2$); G_a = animal age ($a = 1-2$); and $e_{irsfvna}$ = residual error.

The package lme4 version 1.1-35.3 (Bates *et al.*, 2015) was used to create the mixed models, and the function 'step' in the package lmerTest version 3.1-3 (Kuznetsova *et al.*, 2017) was used to create a backward-reduced, fixed-effect table. Accordingly, best-fit models for each response variable were selected, in which only significant terms ($P < 0.05$) were retained. The piecewise model knot location was chosen by iterative adjustment of the knot and comparison of the model Akaike information criterion (AIC) after initial placement based on visual assessment. The final model with one optimised knot balanced the goodness of fit and the stability, based on residual plots. The log-likelihood ratio test was performed using the 'lr_test' function in the lmerTest package version 0.9-40 (Zeileis & Hothorn, 2002), to evaluate if the increased complexity of the piecewise model significantly improved fit over the linear model. The regression coefficients, P -values, and conditional and marginal determination coefficients ($R^2_{(c)}$ and $R^2_{(m)}$, respectively) reported for the different models were estimated using the sjPlot's 'tab_model' function in the sjPlot package version 2.8.15 (Lüdtke *et al.*, 2023). The $R^2_{(m)}$ is the variance explained by the fixed effects of the model, and the $R^2_{(c)}$ is the variance explained by both the fixed and random effects of the model (Nakagawa & Schielzeth, 2013).

In addition, goodness of fit statistics, including the AIC, weighted root mean squared error (RMSE), and Lin's concordance correlation coefficient (CCC) were also reported for each model. The RMSE is a measure of the prediction precision that quantifies the magnitude of errors by squaring them to produce a positive value. As a rule of thumb, the RMSE should be less than half of the standard deviation (SD) (Singh *et al.*, 2005; Moriasi *et al.*, 2007). In other words, the ratio of the RMSE to an observation's SD should be less than 0.5 (Moriasi *et al.*, 2007). This ratio is called the RMSE-to-observations SD ratio (RSR) (Singh *et al.*, 2005; Moriasi *et al.*, 2007). The lower the RSR, the lower the RMSE and the better the model performance (Moriasi *et al.*, 2007). Lin's CCC was also calculated for all the models to measure the agreement between the observed and the model-predicted values. According to Altman (1990), a CCC value higher than 0.80 can be interpreted as an excellent agreement.

The joint test generates an F -ratio and a P -value for each term in the model. In general, the higher the value of the statistic, the higher the ranking of the term. When found to be significant in a model, the TE sources were pairwise compared using Tukey's contrast, determined using the 'emmeans' function in the emmeans package version 1.8.1-1 (Lenth, 2022). Data observations were weighted by the inverse of their relative variance ($1/RV$). The relative variance (RV) was calculated by dividing the squared standard error of the mean (SEM^2) by the mean of each study. Then, each weight was divided by the mean of all weights, and the resulting values were used as weighting factors in the analysis to maintain the expressions of dispersion in the original scale of the measurements (St-Pierre, 2001). The presence of outliers was identified by examining studentised residuals, with the influence plot provided within the car package version 3.1-2 (Fox & Weisberg, 2019). Values beyond ± 3 SD were considered outliers and were thus removed from the dataset. The figures were visualised using the Matplotlib Python package version 3.12.6 (Hunter, 2007).

Results and discussion

Data were compiled from 44 scientific publications, including results for 580 sheep and 742 cattle. Tables 2, 3, and 4 present the linear and piecewise best-fit models for the responses of various parameters to TE inclusion, with goodness of fit measures. The responses evaluated included the DMI, OMD, and CPD (Table 2), the NDFD, ADFD, and weight gain (Table 3), and the faecal nitrogen, urinary nitrogen, and retained nitrogen (Table 4). The results showed that the piecewise regression model consistently outperformed the linear model across all response variables. The piecewise model demonstrated a better fit for the data, with higher $R^2_{(c)}$ values (piecewise: 0.73–0.99, linear: 0.72–0.91), lower RMSE values (piecewise: 1.03–9.98, linear: 1.76–10.57), lower RSR values (piecewise: 0.09–0.48, linear: 0.21–0.50), higher CCC values (piecewise: 0.83–1.0, linear: 0.83–0.96), and lower AIC values (piecewise: 238.70–433.16, linear: 244.20–438.79). Furthermore, the log-likelihood ratio tests showed significant differences between the linear and piecewise models for DMI ($P < 0.001$), OMD ($P = 0.028$), CPD ($P = 0.006$), NDFD ($P = 0.007$), ADFD ($P = 0.033$), faecal nitrogen ($P = 0.009$), urinary nitrogen ($P = 0.003$), and retained nitrogen ($P = 0.006$).

Table 2 Regression parameters and goodness of fit measures for the linear and piecewise best-fit models used to predict dry matter intake (DMI), organic matter digestibility (OMD), and crude protein digestibility (CPD) responses (%) to tannin extract inclusion in g/100 g dry matter (DM) in sheep and cattle diets

	DMI			OMD			CPD		
Model type	Linear	Piecewise		Linear	Piecewise		Linear	Piecewise	
MODEL REGRESSION									
Intercept									
Estimate	2.32	−0.62		3.91	1.99		−1.48	−3.66	
SE	1.20	1.23		2.02	2.26		1.39	1.63	
P-value	0.058	0.616		0.059	0.383		0.294	0.031	
Tannin extract level (g/100 g of DM)									
		Knot			Knot			Knot	
		<1.6	≥1.6		<2.1	≥2.1		<2.1	≥2.1
Estimate	−2.14	2.24	−1.56	−1.55	0.24	−1.19	−3.43	−1.27	−2.86
SE	0.31	0.83	0.29	0.29	0.96	0.34	0.36	0.83	0.39
P-value	<0.001	0.009	<0.001	<0.001	0.803	0.001	<0.001	0.133	<0.001
Animal species (sheep vs cattle)									
Estimate	NS	NS		NS	NS		4.40	3.89	
SE	–	–		–	–		1.82	1.91	
P-value	–	–		–	–		0.021	0.049	
Tannin extract source (black wattle vs chestnut)									
Estimate	NS	NS		−4.81	−4.95		NS	NS	
SE	–	–		1.85	1.82		–	–	
P-value	–	–		0.012	0.009		–	–	
Tannin extract source (quebracho vs chestnut)									
Estimate	NS	NS		−5.00	−4.78		NS	NS	
SE	–	–		2.36	2.39		–	–	
P-value	–	–		0.039	0.050		–	–	
MODEL GOODNESS OF FIT									
R ² _m /R ² _c	0.45/0.86	0.49/0.93		0.33/0.81	0.30/0.83		0.70/0.91	0.66/0.93	
RMSE	1.76	1.24		2.01	1.85		1.82	1.45	
RSR	0.35	0.29		0.37	0.34		0.29	0.23	
AIC	372.91	350.19		368.37	365.55		244.20	238.70	
CCC	0.93	0.97		0.91	0.93		0.96	0.97	
N	61/27	61/27		63/28	63/28		43/21	43/21	

