Research article

Modulatory effects of dietary tannins on polyunsaturated fatty acid biohydrogenation in the rumen: A meta-analysis

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ABSTRACT

Background: Tannins are a group of phenolic compounds that can modify the rumen biohydrogenation (BH) of polyunsaturated fatty acids (PUFA), but to date results obtained have been inconsistent. This study therefore aims to conduct a meta-analysis of the scientific literature related to the effects of tannins on rumen BH and fermentation.

Methods: A total of 28 articles were collected from various scientific databases, such as Scopus, Science Direct and Google Scholar, and the data were analysed using a random effects model and meta-regression for rumen BH. The publication bias on the main variables of rumen fermentation was assessed using a funnel plot and Egger's test.

Results: An increase in tannin levels significantly reduced methane production ($p < 0.001$) and the population of Butyrivibrio fibrisolvens ($p < 0.05$). Dietary tannins also decreased the SFA proportion ($p < 0.001$) and increased ($p < 0.001$) the rumen monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) proportions. In addition, there were negative relationships between dietary tannin levels and BH rates of C18:2 n-6 and C18:3 n-3 ($p < 0.05)$.

Conclusion: Dietary tannins modulate the rumen fermentation profile, mitigate methane emissions, and inhibit rumen BH of PUFA.

1. Introduction

Polyunsaturated fatty acids (PUFA) are part of the essential fatty acids, and therefore need to be supplied through diets rich in the substances. The provision of PUFA at elevated levels in animal products (meat, milk, etc.) has gained attention due to their beneficial effects for human health [1]. As the proportion of PUFA in animal products increases, the SFA content decreases, which causes the PUFA/SFA ratio in meat to increase. According to Poulson et al. [2], the content of C18:2 c9 t11 (rumenic acid) in the longissimus and semitendinosus muscles increases by 200%–400% during the pasture-based finisher period. A previous study demonstrated that the intake of PUFA plays an important role in maintaining human health through its metabolic role as an anticarcinogen [3].

Most of the PUFA consumed by ruminants pass through metabolic processes by rumen microbes from the genus Butyrivibrio sp. Accordingly, in the rumen system lipolysis and biohydrogenation (BH) processes convert PUFA to SFA, especially C18:0 (stearic acid) and a small proportion of C18:1 t11 (vaccenic acid). Extensive BH activity causes ineffective deposition of PUFA in animal products. The presence of plant secondary metabolites such as phenols and tannins may affect the lipolysis and BH of PUFA in the rumen. It has been shown that tannins reduce PUFA BH and increase PUFA concentration in the rumen [4, 5, 6, 7, 8], but the results have varied.

A meta-analysis of the effect of dietary tannins on the BH activity of PUFA in the rumen has yet to be conducted. This indicates that further verification of the strategic function of tannins as modulators of rumen

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lipid metabolism is required. The *in vitro* and *in sacco* studies conducted by Jayanegara et al. [6], Jafari et al. [9] and Jafari et al. [10] showed the potential of various tropical forage species as a tannin source in modulating BH to increase the flow of C18:3 n-3 and C18:2 n-6 to the duodenum. Several studies have also shown that some tropical forage species can improve production performance by providing bypass protein for ruminants [11, 12]. Therefore, the meta-analysis is expected to provide a comprehensive evaluation of the effects of dietary tannins on the fermentation, fatty acid profile and PUFA BH activity in the rumen based on various scientific literature sources.

