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Effect of *Acacia mearnsii* as a source of tannins on rumen fermentation *in vivo* and gas production kinetics *in vitro*

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Abstract

This study investigated the effect of *Acacia mearnsii* forage (AMF) inclusions in maize silage on the rumen volatile fatty acids (VFAs) (acetic, propionic and butyric acid); and AMF inclusions in maize silage and *A. mearnsii* tannin extract (ATE) in pellets on *in vitro* gas production kinetics. To determine the VFA profile, twenty-four crossbred Holstein-Friesian and Jersey cows were equally assigned to four treatments; 0, 5, 15 or 25 % (as fed) AMF in maize silage in a completely randomized study design. Cows were subjected to 14 d adaptation period to treatments then 21 d of weekly rumen fluid sampling per treatment followed. The above-mentioned treatments were assessed for their effect on the *in vitro* gas production kinetics along with 0, 0.75 , 1.5 or 3 % (as fed) ATE inclusion in concentrate pellets and incubated for 0, 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h. Dietary AMF inclusions did not affect ($P > 0.05$) total rumen volatile fatty acids but linearly increased ($P < 0.05$) propionic and butyric acid. Similarly, it did not change ($P > 0.05$) the *in vitro* gas production kinetics, while ATE decreased the gas production rate linearly ($P < 0.05$). In conclusion, AMF and ATE slightly affected rumen fermentation since AMF inclusions increased propionic and butyric acid linearly up to the 25 % inclusion while ATE inclusions decreased the gas production rate.

Keywords: methane, tannins, rumen volatile fatty acids

1 Introduction

Ruminant products are necessary to meet protein requirements of the growing world human population. To meet the protein requirements of humans, ruminant production has been gradually intensifying. However, ruminant production intensification would enhance methane (CH_4) emissions which is a greenhouse gas that is linked to ruminant production (Mhlongo *et al*., 2023). Intensification of dairy production maximizes milk yield, which positively correlates with CH_4 production (Breider *et al*., 2019). CH_4 production also can decrease dairy performance as its production requires dietary metabolizable energy which decreases intake of this nutrient for milk production (Krueger *et al*., 2010; Moate *et al*., 2020). Climate change effect of CH_4 production could lengthen dry periods which could limit dairy performance by decreasing feed quality. Lengthy dry periods could force farmers to source expensive conventional feed supplements. Thus, the identification of cheap non-conventional feeds containing anti-methane and nutritive properties could address the above-mentioned challenges (Mhlongo *et al*., 2023).

Tanniferous feeds are a suitable solution to depress CH_4 emissions in ruminants and act as feed supplements. *Acacia mearnsii* forage (AMF) has a moderate protein content, contains tannins and grows all year round in South Africa which potentiate its use for controlling CH_4 and as dietary supplement (Adejoro *et al*., 2019; Alves *et al*., 2017; Mkhize *et al*., 2014). *A. mearnsii* is an Australian tree species that is commercially grown in South Africa for tannin and woodchip production (Chan *et al*., 2015) while it is rarely considered as animal feed. Hence, there is scarce understanding of the response of rumen volatile fatty acids as well as rumen fermentation kinetics to dietary addition of AMF.

A. mearnsii tannin extracts (ATE) affect rumen fermentation kinetics inconsistently (Nascimento *et al*., 2021). There

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is a need to test ATE and AMF at various inclusion levels, both *in vitro* and *in vivo*, to identify optimal inclusion levels that do not depress methane and ruminant performance (Cieslak *et al*., 2016; Gemeda & Hassen, 2015). The objective of this study was to investigate the effect of AMF inclusion on *in vivo* rumen fermentation and ATE and AMF inclusions on *in vitro* gas production kinetics. It was hypothesised that the inclusion of AMF in maize silage diets would decrease volatile fatty acid (VFA) production, while *in vitro* gas production kinetics would be negatively affected by AMF and ATE inclusions.

2 Materials and methods

The KwaZulu-Natal-Department of Agriculture and Rural Development's Research Committee (ref: 12/11/1/15 (2387JD)) and University of KwaZulu-Natal's Animal Research Ethics Committee (ref: AREC/00003470/2021) approved this study.

2.1 In vivo rumen volatile fatty acid determination

For the *in vivo* rumen VFA determination, rumen fluid was sourced from 24 crossbred Holstein-Friesian and Jersey cows of the Springfontein commercial dairy farm (Kokstad, South Africa, coordinates 30°15'52.0" S and 29°10'15.0" E, 1450 m a.s.l.). Cows were spray painted for identification according to their assigned treatments. The cows that were 211±16.4 d in milk were equally divided into four groups as described by Mhlongo *et al*. (2023) where each cow was an experimental unit.