SE: standard error, NS: non-significant, R²_m: marginal determination coefficient, R²_c: conditional determination coefficient, RMSE: root mean squared error, RSR: ratio of RMSE to the standard deviation of the observations, AIC: Akai information criterion, CCC: Lin's concordance correlation coefficient, N: number of observations/studies. For each predictor, the second category is the reference level.

This study examined several potential predictors, including the TE inclusion level, TE source, animal species, supplementation period, dietary CP level, dietary NDF level, and animal age. The TE type (hydrolysable versus condensed tannin) was also included as an alternative predictor for the TE source.

The results showed that, other than the TE inclusion level, none of the potential predictors had a significant effect on the DMI. Table 2 presents the linear and piecewise best-fit models for the DMI response to TE inclusion, and Figure 1a illustrates the piecewise model's predicted DMI response to TE inclusion (g/100 g DM), along with the weighted observations. The model showed a positive DMI response below the model knot (<1.6 g TE/100 g DM) and a negative DMI response above the model knot (≥ 1.6 g TE/100 g DM), decreasing at a rate of 1.56% per additional gram of TE. This resulted in a 5% reduction in the DMI at 3 g TE/100 g DM. This level (3 g TE/100 g DM) may thus represent a threshold for the inclusion of TE from chestnut, quebracho, and black wattle, in terms of their effects on the DMI in sheep and cattle, for use when incorporating dietary TE for various applications and associated benefits. A 5% reduction in the DMI may be acceptable, as it is reported to be statistically insignificant in a number of studies (Frutos *et al.*, 2004; Piñeiro-Vázquez *et al.*, 2018; Avila *et al.*, 2020). These results indicate that the concentration of TE in the feed is the primary factor determining the animal's response in terms of increasing or decreasing DMI, and this effect is most probably caused by the impact of TE on feed palatability. It has been documented that ruminant animals (goats, sheep, and cattle) may exhibit a preference for bitter tastes at lower concentrations (Goatcher & Church, 1970; Lamy *et al.*, 2011); however, high TE levels likely decrease the DMI partly because of the astringent effect and bitterness of TE in the mouth, and partly because of the adverse post-ingestive influences of TE on the oral cavity and foregut (Silanikove *et al.*, 2001; Attia *et al.*, 2013; Bhatt *et al.*, 2023). Furthermore, higher TE levels may disrupt digestive processes (for example, through the inhibition of digestive enzymes and binding to proteins), leading to reduced nutrient digestibility and decreased feed intake (Kan *et al.*, 2020; Tretola *et al.*, 2023).

Our finding of a significant relationship between the TE inclusion level and feed intake agrees with the meta-analysis by Yanza *et al.* (2021), which showed decreased ruminant DMI (kg/d) with dietary TE inclusion. While they did not employ a piecewise model, Yanza *et al.* (2021) suggested that a quadratic model fit better than a linear model. In contrast, other recent meta-analyses reported that dietary tannin supplementation had no effect on the DMI of beef cattle (Orzuna-Orzuna *et al.*, 2021a) and sheep (Orzuna-Orzuna *et al.*, 2021b). The apparent contradiction of these reports with our findings may be partly attributed to our selection criteria, which allowed the TE inclusion to manifest its effect on the DMI by only including animals fed the TE-containing feed *ad libitum*. The difference in the approach used to calculate the DMI response to TE inclusion could also be a contributing factor.

Our results further indicate that sheep and cattle seem to have similar DMI responses to TE inclusion. While it may be argued that sheep are more selective eaters than cattle, and this may influence the perceived palatability of the diet provided, there is a distinction between providing ample choices and providing a selected diet (Forbes & Mayes, 2002). Previous research on the responses of ruminants to bitterness identified a substantial difference between goats and sheep, and goats and cattle, but found it hard to distinguish between sheep and cattle in their responses to bitterness (Goatcher & Church, 1970; Lamy *et al.*, 2011).

Apart from the TE inclusion level and TE source, no other potential predictors significantly affected the OMD. Table 2 presents the linear and piecewise best-fit models for the OMD response to TE inclusion. The TE inclusion level had the highest rank among the OMD fixed effects ($F = 19.9$, $P < 0.001$), followed by the TE source ($F = 3.6$, $P = 0.046$). Figure 1b depicts the piecewise model-predicted influence of the TE level and TE source on the OMD response, with the weighted observations. In the case of chestnut TE, the model showed a positive OMD response below the model knot (<2.1 g chestnut TE/100 g DM). However, a negative response was observed above the knot (≥ 2.1 g chestnut TE/100 g DM), and a response rate of 1.19% per additional gram of chestnut TE resulted in a 5% reduction at 6 g chestnut TE/100 g DM. This 6 g TE/100 g DM can thus be considered the threshold for chestnut TE, in terms of the effect on OMD in sheep and cattle. For quebracho and black wattle TE at levels below 2.1 g TE/100 g DM, there was a minor negative OMD response. However, at 2.1 g TE/100 g DM, both resulted in a 5% OMD reduction, indicating that this is the dietary threshold for quebracho and black wattle TE inclusion, in terms of the effect on OMD (Figure 1b). The pairwise test showed a significant OMD response difference between chestnut and black wattle TE ($P = 0.029$), but not between chestnut and quebracho TE ($P = 0.130$) or black wattle and quebracho TE ($P = 0.996$).