2. Materials and methods

2.1. Database development

A database was developed from studies on the use of dietary tannins on the profile of rumen fermentation, fatty acids, and the BH of PUFA. The scientific literature search engines used were Scopus, Google Scholar, and Science Direct with the keywords “tannin”, “*in vitro*”, “rumen”, “fatty acid”, and/or “biohydrogenation”. The inclusion criteria for articles in the meta-analysis study were: (1) those published in English; (2) inclusion of control treatment in the experiment (no addition of tannin); (3) the presence of tannin sources in the basal diet or as additives; and (4) the experiment was evaluated through the rumen *in vitro* system. The literature search and selection process in the meta-analysis are shown in Figure 1. A total of 51 full-text articles were selected according to the inclusion criteria, but 23 were removed due to irrelevant experimental data and sampling methods that did not meet the criteria set. The number of studies that finally met the criteria was 28 (Table 1), with the process based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) protocol [40]. The tabulated *in vitro* rumen fermentation techniques consisted of the Hohenheim gas test \((n = 5)\), the *in vitro* gas production system \((n = 6)\), batch culture incubation \((n = 1)\), the pressure transducer technique \((n = 3)\), glass bottle
| No. | Reference | Incubation method | Incubation time (h) | Rumen donor | Basal feed | Tannin type | Tannin source | Tannin level (% DM) |
|-----|-----------|-------------------|---------------------|-------------|------------|-------------|---------------|-------------------|
| 1   | Abo-Donia et al. [13] | HGT | 6, 12, 24 | Goat (Liuyang black) | Maize stover and concentrate (45:55) | Hydrolysable tannin | Gallnut | 0-0.9 |
| 2   | Alman-Zakaria et al. [14] | IGPS | 24 | Goat (Kacang crossbred) | Alfalfa hay and concentrate (50:50) | Condensed tannin | Elaeis guineensis leaf | 0-10 |
| 3   | Al-Jumaill et al. [15] | IGPS | 24 | Goat (Kacang crossbred) | Alfalfa hay and concentrate (50:50) | Tannic acid | Commercial tannic acid | 0-20 |
| 4   | Bichara [16] | GBI | 3, 6, 9, 24 | Cow (Holstein) | Grass silage, linseed oil | Condensed tannin | Inga edulis, Desmodium ovalifolium | 0-8.15 |
| 5   | Buccioni et al. [17] | GBI | 6, 12, 18 | Sheep (Iwes) | Wheat straw and concentrate | Tannin | Quebracho, chestnut | 0-8.2 |
| 6   | Cappucci et al. [18] | PTT | 6,12, 24 | Sheep (Massese ewes) | Barley, wheat bran, dehydrated alfalfa and concentrate | Tannin | Acacia dealbata, Uncaria gambir, Casalpina spinosa, Castanea sativa | 0-4 |
| 7   | Carreño et al. [19] | GBI | 12, 24 | Sheep (Merino) | Alfalfa:concentrate (50:50) | Tannin | Schinopsis lorentzii, Vitis vinifera, Castanea sativa | 0-8 |
| 8   | Costa et al. [20] | GBI | 6 | Sheep | Dehydrated alfalfa, wheat grain, soybean meal, sunflower oil | Tannin | Chestnut, quebracho, grape seed, rockrose | 0-10 |
| 9   | Fatahnia et al. [21] | GBI | 24 | Cow (Holstein) | Alfalfa hay: wheat straw (70:30) | Condensed tannin | Cynips ladanifer | 0-0.07 |