The treatments were: 0 % (0AMF), 5 % (5AMF), 15 % (15AMF) and 25 % (25AMF) (as fed) AMF in maize silage diets (Table 1); AMF inclusions of the current study are described by Bhatta *et al*. (2015). After adapting to treatments (14 d), cows were subjected to a data collection period (21 d) (Griffiths *et al*., 2013). Twenty-three cows were used to collect rumen fluid samples due to the removal of one cow that was sick in the control group. Weighed daily, fresh maize silage from the maize silage pit was manually mixed with different AMF inclusions in labelled feed bags and fed to each cow. Whole plant AMF used in this study was milled (Tomcat 150 CDE Wood-Chipper, Worcester, South Africa) and shed-dried (7 days) before use for protection against rainfall damage. Feeding of the experimental diets was realized for 2 hours after the afternoon milking session (2:00 pm). Cows were individually housed in wooden pens (2.4 m, length, 3.12 m, width and 1.03 m, height) locked with barb wires and iron standards gates. Each pen had a plastic tub (20 L) full of fresh water and plastic feeders attached to the front wide enough for each cow (51 cm apart) to access feed.

Between the morning (5:00 am) and afternoon (2:00 pm) milking sessions, cows grazed (white clover + perennial ryegrass). During each milking session, cows were offered concentrate (15 % CP) (AFGRI, Pietermaritzburg, South Africa) per individual milk yield by an automated feeder and were teat dipped on each quarter.

Pasture was manually sampled diagonally in grazing camps weekly during data collection. Using the same sampling frequency, lucerne and veld hay were sampled from the top and bottom part of each hay bale. Feed samples collected weekly to form one pooled feed sample were sent to the feed analytical laboratory (Cedara college, 1 Cedara Road, Pietermaritzburg 3200) for chemical analysis. More details and the methods of the chemical analyses are presented in section 2.3. The chemical composition of the pooled weekly feed samples are given in Table 1.

Rumen fluid was collected weekly at the end of the morning milking session to prevent stressing the experimental animals. Rumen fluid was collected by immobilising cows in

Table 1: *Chemical composition of different feeds.*

Feeds (g kg^{-1} DM)	*DM*	*Ash*	*EE*	*ADF*	*NDF*	*CP*	*ADIN*	*CT*
Lucerne hay	967.3	108.0	28.7	304.2	406.4	119.6	9.3	-
AMF	879.7	44.7	31.8	658.3	753.3	157.0	16.5	31.0
Veld hay	940.4	63.3	14.8	522.2	844.1	69.1	5.4	-
Ryegrass + white clover	964.5	121.8	18.5	293.1	536.5	219.1	12.6	-
Maize silage								
0AMF	964.6	56.5	25.8	339.8	607.4	81.0	6.6	-
5AMF	966.8	201.0	20.1	289.6	476.8	67.2	-	1.56
15AMF	969.2	49.4	24.4	37.73	615.1	75.4	-	4.65
25AMF	964.0	46.0	29.3	454.7	641.3	97.9	-	7.75

0AMF, 5AMF, 15AMF and 25AMF were 0, 5, 15 and 25 % *Acacia mearnsii* forage (AMF) inclusion in maize silage, respectively; DM: dry matter; EE: ether extract, ADF: acid detergent fibre; NDF: neutral detergent fibre; CP: crude protein; CT: condensed tannins; ADIN: acid detergent insoluble nitrogen.

a headgate and injecting a needle attached to a syringe (60 ml) in the *paralumbar fossa* section of each cow. Five ml of rumen fluid per cow per week was collected. Collected rumen fluid was stored in a vial containing 25 % metaphosphoric acid and frozen until analysis. Frozen rumen fluid samples were submitted to the central analytical facility (Stellenbosch University's GC-MS division, Matieland, South Africa) for rumen volatile fatty acids (VFAs: acetic acid, propionic acid and butyric acid) analysis as described by Tayengwa, (2020).