The results of this study imply that chestnut TE had a significantly less negative effect on OMD than black wattle TE at the same inclusion level (Table 2). This aligns with the results of Deaville *et al.* (2010), which showed a higher OMD for chestnut versus black wattle TE inclusion in sheep silage, at equal inclusion levels. Previous research has also found that condensed tannins reduce the *in vitro* OMD more than hydrolysable tannins (Makkar *et al.*, 1995; Jayanegara *et al.*, 2015). The greater effect of the black wattle TE on OMD, compared to the chestnut TE, thus likely reflects the higher condensed tannin content of black wattle. The greater negative effect of condensed tannins on OMD than hydrolysable tannins is also reported in this study.

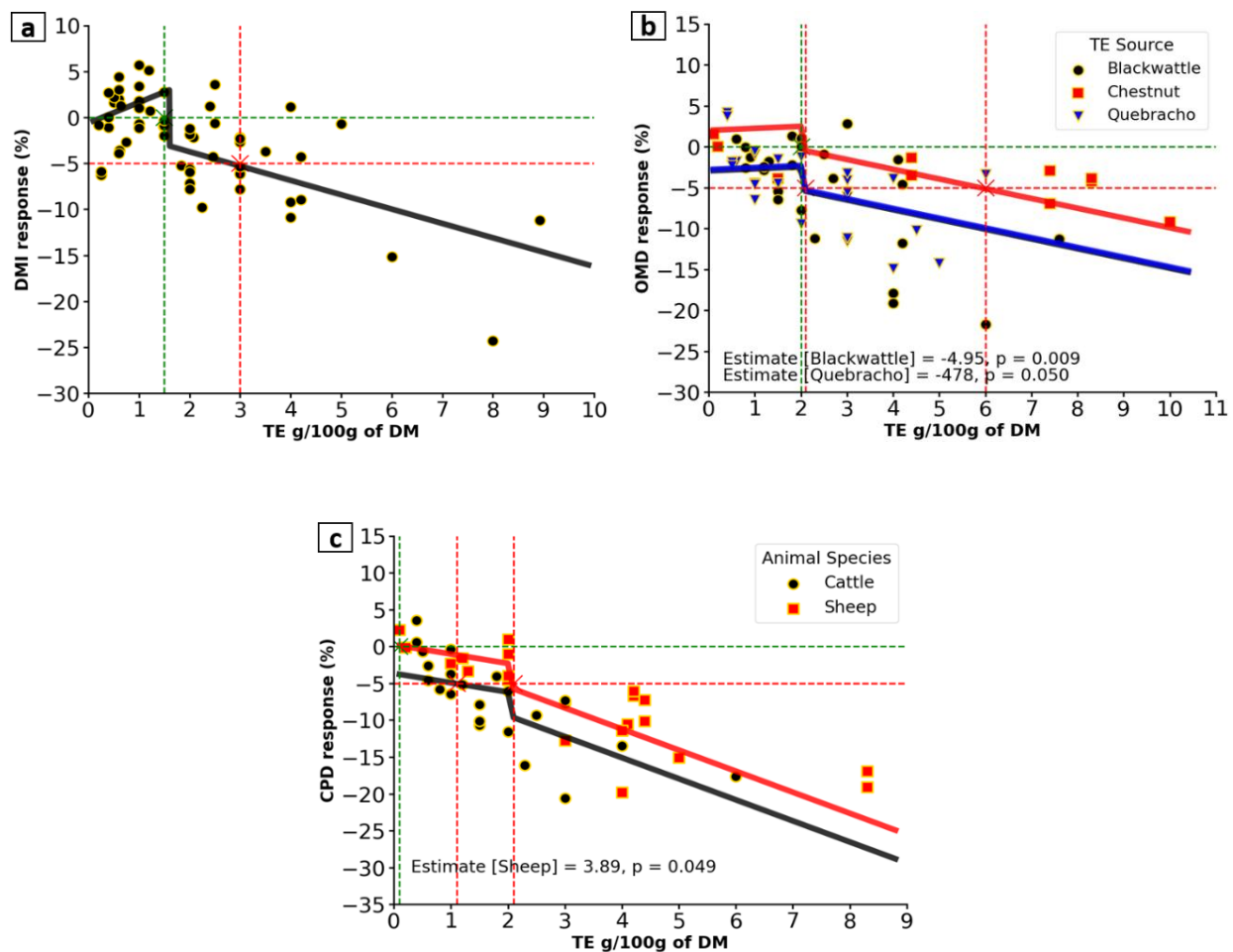


Figure 1 Weighted observations and predicted lines for the responses (%) of (a) dry matter intake (DMI), (b) organic matter digestibility (OMD), and (c) crude protein digestibility (CPD) to tannin extract (TE) inclusion rates in g/100 g of dry matter (DM), determined using a piecewise model. The green cross on each graph indicates the upper end of the optimum inclusion level, while the red cross on each graph indicates the proposed threshold level.

The significant relationship between the TE inclusion level and the OMD response found in this study agrees with the results of the meta-analysis by Jayanegara & Palup (2010), who found that ruminant OMD (mg/g DM) decreased linearly with an increase in the condensed tannin inclusion level. The negative effect of high levels of TE on OMD could be due to tannin's ability to bind with exogenous enzymes and feed particles like proteins or fibres. This binding can reduce enzyme activity and alter the structural integrity of the particles, limiting enzyme accessibility (Duodu *et al.*, 2003; Taylor *et al.*, 2009; Giuberti *et al.*, 2020).

and hindering microbial attachment (Kelln *et al.*, 2020; Rira *et al.*, 2022), and thereby reducing digestion. Additionally, tannins may exhibit toxic effects on ruminal microorganisms such as fibre-degrading bacteria (Cipriano-Salazar *et al.*, 2018; Nawab *et al.*, 2020).