| 10  | Guerreiro et al. [22] | HT | 6 | Sheep (Merino Branco) | Dehydrated alfalfa, wheat grain, soybean meal, sunflower oil | Condensed tannin | Cynips ladanifer | 0-10 |
| 11  | Guerreiro et al. [23] | GBI | 24 | Sheep (Merino Branco) | Oat hay and concentrate | Condensed tannin | Cynips ladanifer | 0-10 |
| 12  | Irawan et al. [24] | HGT | 24, 48 | Cattle (Bali) | Forage:concentrate (75:25), corn oil | Hydrolysable tannin | Lescaena leucocephala | 0-4 |
| 13  | Ishlak et al. [25] | BCI | 24 | Cow (Holstein) | Grass hay: concentrate (44:56) | Condensed tannin | Quebracho | 0-10 |
| 14  | Jafari et al. [9] | IGPS | 24 | Goat (Kacang crossbred) | Alfalfa hay:concentrate (60:40) | Tannin | Carica papaya leaf | 0-6 |
| 15  | Jafari et al. [26] | IGPS | 24 | Goat (Kacang crossbred) | Alfalfa hay:concentrate (50:50) | Condensed tannin | Carica papaya leaf | 0-6 |
| 16  | Jafari et al. [27] | IGPS | 24 | Goat (Kacang crossbred) | Alfalfa hay:concentrate (50:50) | Condensed tannin | Carica papaya leaf | 0-15 |
| 17  | Khiasa-ard et al. [28] | RUSITEC | 24 | Cow (Brown Swiss) | Grass-clover hay | Condensed tannin | Acacia mearnsii, Onobrychis vicifolia | 0-7.9 |
| 18  | Mandal et al. [29] | GBI | 24 | Goat | Barseem hay:concentrate mixture (40:60), sunflower oil | Tannin | Artocarpus heterophyllus, Ficus benghalensis, Ficus glomerata | 0-1 |
| 19  | Menci et al. [30] | PTT | 24 | Sheep (Texel breed) | TMR, hay:concentrate (80:20) | Condensed tannin, mixture hydrolysable and condensed tannin | Castanea sativa, Schinopsis lorentzii | 0-3 |
| 20  | Minieri et al. [31] | HGT | 6, 12, 18 | Sheep (Iwes) | Grass hay and concentrate | Condensed tannin | Schinopsis lorentzii | 0-4.9 |
| 21  | Mirmi et al. [32] | HGT | 24 | Cow | Hay:concentrate (50:50) | Tannin | Asadradchta indica, Allium sativum, Cuminum cyminum, Terminalia chebula | 0-40.1 |
| 22  | Natalello et al. [33] | GBI, PTT | 12, 24 | Sheep (Merino) | TMR, forage:concentrate (50:50) | Tannin | Whole pomegranate by-product | 0-2 |
| 23  | Odhab and Sazili [34] | HGT | 24 | Sheep (Dorper) | Ammoniated rice straw:concentrate (60:40) | Tannin | Nigella sativa seeds, Rosmarinus officinalis leaves | 0-2 |
| 24  | Shokryzadan et al. [35] | IGPS | 24 | Goat | Alfalfa:concentrate (60:40) | Condensed tannin | Garcinia mangostana | 0-8.4 |
| 25  | Szczesniak et al. [36] | RUSITEC | 24 | Cow (Polish Holstein-Friesian) | TMR, maize silage, lucerne silage, concentrate | Condensed tannin | Vaccinium vitis idaea | 0-0.45 |
| 26  | Thanh et al. [37] | IGPS | 24, 48 | Cow (Holstein-Friesian) | Forage:concentrate (60:40), soybean oil | Condensed tannin | Grape seed | 0-0.8 |
| 27  | Toral et al. [38] | GBI | 6, 24 | Sheep (Iwes) | Alfalfa hay, saifoin hay | Condensed tannin | Onobrychis vicifolia | 0-3.5 |
| 28  | Vasta et al. [39] | GBI | 12 | Cow (Friesian-Holstein) | Hay, hay plus concentrate | Tannin | Ceratonia siliqua, Acacia cyanophylla, Schinopsis lorentzii | 0-0.1 |
incubation (n = 11), the rumen simulation technique (n = 2), and the Hungate tube (n = 1). The rumen fluid donors were taken from cows, goats and sheep. Articles were published over the period 2009 to 2022, with a tannin level ranging from 0 to 40.1% DM.