2.2 *In vitro gas production kinetics' determination*

The *in vitro* gas production experiment was conducted at the North-West University research farm (Molelwane; coordinates 25°47'23" S and 25°37'15" E, South Africa) using a procedure described by Mhlongo *et al*. (2021). The AMF treatments of this study included 0AMF, 5AMF, 15AMF, and 25AMF (Table 1), and the ATE treatments were 0.75 % (0.75ATE), 1.5 % (1.5ATE), and 3 % (3ATE) (as fed) ATE in pellets (Table 2) prepared from PSP (Kokstad, KwaZulu-Natal, South Africa). The ATE inclusions used in the current study have been described by Tseu *et al*., (2021). The ATE (68 % purity) was prepared at the NTE house (Pietermaritzburg, KwaZulu-Natal, South Africa). Commercial pellets (15 % CP) represented the control (0ATE) (AFGRI, Pietermaritzburg, South Africa). Before morning feeding, rumen fluid was collected from a fistulated donor cow (Bonsmara; 550 kg). The donor cow had unlimited access to lucerne hay and blue buffalo (*Cenchrus ciliaris*) pasture, and clean water.

Table 2: *Feed ingredient composition for different (*Acacia mearnsii *tannin extract (ATE) levels.*

Item (in kg)	*3ATE*	*1.5ATE*	*0.75ATE*
Crushed maize	498	498	498
Hominy chop	275	275	275
Wheat bran	100	100	100
Molasses liquid	50	50	50
Soya oil cake	30	30	30
Urea	15	15	15
Lime powder	12.5	12.5	12.5
Salt	10	10	10
Monoc. P. 21*	5	5	5
Mineral premix	5	5	5
Condensed tannins†	44	22	11

*Monocalcium phosphate 21; †Condensed tannins extract powder; 0.75 % (0.75ATE), 1.5 % (1.5ATE), and 3 % (3ATE) (as fed) ATE (*Acacia mearnsii* tannin extract) inclusion in pellets.

During the *in vitro* gas production experiment, triplicates of the treatments were milled (1 mm sieve), weighed (1 g) and transferred to serum bottles (125 ml)(Table 3). ANKOM buffer solution (90 ml) and rumen fluid (10 ml) were also added to the treatments to the serum bottles. The rumen fluid was extracted by squeezing the rumen digesta into a pre-warmed (39 °C) carbonated thermos flask. Rumen fluid was transferred into thermos flasks by passing the digesta through two layers of muslin cloth. Air-tight-rubber stoppers were used to close serum bottles. The treatment and blank serum bottles were incubated (39 °C) (ECR Manufacturing; model no: SU131H; serial no:128538; fans: 15 amps; elementary 0.0k) for 0, 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h by inserting the needle (23 g) into the gas pressure transducer (4200-015GI, Omega Engineering Inc., Canada), the gas pressure (psi) was measured for each incubation period using the microprocessor. The gas pressure readings were converted from psi to gas volume in millilitres by fitting in the site-specific the equation given by Mhlongo *et al*., (2021) and Ouda *et al*., (2006). At 96-hour post-incubation, the bottles were cooled (at 5 °C for 1 h) in the cold room to end fermentation. Ash-free ANKOM F57 filter bags that were filled with pre-weighed residues in the crucibles and incinerated in the muffle furnace (600 °C for 12 h) were used to determine the *in vitro* organic matter degradability (ivOMD). Partition factors which determine fermentation efficiency were calculated as the cumulative gas production to ivOMD ratio (Mhlongo *et al*., 2021).

Table 3: *Dry matter (DM) and organic matter (OM) of AMF enriched maize silage and ATE pellets.*

Treatment	*DM* $g\ kg^{-1}$	*OM* $g\ kg^{-1}$
0ATE	916.8	848.9
0.75ATE	921.3	856.4
1.5ATE	914.9	867.2
3ATE	911.6	858.7
0AMF	933.7	885.8
5AMF	938.7	885.2
15AMF	931.6	886.6
25AMF	934.7	891.4

A. mearnsii tannin extract (ATE), included at 0 (0ATE), 0.75 (0.75ATE), 1.5 (1.5ATE) or 3 % (3ATE) in pellets; *A. mearnsii* forage (AMF), included at 0 (0AMF), 5 (5AMF), 15 (15AMF) or 25 % (25AMF) in maize silage.