The TE inclusion level and animal species both significantly affected the CPD, while none of the other potential predictors had any significant effect. Table 2 shows the linear and piecewise best-fit models for CPD response to TE inclusion and their goodness of fit measures. The TE inclusion level had the highest rank among the CPD fixed effects ($F = 110.3$, $P < 0.0001$), followed by animal species ($F = 4.3$, $P = 0.056$). Figure 1c illustrates the impact of the TE level and animal species on the CPD response to TE inclusion, as determined by the piecewise model. In sheep, the model showed a positive CPD response below the model knot (< 2.1 TE g/100 g DM), with this decreasing gradually to a minor negative response in CPD. However, above the model knot (≥ 2.1 g TE/100 g DM), the CPD decreased by 2.86% per additional gram of TE, reaching a 5% reduction at 2.1 g TE/100 g DM. In cattle, the 5% reduction in CPD occurred at a lower TE inclusion level of 1.1 g TE/100 g DM. We therefore suggest threshold inclusion levels for TE from chestnut, quebracho, and black wattle of 2.1 g TE/100 g DM in sheep and 1.1 g TE/100 g DM in cattle, in terms of the effects on CPD, when the TE is incorporated for various applications and associated benefits. A 5% reduction in CPD may be acceptable, given the lack of statistical significance for this level of reduction reported in several studies (Benchaar *et al.*, 2008; AL-Dobaib, 2009).

The negative effect of high levels of TE on CPD could be due to tannin's ability to form indigestible tannin-protein complexes (Jones & Mangan, 1977; Aschfalk *et al.*, 2000; Osakwe *et al.*, 2004), inhibit digestive enzymes (Blytt *et al.*, 1988; Hagerman, 1989; Huang *et al.*, 2022), and interfere with rumen microbial activity (Min *et al.*, 2005; Huang *et al.*, 2022). The results suggest that sheep are slightly more able to digest CP than cattle are ($3.89 \pm 1.91\%$, $P = 0.049$) at the same TE inclusion level (Table 2, Figure 1c). This difference in response between the two species may be due to their differences in CPD, as sheep have been previously shown to have better CPD levels than cattle (Südekum *et al.*, 1995; Woods *et al.*, 1999). For instance, Woods *et al.* (1999) reported 4.62% higher digestibility of soya hulls in sheep than in cattle, and up to 16.73% higher digestibility of palm kernel meal in sheep than in cattle (Woods *et al.*, 1999). Moreover, a recent meta-analysis that examined species differences in ruminal fermentation control found that cattle had a significantly lower CPD than sheep, despite no significant difference being observed in the overall OMD (Pfau *et al.*, 2023). Some researchers have suggested that sheep may have lower faecal metabolic losses than cattle, resulting in their higher CPD (Van Soest, 1988; Van Soest, 1994; Woods *et al.*, 1999). However, others have noted that the ratio of the mean retention time of particles to the mean retention time of fluid in the reticulorumen is higher in cattle than in sheep, suggesting that the liquid washout of particulate digesta is higher in cattle than in sheep (Pfau *et al.*, 2023). This may facilitate a higher microbial yield from the reticulorumen, but would increase the metabolic losses of faecal nitrogen of microbial origin, lowering CPD values (Pfau *et al.*, 2023). Nonetheless, further investigation is needed to improve our understanding of the mechanisms involved in this species difference in CPD.

Of the potential predictors tested, only the TE inclusion level and TE source significantly affected the NDFD response. Table 3 presents the linear and piecewise best-fit models for the NDFD response to TE inclusion. The results indicated that the TE inclusion level had the highest rank among the fixed effects for the effects on NDFD ($F = 54.7$, $P < 0.001$), followed by the TE source ($F = 4.6$, $P = 0.025$). Figure 2a illustrates the effect of the TE inclusion level and TE source on the predicted NDFD, as determined by the piecewise model. The model showed an optimal positive NDFD response to chestnut and quebracho TE inclusion below the model knot (< 2.1 g TE/100 g DM). However, inclusion rates at or above the knot value (≥ 2.1 g TE/100 g DM) produced a negative response, with the NDFD decreasing at a rate of 3.23% per additional gram of chestnut or quebracho TE, resulting in a 5% reduction at the same knot value (2.1 g TE/100 g DM). This particular inclusion level (2.1 g TE/100 g DM) can thus be considered the threshold for chestnut and quebracho TE inclusion in sheep and cattle diets, in terms of the effect on the NDFD. In contrast, black wattle TE inclusion significantly decreased the NDFD, with an approximately 7% decrease when 1 g TE/100 g DM was included (Figure 2a). Pairwise tests indicated non-significant differences in the NDFD responses to chestnut and quebracho TE ($P = 0.998$), and to chestnut and black wattle TE ($P = 0.107$), but a significant difference in the NDFD responses to the inclusion of quebracho and black wattle TE ($P = 0.034$).

Table 3 Regression parameters and goodness of fit measures for the linear and piecewise best-fit models used to predict neutral detergent fibre digestibility (NDFD), acid detergent fibre digestibility (ADFD), and weight gain responses (%) to tannin extract inclusion in g/100 g dry matter (DM) in sheep and cattle diets

	NDFD			ADFD			Weight gain		
Model type	Linear	Piecewise		Linear	Piecewise		Linear	Piecewise	
MODEL REGRESSION									
Intercept									
Estimate	4.29	1.56		4.63	3.93		5.27	1.42	
SE	3.46	3.69		4.20	4.55		3.31	3.29	
P-value	0.220	0.674		0.279	0.394		0.123	0.671	
Tannin extract level (g/100 g of DM)									
		Knot			Knot			Knot	
		<2.1	≥2.1		<1.4	≥1.4		<1.6	≥1.6
Estimate	-3.95	-0.71	-3.23	-3.03	-1.37	-2.90	-3.63	3.49	-3.01
SE	0.45	1.41	0.52	0.73	3.91	0.80	0.93	1.87	0.90
P-value	<0.001	0.614	<0.001	<0.001	0.729	0.001	0.001	0.074	0.003
Animal species (sheep vs cattle)									
Estimate	NS	NS		NS	NS		NS	NS	
SE	—	—		—	—		—	—	
P-value	—	—		—	—		—	—	
Tannin extract source (black wattle vs chestnut)									
Estimate	-7.42	-8.62		-16.96	-16.83		NS	NS	
SE	3.96	4.07		5.98	6.06		—	—	
P-value	0.066	0.039		0.008	0.010		—	—	
Tannin extract source (quebracho vs chestnut)									
Estimate	0.35	-0.27		-4.38	-4.33		NS	NS	
SE	4.09	4.17		4.87	4.93		—	—	
P-value	0.933	0.949		0.376	0.388		—	—	
MODEL GOODNESS OF FIT									
R ² _m / R ² _c	0.60/0.90	0.59/0.92		0.53/0.85	0.53/0.85		0.38/0.97	0.44/0.99	
RMSE	2.82	2.56		3.67	3.65		1.48	1.03	
RSR	0.26	0.23		0.33	0.33		0.13	0.09	
AIC	417.97	412.65		246.23	243.69		226.22	214.4	
CCC	0.96	0.97		0.94	94		0.98	1.00	
N	62/28	62/28		35/17	35/17		32/15	32/15	