2.2. Data analysis

The data were analysed using the random effects meta-analysis method. The effect size calculation (d) in Eq. (1) was based on the standardised mean difference of Hedges' d [41]:

\[ d = \frac{(X^E - X^C)}{S} \]

(1)

where \(X^E\) is the mean of the experimental or tannin group; \(X^C\) is the control group; \(S\) the pooled standard deviation; and \(J\) the correction factor for the small sample size. The mathematical modeling of the one-way random effect is stated in Eq. (2).

\[ y_i = \theta + v_i + e_i \]

(2)

where \(y_i\) is the value of the effect size (in Hedge’s d); \(\theta\) the i-th observation (the general parameter of the combined effect size; \(v_i\) the real variation of the effect size; and \(e_i\) the error of the i-th observation.

In Eq. (3), the estimation of the variance between studies (\( \tau^2 \)) was based on the DerSimonian and Laird [42] method:

\[ \tau^2 = \frac{Q}{C} \]

(3)

where \(Q\) is the weighted sum square; \(df\) the degrees of freedom; and \(C\) the value of C. The meta-analysis was conducted using the OpenMEE platform (http://www.cebm.brown.edu/openmee/) for rumen fermentation.

### Table 2. Descriptive statistics of the database.

| Variables          | Unit       | NC | Mean            | MIN | MAX          | SD |
|--------------------|------------|----|-----------------|-----|--------------|----|
| Rumen fermentation |            |    | Control Tannin  |     | Control Tannin |    |
| pH                 |            | 69 | 6.86           | 6.85 | 7.40         | 0.27 |
| NH3                | mg/dL      | 66 | 23.24          | 19.81 | 70.90       | 11.89 |
| C2                 | mM         | 64 | 49.47          | 50.73 | 83.74       | 22.88 |
| C3                 | mM         | 64 | 22.62          | 23.17 | 33.46       | 7.09  |
| C4                 | mM         | 64 | 11.19          | 11.37 | 23.57       | 7.74  |
| iso-C4             | mM         | 32 | 1.43           | 1.17  | 5.45        | 2.12  |
| C5                 | mM         | 32 | 2.50           | 2.18  | 7.50        | 2.33  |
| iso-C5             | mM         | 32 | 2.00           | 1.75  | 5.45        | 2.12  |
| C2/C3              |            | 64 | 2.55           | 2.54  | 3.52        | 0.66  |
| Total VFA mM       |            | 64 | 67.06          | 69.43 | 126.36      | 34.61 |
| CH4 24 h ml/g DM   |            | 28 | 7.55           | 5.89  | 11.12       | 2.27  |
| Total bacteria Log |           | 14 | 10.21          | 10.06 | 11.18       | 0.78  |
| Total protozoa Log |           | 14 | 6.38           | 5.43  | 7.48        | 1.00  |
| Methanogens Log     |           | 14 | 8.13           | 7.38  | 9.06        | 0.80  |
| B. fibrisolvens Log |           | 11 | 4.47           | 3.93  | 5.33        | 0.88  |
| Rumen FA profile   |            |    |                |      |              |     |
| C14:0              | % total FA | 16 | 2.81           | 2.01  | 5.69        | 1.89  |
| C15:0              | % total FA | 56 | 1.03           | 0.94  | 2.72        | 0.64  |
| C16:0              | % total FA | 16 | 16.71          | 15.72 | 19.62       | 6.09  |
| iso-C16:0          | % total FA | 34 | 2.36           | 1.93  | 15.50       | 4.89  |
| C16:1 n-7          | % total FA | 32 | 0.97           | 0.79  | 1.66        | 0.39  |
| C17:0              | % total FA | 27 | 4.76           | 3.88  | 37.00       | 11.21 |
| iso-C17:0          | % total FA | 32 | 2.30           | 2.04  | 13.70       | 4.38  |
| C18:0              | % total FA | 58 | 33.06          | 30.37 | 61.90       | 18.39 |
| C18:1 n-9          | % total FA | 64 | 7.64           | 8.99  | 35.80       | 8.21  |
| C18:1 t10          | % total FA | 16 | 2.29           | 2.27  | 4.58        | 1.77  |
| C18:1 t11          | % total FA | 30 | 11.89          | 15.93 | 22.70       | 6.13  |
| C18:2 n-6          | % total FA | 25 | 5.28           | 6.82  | 10.85       | 5.99  |
| C18:2 c9 t11       | % total FA | 76 | 0.56           | 0.60  | 4.38        | 1.11  |
| C18:2 t10 c12      | % total FA | 60 | 0.50           | 0.54  | 4.30        | 0.89  |
| C18:3 n-3          | % total FA | 70 | 0.82           | 1.07  | 3.90        | 1.01  |
| C20:4 n-6          | % total FA | 25 | 0.86           | 0.82  | 2.73        | 0.81  |
| C20:5 n-3          | % total FA | 21 | 0.85           | 1.12  | 2.80        | 0.60  |
| EPA                 | % total FA | 44 | 57.66          | 52.80 | 82.97       | 14.89 |
| MUFA                | % total FA | 36 | 16.48          | 19.84 | 26.20       | 5.94  |
| PUFA                | % total FA | 69 | 9.48           | 12.62 | 21.57       | 8.73  |

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variables (15 items), rumen fatty acids (20 items), and C18 UFA BH (3 items). A cumulative forest plot (95% confidence interval) and meta-regression of the tested variables were constructed using MedCalc software (https://www.medcalc.org/). Subsequently, a funnel plot and Egger’s test were employed to detect publication bias both visually and quantitatively, performed using JASP software (https://jasp-stats.org/).