2.3 *Chemical analyses*

Feed samples of both experiments were oven-dried (60 °C for 72 h), ground to pass through a 2 mm sieve, packed in

labelled zip lock bags and sent to Cedara Analytical Laboratory (1 Cedara road, KwaZulu-Natal, South Africa) for proximate analysis. Feed samples were analysed for nitrogen content using the Leco Truspec nitrogen analyser and nitrogen content was multiplied by 6.25 to estimate crude protein (CP, ID 968.06) (Leco FP200, LECO, Pretoria, South Africa). Dry matter (DM, ID 934.01) and ash (ID 942.05) were analysed using the AOAC methods (AOAC, 2000). ANKOM 220 Fibre analyser was used to determine acid detergent fibre (ADF) and neutral detergent fibre (NDF) using the filter bag technique (ANKOM Technology, 2011). The Soxhlet Buchi 810 Fat analyser (Soxhlet Buchi, Flawil, Switzerland) was used to estimate the content of ether extract (EE).

The AMF and ATE samples contained in a medium-sized zip-lock bags were sent to MIMOSA extract company (NTE House, Pietermaritzburg, South Africa) for tannin content analysis (SLTC, 1965). Briefly, tannin content analysis was done by heating the samples under reflux with water to extract tannins. Extract liquor was filtered off twice to extract more tannins and concentrated to dryness under vacuum to a brown liquid. Hide powder was mixed with the brown liquid, stirred, left to stand filtered. The filtrate was evaporated to determine non-tannins while the tannin content was determined as the difference between soluble solids and non-tannins (SLTC, 1965).

2.4 Mathematical calculations and statistical analysis

Total VFA were calculated as the sum of acetic, propionic and butyric acids. The acetic to propionic ratio was calculated as the acetic acid divided by propionic acid. The VFA proportions were fitted in the following formula to predict CH_4 ($mg\,l^{-1}$): $CH_4 = 0.45$ (Acetate) – 0.27 (Propionate) + 0.40 (Butyrate) (Moss *et al*., 2000). The *in vitro* gas production kinetics were determined using the following equation (Ørskov & McDonald, 1979):

$$P = a + b\,(1 - e^{c(t-lt)})$$

where, P is the dependent variable; a, quickly degradable fraction's gas production; b, slowly degradable fraction's gas production; c, gas production rate; lt, lag time and t, time. Potential gas and effective gas production were calculated as: $PGas = a + b$ while $EGas = a + (bc)/(k + c)$ and k is the rumen outflow rate assumed to be $0.02\,h^{-1}$ (Buthane *et al*., 2021).

The general linear model procedure of statistical analysis software (SAS, version 9.4) was used to determine the effect of the independent variables on the dependent variables in the current study. The data were analysed using the following model:

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$$

where μ is the dependent variable, α_{ij} is the treatment effect and ϵ_{ij} is the error term. Tukey posthoc test was used to test the differences between pairs of treatment means. The results were reported as least square means and standard error of means. The orthogonal contrast statement was used to determine the linear and quadratic effects (Adejoro *et al*., 2019). Statistical significance was declared at $P < 0.05$.

3 Results

Rumen VFA at different inclusions of AMF in maize silage diets are presented in Table 4. Inclusions of AMF in maize silage did not affect the overall production of ruminal VFA and the predicted CH_4 in comparison to the control diet. However, propionic and butyric acid linearly increased and were both the highest at 25AMF, while the other VFA lacked either a linear or quadratic effect (Table 4).

Table 4: *Effect of dietary inclusions of* Acacia mearnsii *in maize silage diets on rumen fermentation of dairy cows.*

						Significance		
Fermentation parameters	*0AMF*	*5AMF*	*15AMF*	*25AMF*	*SEM*	*Treatment*	*Linear*	*Quadratic*
Acetic acid ($mg\,l^{-1}$)	269.42	260.91	395.33	451.26	94.44	0.35	0.11	0.72
Propionic acid ($mg\,l^{-1}$)	10.31	13.03	20.00	20.37	3.40	0.10	0.02	0.72
Butyric acid ($mg\,l^{-1}$)	24.29	31.96	43.13	48.28	8.96	0.22	0.04	0.89
Acetic : propionic acid ratio	25.16	19.90	19.67	22.23	2.64	0.41	0.43	0.13
Propionic : butyric acid ratio	0.44	0.41	0.49	0.45	0.02	0.08	0.28	0.75
Total VFAs ($mg\,l^{-1}$)	304.01	305.92	458.45	519.91	105.18	0.32	0.09	0.77
Predicted CH_4 ($mg\,l^{-1}$)	114.65	113.57	169.88	194.21	40.04	0.35	0.10	0.74

SEM; standard error of means and significant difference; $P < 0.05$, AMF; *A. mearnsii* forage; 0 % (0AMF), 5 % (5AMF), 15 % (15AMF) and 25 % (25AMF) (as fed) AMF in maize silage diets.