SE: standard error, NS: non-significant, R²_m: marginal determination coefficient, R²_c: conditional determination coefficient, RMSE: root mean squared error, RSR: ratio of RMSE to the standard deviation of the observations, AIC: Akai information criterion, CCC: Lin's concordance correlation coefficient, N: number of observations/studies. For each predictor, the second category is the reference level.

Similarly, other than the TE inclusion level and TE source, none of the potential predictors were significantly associated with the ADFD response. Table 3 presents the linear and piecewise best-fit models for the ADFD response to TE inclusion. Among the fixed effects of the ADFD model, the TE inclusion level

had the highest rank ($F = 15.6$, $P = 0.003$), followed by the TE source ($F = 5.2$, $P = 0.045$). Figure 2b illustrates the effect of the TE inclusion level and TE source on the predicted ADFD response, based on the piecewise model. The model showed an optimal positive response in ADFD to chestnut TE inclusion below the model knot (<1.4 g TE/100 g DM). Beyond this inclusion level, a negative ADFD response was observed, with a 2.9% reduction in ADFD per additional gram of chestnut TE, resulting in a 5% reduction at 3.1 g TE/100 g DM. In the case of quebracho TE, the 5% reduction was reached earlier, at an inclusion level of 1.6 g TE/100 g DM. These inclusion levels of 3.1 g chestnut TE/100 g DM and 1.6 g quebracho TE/100 g DM may thus serve as the ADFD threshold inclusion levels, at which a 5% decrease in ADFD occurs. In contrast, black wattle TE inclusion considerably decreased the ADFD, with a reduction of approximately 14% observed at an inclusion level of 1 g black wattle TE/100 g DM (Figure 2b). Pairwise tests indicated no significant difference in ADFD responses to chestnut and quebracho TE inclusion ($P = 0.657$), a tendency for differences in ADFD responses to quebracho and black wattle TE inclusion ($P = 0.081$), and a significant difference in ADFD responses to chestnut and black wattle TE inclusion ($P = 0.044$).