3. Results

3.1. Rumen fermentation

Descriptive statistics of various parameters in the database are presented in Table 2. The results of the meta-analysis in Table 3 show that, in comparison to the control, dietary tannins significantly decreased ($p < 0.001$) the concentrations of ammonia, (NH$_3$), valerate (C$_5$), iso-butyrate (iso-C$_4$), and iso-valerate (iso-C$_5$). Furthermore, tannins significantly reduced ($p < 0.05$) the population of protozoa, methanogens, and B. fibrisolvens bacteria in the rumen. Methane formation under in vitro conditions also decreased significantly ($p < 0.001$) in the tannin group. However, dietary tannins did not significantly alter pH, several VFA items (total VFA, C$_3$, C$_4$, and C$_2$/C$_3$ ratio), and total bacteria. The cumulative forest plot results for each effect size of rumen fermentation profiles are shown in Figure 2.

3.2. Rumen fatty acids

Dietary tannins fell ($p < 0.05$) C$_{16}:1$ n-7 and iso-C$_{17}:0$ (Table 4), while the presence of tannins increased ($p = 0.002$) the intermediate fatty acid of rumen BH, i.e., C$_{18}:1$ t$_{11}$. There was also an increase in the PUFA group, i.e., C$_{18}:3$ n-3 and C$_{20}:5$ n-3 ($p < 0.001$ and $p = 0.016$, respectively) due to tannins, while C$_{18}:1$ n-9 and C$_{18}:2$ n-6 were similar in the control and tannin groups. Furthermore, tannins in diets significantly reduced ($p < 0.001$) SFA composition and simultaneously increased ($p < 0.001$) MUFA and PUFA in the rumen. An illustration of the cumulative forest plots of the effect size of various types of rumen fatty acids is shown in Figure 3.

| Variable | Unit | NC | Estimate | Lower bound | Upper bound | Std. error | p-Value | $\tau^2$ | Q | Het. p-value | $I^2$ |
|----------|------|----|----------|-------------|-------------|------------|---------|--------|----|-------------|-------|
| pH       | –    | 69 | –0.119   | –0.314      | 0.077       | 0.100      | 0.235   | 0.220  | 113.335 | <0.001      | 40.001 |
| NH$_3$   | mg/dL | 66 | –1.156   | –1.566      | –0.747      | 0.209      | <0.001  | 2.087  | 330.919 | <0.001      | 80.358 |
| C$_2$    | mM   | 64 | 0.601    | 0.145       | 1.057       | 0.233      | 0.010   | 2.790  | 483.613 | <0.001      | 86.973 |
| C$_3$    | mM   | 64 | –0.116   | –0.422      | 0.190       | 0.156      | 0.458   | 1.246  | 305.428 | <0.001      | 77.081 |
| C$_4$    | mM   | 64 | –0.007   | –0.247      | 0.233       | 0.122      | 0.953   | 0.641  | 194.343 | <0.001      | 62.438 |
| iso-C$_4$| mM   | 32 | –0.706   | –1.031      | –0.382      | 0.166      | <0.001  | 0.613  | 137.151 | <0.001      | 77.397 |
| C$_5$    | mM   | 32 | –0.714   | –1.022      | –0.406      | 0.157      | <0.001  | 0.630  | 152.239 | <0.001      | 75.696 |
| iso-C$_5$| mM   | 32 | –0.936   | –1.330      | –0.541      | 0.201      | <0.001  | 0.988  | 192.446 | <0.001      | 83.892 |
| C$_2$/C$_3$|      | 64 | 0.035    | –0.205      | 0.275       | 0.122      | 0.774   | 1.058  | 519.834 | <0.001      | 81.340 |
| Total VFA| mM   | 64 | 0.049    | –0.213      | 0.311       | 0.134      | 0.715   | 0.776  | 252.441 | <0.001      | 75.044 |
| CH$_4$ 24 h| ml/g DM | 28 | –1.058   | –1.370      | –0.745      | 0.160      | <0.001  | 0.354  | 56.116  | <0.001      | 51.885 |
| Total bacteria | Log$_{10}$ cells/L | 14 | –0.114   | –0.370      | 0.142       | 0.131      | 0.384   | 0.000  | 12.115  | 0.518       | 0.000  |
| Total protozoa | Log$_{10}$ cells/L | 14 | –0.672   | –0.979      | –0.366      | 0.156      | <0.001  | 0.091  | 17.729  | 0.168       | 26.675 |
| Methanogens | Log$_{10}$ cells/L | 14 | –1.178   | –1.466      | –0.890      | 0.147      | <0.001  | 0.033  | 14.577  | 0.335       | 10.816 |
| B. fibrisolvens | Log$_{10}$ cells/L | 11 | –0.576   | –1.130      | –0.023      | 0.282      | 0.041   | 0.589  | 32.125  | <0.001      | 68.872 |