Table 5: *Effect of dietary inclusions of* Acacia mearnsii *in maize silage diets on rumen fermentation.*

						Significance		
Fermentation parameters	*0AMF*	*5AMF*	*15AMF*	*25AMF*	*SEM*	*Treatment*	*Linear*	*Quadratic*
a, ml	16.09	15.22	14.29	15.02	1.25	0.78	0.47	0.53
b, ml	127.35	136.09	131.67	121.31	12.48	0.86	0.69	0.46
c, % h^{-1}	0.03	0.03	0.02	0.03	<0.01	0.67	0.40	0.53
Lt, h	1.77	2.05	2.36	1.85	0.43	0.76	0.78	0.36
PGas, ml g^{-1} OM	143.44	151.30	145.96	136.33	12.88	0.87	0.65	0.51
EGas, ml g^{-1} OM	95.89	95.61	83.50	81.13	11.85	0.73	0.31	0.93

Significant difference, $P < 0.05$ and SEM, standard error of means; a, quickly degradable fraction's gas production; b, slowly degradable fraction's gas production; c, gas production rate of the (b) fraction; lt, lag time, PGas, potential gas, EGas, effective gas production. AMF; *A. mearnsii* forage; 0 % (0AMF), 5 % (5AMF), 15 % (15AMF) and 25 % (25AMF) (as fed) AMF in maize silage diets.

Table 6: *Effect of dietary inclusions of* Acacia mearnsii *in maize silage diets on rumen fermentation.*

						Significance		
Fermentation parameters	*0ATE*	*0.75ATE*	*1.5ATE*	*3ATE*	*SEM*	*Treatment*	*Linear*	*Quadratic*
a, ml	20.13	14.03	18.39	22.84	2.91	0.12	0.32	0.06
b, ml	156.83	126.85	149.69	155.67	18.41	0.50	0.80	0.29
c, % h^{-1}	0.073	0.04	0.04	0.03	0.01	0.17	0.045	0.38
Lt, h	2.08	1.64	1.79	1.30	0.44	0.60	0.25	0.95
PGas, ml g^{-1} OM	176.96	140.87	168.08	178.50	20.43	0.40	0.71	0.22
EGas, ml g^{-1} OM	141.86	97.56	119.09	118.77	21.04	0.45	0.59	0.26

ATE, *Acacia mearnsii* tannin extract; significant difference, $P < 0.05$ and SEM, standard error mean; a, quickly degradable fraction's gas production; b, slowly degradable fraction's gas production; c, gas production rate of (b) fraction; lt, lag time, PGas, potential gas, and EGas, effective gas production and 0.75 % (0.75ATE), 1.5 % (1.5ATE), and 3 % (3ATE) (as fed) ATE in pellets.

The effect of AMF inclusion to maize silage diets on the *in vitro* gas production is shown in Table 5. Inclusions of AMF did not affect any of the gas production kinetics. Additionally, linear and quadratic effects did not affect the gas production kinetics (Table 5).

The effect of ATE inclusions to pellets on the *in vitro* gas production kinetics is shown in Table 6. The ATE inclusions and quadratic effect did not affect the gas production kinetics, while the rate of gas production decreased linearly with ATE inclusions being the least for 3ATE.

4 Discussion

4.1 Effect of AMF inclusions on rumen volatile fatty acids and gas production kinetics

Ruminant production can reduce feed costs by replacing conventional feeds with non-conventional feeds that contain moderate levels of protein sources such as AMF (Beigh *et al*., 2017). Non-conventional feeds such as AMF can also be used to control CH_4 production because they contain tannins that suppress CH_4 (Rira *et al*., 2015). However, the effects of these feeds on rumen fermentation and degradation kinetics are inconsistent and require further research to determine their optimal inclusion level (Mhlongo *et al*., 2025). The present study found that AMF inclusions in maize silage only linearly increase propionic and butyric acids, without affecting the other VFAs. The current study also demonstrated that the presence of AMF in maize silage does not influence any of the fermentation parameters. Consequently, the hypothesis was rejected in relation to the objectives of this study.