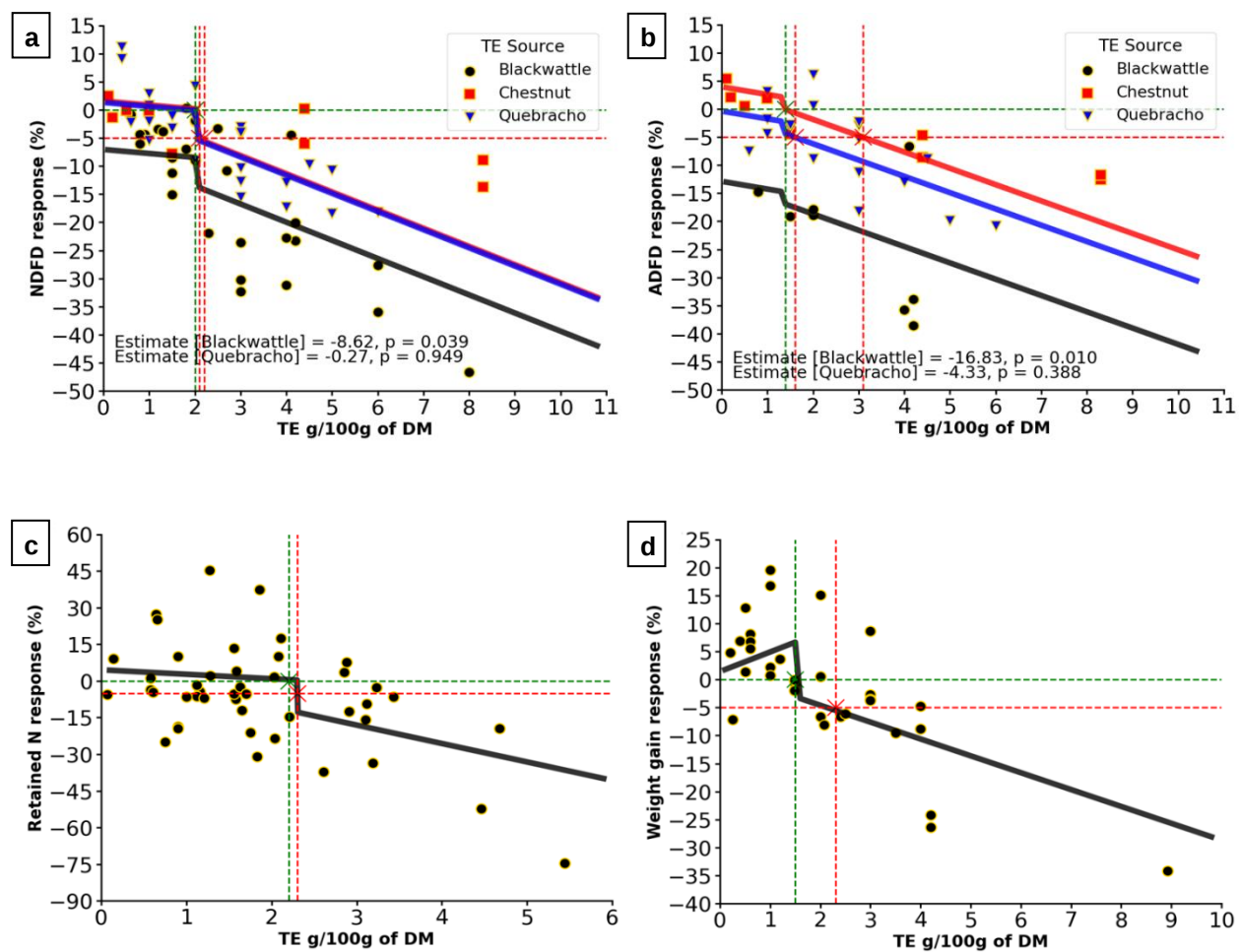


Figure 2 Weighted observations and predicted lines for the responses (%) of (a) neutral detergent fibre digestibility (NDFD), (b) acid detergent fibre digestibility (ADFD), (c) retained nitrogen, and (d) weight gain to tannin extract (TE) inclusion using the piecewise model. The green cross on each graph indicates the upper end of the optimum inclusion level, while the red cross on each graph indicates the proposed threshold level.

These results suggest that the inclusion of black wattle TE in the diet has a notable negative effect on both the NDFD ($-8.62 \pm 4.07\%$) and ADFD ($-16.96 \pm 5.98\%$), relative to the effects of including chestnut TE at the same levels (Table 3). However, the results suggest no significant difference in NDFD and ADFD responses to quebracho and chestnut TE inclusion (Table 3).

The differences between TE sources in their effects on the NDFD and ADFD may be attributed to the differing chemical structures of the tannins from these sources (Kardel *et al.*, 2013; Dos Santos Grasel *et al.*, 2016), which enable varying degrees of complex formation with the feed NDF or ADF content. For example, while both black wattle and quebracho are sources of condensed tannins (Das *et al.*, 2020), their chemical composition differs in terms of the variety of starter and extender units present and the distribution of oligomers, potentially affecting their impact on fibre digestibility (Venter *et al.*, 2012). This meta-analysis suggests that black wattle TE has the most notable negative impact on fibre digestibility, when compared to chestnut and quebracho TE. The inhibitory effects of tannins on fibre digestibility could be attributed to their interference with the attachment and development of feed-associated microbial communities, notably by fibrolytic bacteria in the rumen, which are crucial for the degradation of fibrous feeds (Salami *et al.*, 2018; Kelln *et al.*, 2020; Rira *et al.*, 2022). For example, tannins can inhibit the fibre-digesting activity of *Fibrobacter succinogenes*, a fibrolytic bacterium, by inactivating extracellular enzymes and interfering with adhesion to cellulose fibres (Bae *et al.*, 1993; Min *et al.*, 2005).

The TE level, TE source, dietary protein level, and supplementation period were the only potential predictors to significantly affect the faecal nitrogen response. Table 4 presents the linear and piecewise best-fit models for the faecal nitrogen response to TE inclusion. Of the model fixed effects, the TE inclusion level had the highest rank ($F = 89.1$, $P < 0.001$), followed by the TE source ($F = 19.4$, $P < 0.004$), dietary protein level ($F = 9.3$, $P = 0.024$), and supplementation period ($F = 8.3$, $P = 0.010$). The piecewise model showed a negative faecal nitrogen response to chestnut TE inclusion below the model knot (< 1 g TE/kg MBW). However, above this knot (≥ 1 g TE/kg MBW), the response became positive, with a 13.74% increase in faecal nitrogen per additional gram of chestnut TE. For quebracho TE, the positive response occurred at a higher inclusion level (1.3 g TE/kg MBW). The dietary inclusion of black wattle TE considerably increased the faecal nitrogen response, with an 18.86% increase at 1 g TE/kg MBW. Black wattle TE inclusion increased the faecal nitrogen level $17.39 \pm 3.09\%$ more than chestnut TE inclusion at the same level, and 21.91% more than quebracho TE inclusion at the same level (Table 4). Pairwise tests showed a non-significant difference between the effects of chestnut and quebracho TE inclusion ($P = 0.693$) on the faecal nitrogen response, but differences between the effects of quebracho and black wattle TE inclusion ($P = 0.018$), and between chestnut and black wattle TE inclusion ($P < 0.001$). These results indicate that black wattle TE had the most notable effect on the faecal nitrogen content, when compared to chestnut and quebracho TE.

The observed increase in the faecal nitrogen response following the inclusion of higher levels of TE can be attributed to the formation of indigestible tannin-protein complexes (Aschfalk *et al.*, 2000; Osakwe *et al.*, 2004; Jayanegara & Palupi, 2010), the inhibition of digestive enzymes (Hagerman, 1989; Huang *et al.*, 2022), the disruption of rumen microbial activity (Min *et al.*, 2005; Huang *et al.*, 2022), and a shift in nitrogen excretion from urine to faeces, as a result of reduced amino acid absorption (Silanikove *et al.*, 2001; Patra & Saxena, 2011; Bunglavan & Dutta, 2013). Collectively, these factors may decrease protein digestion and absorption, resulting in higher levels of undigested proteins in the faeces. Therefore, proper management of tannin levels in the diet is crucial to optimise protein utilisation and minimise excessive nitrogen excretion in the faeces, without enhancing urinary nitrogen excretion. The differences in faecal nitrogen responses between the TE sources could be due to their chemical structure variations, which may influence the extent of complex formation with feed proteins, as well as their interactions with rumen microbes and digestive enzymes (Kardel *et al.*, 2013; Dos Santos Grasel *et al.*, 2016).