Figure 2. Cumulative forest plot for effect size of the rumen fermentation variable.
3.3. Rumen biohydrogenation of PUFA

The BH levels of C18:1 n-9, C18:2 n-6, and C18:3 n-3 in the tannin treatments were 42.38, 64.56, and 70.04%, respectively (Table 2). Meanwhile, the results of the meta-analysis in Table 4 show that dietary tannins inhibited the rumen BH activity of PUFA, as indicated by the lower C18:1 n-9 ($p = 0.008$), C18:2 n-6 ($p = 0.005$), and C18:3 n-3 ($p < 0.001$) in comparison to the control group.

### Table 4. Effect of dietary tannin on the in vitro rumen fatty acid profile and BH C18 UFA.

| Variable                  | NC | Estimate | Lower bound | Upper bound | Std. error | p-Value | $\tau^2$ | Q     | Het. p-value | $I^2$ |
|---------------------------|----|----------|-------------|-------------|------------|---------|---------|-------|-------------|-------|
| Rumen FA profile (% total FA) |    |          |             |             |            |         |         |       |             |       |
| C14:0                     | 16 | 0.093    | -0.325      | 0.511       | 0.213      | 0.662   | 0.450   | 59.325| <0.001      | 74.716|
| C15:0                     | 56 | -0.247   | -0.519      | 0.025       | 0.139      | 0.075   | 0.820   | 359.701| <0.001      | 84.710|
| C16:0                     | 16 | -0.082   | -0.288      | 0.124       | 0.105      | 0.434   | 0.022   | 17.235| 0.305       | 12.966|
| iso-C16:0                 | 34 | -0.069   | -0.243      | 0.105       | 0.089      | 0.437   | 0.034   | 38.493| 0.235       | 14.269|
| C16:1 n-7                 | 32 | -0.284   | -0.499      | -0.070      | 0.109      | 0.009   | 0.000   | 20.015| 0.935       | 0.000 |
| C17:0                     | 27 | -0.193   | -0.408      | 0.021       | 0.109      | 0.078   | 0.032   | 29.279| 0.299       | 11.198|
| iso-C17:0                 | 32 | -0.381   | -0.676      | -0.087      | 0.150      | 0.011   | 0.390   | 85.719| <0.001      | 63.835|
| C18:0                     | 58 | -0.195   | -0.479      | 0.089       | 0.145      | 0.178   | 0.814   | 233.476| <0.001      | 75.584|
| C18:1 n-9                 | 64 | 0.117    | -0.099      | 0.334       | 0.110      | 0.287   | 0.265   | 108.632| <0.001      | 43.847|
| C18:1 t10                 | 16 | 0.027    | -0.147      | 0.200       | 0.088      | 0.763   | 0.000   | 13.015| 0.601       | 0.000 |
| C18:1 t11                 | 30 | 0.601    | 0.218       | 0.984       | 0.195      | 0.002   | 0.824   | 151.394| <0.001      | 80.845|
| C18:2 n-6                 | 25 | 0.271    | 0.033       | 0.510       | 0.122      | 0.026   | 0.132   | 41.561| 0.041       | 42.253|
| C18:2 c9 t11              | 76 | 0.080    | -0.166      | 0.327       | 0.126      | 0.523   | 0.765   | 414.743| <0.001      | 81.917|
| C18:2 t10 c12             | 59 | -0.117   | -0.329      | 0.095       | 0.108      | 0.278   | 0.289   | 110.528| <0.001      | 47.525|
| C18:3 n-3                 | 70 | 0.319    | 0.168       | 0.470       | 0.077      | <0.001  | 0.209   | 179.237| <0.001      | 61.503|
| C20:4 n-6                 | 25 | -0.026   | -0.230      | 0.214       | 0.178      | 0.806   | 0.025   | 26.676| 0.320       | 10.033|
| C20:5 n-3                 | 21 | 0.584    | 0.108       | 1.060       | 0.243      | 0.016   | 0.846   | 72.267| <0.001      | 72.325|
| SFA                       | 44 | -0.778   | -1.109      | -0.448      | 0.169      | <0.001  | 0.786   | 164.466| <0.001      | 73.855|
| MUFA                      | 36 | 0.991    | 0.538       | 1.444       | 0.231      | <0.001  | 1.532   | 251.871| <0.001      | 86.104|
| PUFA                      | 69 | 0.703    | 0.461       | 0.945       | 0.124      | <0.001  | 0.493   | 156.843| <0.001      | 56.645|
| C18:1 UFA biohydrogenation (%) |    |          |             |             |            |         |         |       |             |       |
| C18:1 n-9                 | 46 | -0.549   | -0.957      | -0.141      | 0.208      | 0.008   | 1.359   | 221.482| <0.001      | 79.682|
| C18:2 n-6                 | 53 | -0.621   | -1.051      | -0.191      | 0.219      | 0.005   | 1.898   | 398.590| <0.001      | 86.954|
| C18:3 n-3                 | 61 | -0.693   | -1.097      | -0.289      | 0.206      | <0.001  | 1.786   | 585.457| <0.001      | 89.581|