The increase in propionic and butyric acid with AMF inclusions is consistent with a previous *in vitro* study (Hassanat & Benchaar, 2013). This contradicts previous findings that indicated a decrease in propionic and butyric acid levels with increasing AMF inclusions *in vivo* (Uushona *et al*., 2023). The increase in propionic and butyric may have been due to no decrease in nutrient digestibility, as previously shown that decrease nutrient digestibility in tanniferous diets decreases propionate and butyrate production (Uushona *et al*., 2023). It has been demonstrated that a decrease in tannin inclusions leads to a reduction in dry matter digestibility, resulting in a concomitant decrease in propionate and butyrate production (Hundal *et al*., 2016). An in-

crease in protein content, results in a higher proportion of total VFAs and propionate production in tannin-enriched diets (Zhou *et al*., 2019). It is plausible that in the present study, the protein content of the diets was adequate, thereby increasing propionate levels. Propionic and butyric acid are said to correlate negatively with dietary crude protein content (Getachew *et al*., 2004). Consequently, it is plausible that the increased inclusion of AMF's tannins may have led to a suppression of crude protein availability, subsequently resulting in an elevation of propionic and butyric acid levels (Xia *et al*., 2018). Moreover, increasing fibre level in ATE enriched diets increases propionate and buytyrate (Dschaak *et al*. 2011). Thus, inclusion of AMF must have increased fibre level in the diets as well as propionic and butyric acid.

On the other hand, the observation of no effect of AMF inclusions on gas production kinetics disagrees with previous studies (Kusuma *et al*., 2022). However, these findings correspond with other results (Nascimento *et al*., 2018). It has been previously demonstrated that gas production is positively correlated with crude protein content (Basha *et al*., 2013). It is possible that the inclusion of AMF increased tannin level, which subsequently decreased crude protein level due to the tannin-protein complex and curtailed gas production response (Vargas-Ortiz *et al*., 2022). It has also been demonstrated before that tanniferous diets must possess the ability to inhibit the degradation of dry matter to suppress the kinetic rate of gas production (Suescun-Ospina *et al*., 2022). Therefore, it is likely that the lack of the effect of AMF inclusions on gas production was due to the dry matter degradation not being affected in the current study as dry matter digestibility and gas production positively correlate (Sebata *et al*., 2011). There are further demonstrations that when tanniferous diets do not affect dry matter digestibility, rumen microbes remain not affected as well (Avila *et al*., 2020). Gas production is bound to not be affected as it is a product of microbial activity fermentation of the substrate in the rumen which was the potential case in the current study (Molina-Botero *et al*., 2019).

Our study was limited by the fact that the AMF inclusion effect on VFAs was conducted *in vivo* while inclusions of this forage were conducted *in vitro*. It has been demonstrated that *in vitro* results of the effects of tannins on rumen fermentation are a less reliable predictor than *in vitro* results (Jayanegara *et al*., 2012). Thus, inclusions of AMF used in the current study require further *in vivo* digestibility assessment.

4.2 Effect of tannin extract on gas production kinetics

The dietary ATE inclusions present a potential solution to control and improve ruminant production by suppressing methane (CH_4) emissions but their effects vary (Deuri *et al*., 2020). Further research is necessary to determine their specific impact on gas production, enabling the identification of ATE inclusions that effectively suppress CH_4 and other gases associated with CH_4 production such as H_2 and CO_2 (Bhatta *et al*., 2015). Our study showed that ATE inclusions only suppress the rate of gas production. This result partially agrees with the hypothesis of our objective.

This observation agrees with a previous demonstration by Sarnataro *et al*. (2020). Conversely, these results disagree with other findings by Ibrahim & Hassen (2022). However, these findings may be based on the fact that gas production tends to decrease with decreasing organic and dry matter digestibility in ATE diets (Sujarnoko *et al*., 2020). Thus, ATE inclusions must have complexed the organic nutrients, which led to a lower gas production rate (Naumann *et al*., 2017). The substrate enriched with tannins also affects gas production. For instance, pasture cause more gas production than hay in ATE-enriched diets (Menci *et al*., 2021). The substrate supplemented with ATE in the present study must have influenced the results.

The investigation into the impact of ATE inclusions on gas production parameters might have been constrained by the lack of clarity on the extent to which the ATE tannin level influenced the outcomes. Hence, future studies should evaluate the influence of tannin presence on gas production kinetics using polythene glycol as this product exerts tannin inactivation in the rumen (Basha *et al*., 2013).

5 Conclusions

This study demonstrated that AMF inclusion has no effect on the total rumen volatile fatty acids. Additionally, the results showed neither AMF nor ATE affecting the *in vitro* gas production kinetics. However, dietary AMF or ATE inclusions showed a linear trend on propionic and butyric acids and individual *in vitro* gas production kinetic parameters.

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Conflict of interest

The authors declare no conflict of interest.

Data availability statement

Data available upon reasonable request.

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