Table 4 Regression parameters and goodness of fit measures for the linear and piecewise best-fit models used to predict the responses (%) of faecal nitrogen, urinary nitrogen, and retained nitrogen to tannin extract inclusion in g/kg metabolic body weight (MBW) in sheep and cattle diets

	Faecal nitrogen			Urinary nitrogen			Retained nitrogen		
Model type	Linear	Piecewise		Linear	Piecewise		Linear	Piecewise	
MODEL REGRESSION									
Intercept									
Estimate	-15.20	-12.27		-3.33	-6.88		13.44	4.53	
SE	5.29	5.63		2.67	2.98		6.26	7.73	
P-value	0.007	0.036		0.222	0.027		0.038	0.561	
Tannin extract level (g/kg MBW)									
		Knot			Knot			Knot	
		<1.0	≥1.0		<1.1	≥1.1		<2.3	≥2.3
Estimate	14.35	7.97	13.74	-4.69	1.76	-3.94	-9.91	-1.83	-7.54
SE	1.15	4.28	1.19	0.82	2.89	0.83	2.09	4.68	2.38
P-value	<0.001	0.071	<0.001	<0.001	0.547	<0.001	<0.001	0.698	0.003
Tannin extract source (black wattle vs chestnut)									
Estimate	17.30	17.39		NS	NS		NS	NS	
SE	3.17	3.09		—	—		—	—	
P-value	<0.001	<0.001		—	—		—	—	
Tannin extract source (quebracho vs chestnut)									
Estimate	-5.20	-4.52		NS	NS		NS	NS	
SE	6.20	6.34		—	—		—	—	
P-value	0.407	0.480		—	—		—	—	
Dietary protein level (low vs high)									
Estimate	13.50	12.63		-6.81	-6.67		NS	NS	
SE	4.90	5.05		3.00	2.93		—	—	
P-value	0.009	0.017		0.029	0.029		—	—	
Supplementation period (long vs short)									
Estimate	-11.08	-12.20		5.12	7.15		NS	NS	
SE	4.06	4.09		2.23	2.28		—	—	
P-value	0.010	0.005		0.028	0.004		—	—	
MODEL GOODNESS OF FIT									
R ² _m /R ² _c	0.84/0.95	0.84/0.95		0.46/0.89	0.54/0.92		0.27/0.72	0.25/0.73	
RMSE	4.12	3.86		2.08	1.88		10.57	9.98	
RSR	0.21	0.20		0.30	0.26		0.5	0.48	
AIC	326.89	322.09		266.25	259.57		438.79	433.16	
CCC	0.98	0.98		0.95	0.96		0.83	0.85	
N	44/19	44/19		40/19	40/19		46/19	46/19	

SE: standard error, NS: non-significant, R²_m: marginal determination coefficient, R²_c: conditional determination coefficient, RMSE: root mean squared error, RSR: ratio of RMSE to the standard deviation of the observations, AIC: Akai information criterion, CCC: Lin's concordance correlation coefficient, N: number of observations/studies. For each predictor, the second category is the reference level.

The results also showed that diets with low protein levels (<18%) had $12.63 \pm 5.05\%$ higher faecal nitrogen than diets with high protein levels (>18.0%), when the same TE inclusion level was used. This means that TE inclusion in a low-protein diet resulted in a higher faecal nitrogen to nitrogen intake ratio than TE inclusion in a high-protein diet. This result aligns with those of Seoni *et al.* (2021), who reported a higher faecal nitrogen:nitrogen intake ratio in lambs fed low-protein diets than in lambs fed high-protein diets, when these lambs were supplemented with condensed tannins. The current study also revealed that a longer TE supplementation period (>1 month) may reduce faecal nitrogen by $12.20 \pm 4.09\%$ more than a short supplementation period, when the same TE inclusion level is used. Salem *et al.* (2005), although not directly reporting the faecal nitrogen:nitrogen intake ratio, suggested that lambs given a tannin-containing diet tended to exhibit lower faecal nitrogen:nitrogen intake ratios with longer than shorter supplementation periods. The effect of the supplementation period on the faecal nitrogen response could be attributed to the adaptation of the rumen microbial population to the presence of tannins, thereby allowing better degradation of tannin-protein complexes (Brooker *et al.*, 1999; Odenyo *et al.*, 1999; Makkar, 2003; Mlambo *et al.*, 2015). In addition, physiological adaptations may occur, such as elevated salivary protein synthesis to form soluble tannin-protein complexes (Makkar & Becker, 1998; Brooker *et al.*, 1999; Lamy *et al.*, 2020).

The TE level, supplementation period, and dietary protein level significantly influenced the urinary nitrogen response, while the other potential predictors showed a non-significant effect. Table 4 presents the linear and piecewise best-fit models for the urinary nitrogen response to TE inclusion, and the goodness of fit measures for these models. Of the fixed effects for the urinary nitrogen response, the TE inclusion level had the highest rank ($F = 105.6$, $P < 0.001$), followed by the supplementation period ($F = 12.5$, $P = 0.007$), and the dietary protein level ($F = 6.2$, $P = 0.038$). With a supplementation period longer than one month and a dietary protein content higher than 18%, the piecewise model showed a positive urinary nitrogen response to TE inclusion below the model knot value (<1.1 g TE/kg MBW). However, at inclusion levels above the knot value (≥ 1.1 g TE/kg MBW), the response became negative, with the urinary nitrogen content decreasing by 3.94% per additional gram of TE, and reaching a 5% reduction at 1.5 g TE/kg MBW. The observed decrease in the urinary nitrogen content in response to TE inclusion was likely as a result of TE-protein complex formation (Labieniec *et al.*, 2003; Jayanegara & Palupi, 2010). The results also showed that diets with a low protein content (<18%) had $6.67 \pm 2.93\%$ lower urinary nitrogen levels than high-protein diets (>18.0%), when the same TE inclusion level was used (Table 4). This means that the inclusion of TE in low-protein diets results in a lower urinary nitrogen to nitrogen intake ratio than the inclusion of TE in high-protein diets. This result agrees with the findings of Seoni *et al.* (2021), who reported a tendency towards a lower urinary nitrogen:nitrogen intake ratio in lambs fed low-protein diets supplemented with condensed tannins than in lambs fed high-protein diets. This difference in urinary nitrogen response to dietary protein level may occur because the same TE inclusion level binds a higher proportion of the dietary nitrogen in low-protein diets than in high-protein diets. As a result, the faecal nitrogen:nitrogen intake ratio increases, and the urinary nitrogen:nitrogen intake ratio decreases.