Figure 3. Cumulative forest plot for effect size of the rumen fatty acid profile.
3.4. Meta-regression

There were negative linear relationships between dietary tannin levels and the BH of C18:2 n-6 \( (p = 0.011, R^2 = 0.133) \) and C18:3 n-3 \( (p = 0.001, R^2 = 0.209) \) as shown in Figures 4 and 5 respectively. However, increasing levels of tannins did not alter the BH of C18:1 n-9 \( (p = 0.106, R^2 = 0.06) \), although the trend was also negative (Figure 6).

3.5. Evaluation of publication bias

The funnel plot of total VFA, which is the main parameter in rumen fermentation, showed symmetrical values (Figure 7). This was supported by a statistical assessment of publication bias using Egger’s test, which showed a non-significant result \( (p = 0.905) \), indicating that no publication bias existed in the meta-analysis study.

4. Discussion

The meta-analysis results on the rumen fermentation profile obtained in this study are relatively similar to those of Jayanegara et al. [43]. They show a relationship between an increase in tannin levels with decreased production levels of methane, ammonia, total bacteria, and iso-SCFA, but not in relation to pH parameters in vitro. This was due to the protective effect of the tannin component through the production of stable formations with easily degraded proteins in the rumen [44]. The effect of tannins in the rumen environment also caused abnormalities in the morphology and cell growth of some microbes, which led to a modulation of fermentation [45]. The bioactivity of tannin components such as gallotannins and ellagitannins was able to inhibit the growth of broad-spectrum bacterial species through the disruption of cell regulatory mechanisms [46]. This is in line with a study by Mazhangara et al. [47], which showed the gram-negative and positive antibacterial
potential from a crude extract of *Teucrium trifidum* (CT 77.34–99.40 mg CE/g).

The effect of condensed tannin (CT) on the rumen showed a significant reduction in the accumulation of rumen ammonia compared to its effect on protozoa depopulation [48]. According to Zhou et al. [49], the supplementation level of 16.9 tannic acid/kg DM in combination with dietary protein produced an inhibitory effect on fermentation activity and rumen degradation, which did not affect the population of the genus *Butyrivibrio*. Tannin treatments also showed a consistent trend of population decline for rumen microbes associated with the BH process [50]. Few studies have been conducted on the tannin mechanism in inhibiting *B. fibrisolvens* related to rumen BH. Early indications show that tannin changes the pattern of hydrogen ion production, disintegrates cell walls and liposome, disrupts oxidative phosphorylation metabolic pathways, and reduces substrates for bacterial growth directly related to the rumen FA metabolism [51, 52]. On the other hand, the molecular weight, dose,
and type of tannin supplemented in the diet are amongst factors that determine the effectiveness of tannin in influencing the rumen microbial community selectively. This indicates that there are variations in some rumen fermentation profile variables in certain tannin level categories between the in vitro and in vivo rumen studies. The study by Arcuri et al. [53] found that the B. fibrillosvens and Streptococcus bovis strains isolated from Holstein × Zebu cattle resisted the biological effect of CT Mimosa artemisia extract at low levels (2–3 g L⁻¹). The effect of eliminating the rumen methanogen population using tannin-sourced feeds has a direct impact on reducing the overall level of rumenate methane production. This is because approximately 37% of rumen methane emissions come from an active community of methanogenic bacteria that are in symbiosis with rumen protozoa [54]. In addition, a meta-analysis study of 30 articles on the estimation of rumen methane gas production with different systems showed that the in vitro system experienced measurement bias at the level of tannin addition of above 100 g tannin/kg DM [55]. Therefore, in vitro research is a more appropriate choice to initiate an exploratory study on the potential of feed ingredients in ruminants.