The results also showed that longer TE supplementation periods (>1 month) increased urinary nitrogen by $7.15 \pm 2.28\%$ relative to short supplementation periods, when the same TE inclusion level was used (Table 4). It is relevant to highlight the lack of studies in the literature on the effect of the TE supplementation period on nitrogen balance parameters in sheep and cattle. The effect of the supplementation period on the urinary nitrogen response may indicate that sheep and cattle acclimatise to longer-term TE supplementation. This acclimatisation may involve rumen microbiome adaptation (Brooker *et al.*, 1999; Odenyo *et al.*, 1999; Makkar, 2003; Mlambo *et al.*, 2015), and/or physiological changes such as the increased production of some salivary proteins (Makkar & Becker, 1998; Brooker *et al.*, 1999; Lamy *et al.*, 2020).

The TE inclusion level significantly affected the retained nitrogen response, while the other potential predictors had no significant impact. Table 4 presents the linear and piecewise best-fit models for the response of retained nitrogen to TE inclusion. Figure 2c illustrates the piecewise model's predicted retained nitrogen response to TE inclusion, along with the relevant weighted observations. The model shows a positive optimal response in retained nitrogen to TE inclusion levels below the model knot (<2.3 g TE/kg MBW). However, above the knot (≥ 2.3 g TE/kg MBW), a negative response occurred, with the retained nitrogen decreasing at a rate of 7.54% per additional gram of TE. Notably, the model RMSE (9.98%) and

RSR (0.48) values are the highest of all the models included in this study. As a rule of thumb, the RMSE should be less than half the SD, meaning that the RSR should be less than 0.5 (Singh *et al.*, 2005; Moriasi *et al.*, 2007). This model barely meets this standard (RSR = 0.48), indicating that the model for the retained nitrogen response had the lowest prediction accuracy, compared to the models for the other responses. The high RMSE value found for the retained nitrogen response model could have been caused by the many factors affecting dietary protein utilisation, including the animal's physiological requirements for amino acids, their protein deposition genetic potential, their digestion/absorption of dietary amino acids, and their metabolism/partitioning of absorbed amino acids (Kim & Pluske, 2016). The interactions between these factors may explain the inconsistent results of previous studies for the responses of retained nitrogen levels to dietary tannin inclusion, with some studies finding no significant effect (Jayanegara & Palupi, 2010; Jayanegara *et al.*, 2012), while others (Yanza *et al.*, 2021) reported a quadratic increase (g/100 g N).

The TE inclusion level showed a significant effect on the weight gain response, while the other potential predictors had no significant effects. Table 3 presents the linear and piecewise best-fit models for the response of weight gain to TE inclusion, and Figure 2d displays the piecewise model prediction for the response of weight gain to TE inclusion, along with the weighted observations. The weight gain model demonstrated exceptional predictive accuracy ($R^2_c = 0.99$, CCC = 1.0). The model showed a positive optimal response in weight gain at inclusion levels below the model knot (<1.6 g TE/100 g DM). However, at inclusion levels above the knot (≥ 1.6 g TE/100 g DM), a negative response occurred, with the weight gain decreasing by 3.01% per additional gram of TE from chestnut, quebracho, or black wattle sources. This resulted in a 5% reduction in weight gain at an inclusion level of 2.3 g TE/100 g DM (Figure 2d). The weight gain increase at low TE inclusion levels (<1.6 g TE/100 g of DM) could have been caused by the increase in the DMI, digestibility, and nitrogen retention responses at these inclusion levels, whereas the later decrease in weight gain likely correlates with the decreased DMI, digestibility, and nitrogen retention responses at high TE inclusion levels. In this study, sheep appeared slightly more able to digest CP than cattle, and black wattle TE reduced the OMD, NDFD, and ADFD more than chestnut TE. Nonetheless, these differences between sheep and cattle in the CPD response, and between the TE sources in the OMD, NDFD, and ADFD responses, did not significantly influence either the feed intake or weight gain responses.

Conclusions

While several factors may affect an animal's response to TE inclusion, including the TE source, animal species, dietary protein level, and supplementation period, the TE inclusion level stands out as the most crucial determinant. The TE inclusion level had a stronger relationship with the DMI, digestibility, and weight gain responses when expressed as the g/100 g DM than when expressed as the g/kg MBW, while the opposite was true for the nitrogen balance responses. In this meta-analysis, sheep appeared slightly more able to digest CP than cattle, and black wattle TE reduced the OMD, NDFD, and ADFD more than chestnut TE. Nonetheless, these differences between species in the CPD response and between TE sources in the OMD, NDF, and ADFD responses did not significantly influence either the DMI or weight gain responses. The DMI had an upper-end optimal inclusion level of 1.5 g TE/100 g DM, with a threshold level of 3 g TE/100 g DM. Similarly, the upper-end optimal inclusion level for weight gain was 1.5 g TE/100 g of DM, with a threshold level of 2.3 g TE/100 g DM. Based on the animal weight gain response to TE inclusion, we suggest 1.5 g TE/100 g DM as the maximum inclusion level for chestnut, quebracho, and black wattle TE in sheep and cattle, for sustained animal performance. Furthermore, a threshold of 2.3 g TE/100 g DM could be proposed for an expected 5% reduction in animal weight gain performance, when considering various applications and the environmental benefits of TE inclusion, as higher levels may drastically impair animal performance.

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Authors' contributions

OA: Conceptualisation, methodology, visualisation, writing original draft, manuscript review and editing. AH: Conceptualisation, methodology, supervision, project administration, manuscript review and editing. KL: Conceptualisation, supervision, manuscript review and editing.

Conflict of interest declaration

The authors declare that they have no conflicts of interest.

Data availability statement

In addition to the linear and piecewise models presented in this study, we initially also fitted quadratic models to explore potential curvilinear relationships between the TE inclusion level and animal responses. For some responses, the quadratic term was not statistically significant, while for other responses, the quadratic models showed a statistically better fit than the linear models in model fit metrics like the R^2 and RMSE. However, upon further investigation, we found that the quadratic models produced unrealistic predictions in some cases, and we therefore believe that quadratic models should be used with caution in this type of study. Data for these models can be obtained from the authors, on request.

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