Studies on the effect of bioactive plant components such as tannins are among the promising strategies for avoiding rumen BH of beneficial fatty acid groups. It has also been shown that different sources of tannins are effective in suppressing BH and C18:0 production [19], where various tropical and subtropical plant species have been used effectively [6, 55]. This shows that tannins have an impact on increasing the composition of C18 UFA and C18:1 t11. Furthermore, the mechanism of tannins in suppressing the BH process can take place through the inhibition of the lipolysis process, which is the initial stage of the decomposition of fat fraction into free fatty acids. There is also a toxic effect of tannins on rumen microbes in the terminal stage of BH, which has a positive impact on increasing the percentage of rumen intermediate fatty acids [56]. Similarly, a meta-analysis of 12 articles on natural rumen biomodifiers, for example chitosan, showed similar characteristics to tannin supplementation, such as reduced SFA, and increased rumen CLA and PUFA with in vitro batch culture [57].

Based on the consistency of the findings of previous studies, diets rich in polyphenols have inhibitory effects on rumen BH and methanogenesis [8]. A meta-analysis study of 38 articles showed that increasing the levels of tannin supplementation (0.1–20 g/kg DM) also optimised the accumulation of conjugated linoleic acid (CLA) in in vitro and in vivo observations [58]. The study showed variations in measuring the true production of rumen fatty acids in different observational models. This is a limitation of our study, which only focused on in vitro. Enjalbert et al. [59] found differences in the rates of in vitro and in situ rumen BH and concluded that both methods could still be feasibly used. This was further emphasized by Fievez et al. [60], who measured the rumen BH activity of unprotected fatty acid sources using several approaches. They found that the BH profile of C18 UFA and C18:0 production from simulated continuous cultures for 24 h showed values approximating to those observed in vivo.

Furthermore, Carica papaya leaf supplementation with CT levels of 17.39 mg/g DM to BH levels of C18:2 n-6 and C18:3 n-3 in different rumen systems showed the same significance level [10, 27]. However, these results were not in line with the rumen CLA production level at various incubation periods. Meanwhile, a clearer negative correlation between unsaturated fatty acids also contributes to the extensive BH. For comparison in the second step, the metabolite, the effect of adding tri-terpene saponin extract (500–1000 mg/l) on in vitro BH of C18:2 n-6 was 76.6–78.1%, while C18:3 n-3 was 85.3–86.6% [61]. According to Ebhur and Anya [62], the addition of 300 mg/l essential oil such as anise, lavender, and mixed anise-lavender showed BH levels in vitro (24 h) of C18:2 n-6 of 40–68.1% and C18:3 n-3 of 42.1–70.2%. These findings at least show that the effectiveness of tannins in suppressing rumen BH activity is between saponins and essential oils.

5. Conclusion

This study has revealed that dietary tannins enhance the accumulation of C18:1 t11, C18:3 n-3, C20:5 n-3, MUFA, and PUFA proportions in the rumen. Based on rumen BH activity, an increase in tannin levels linearly reduces the BH of C18:2 n-6 and C18:3 n-3. These patterns suggest that tannins may elevate the PUFA and lower SFA contents in animal products, which, in turn, may improve human health. In addition, tannins provide beneficial effects in relation to a number of rumen fermentation and microbial population parameters, as indicated by reduced ammonia concentration, methane emissions, protozoa population, methanogens, and B. fibrillosvens bacteria. The ability of tannins to lower methane emissions would further help the mitigation of greenhouse gas emissions from the livestock sector and their accumulation in the atmosphere. Further investigation is required to identify tannin types and levels that effectively alter rumen lipid metabolism using a research synthesis approach.

Declarations

Author contribution statement

Malik Makmur: Performed the study; Analyzed and interpreted the data; Wrote the paper.
Mardiati Zain: Contributed analysis tools or data; Wrote the paper.
Muhammad Miftakhus Sholilkin: Performed the study; Analyzed and interpreted the data; Wrote the paper.
Suharлина: Performed the study; Contributed analysis tools or data; Wrote the paper.
Anuraga Jayanegara: Conceived and designed the study; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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