

ORIGINAL ARTICLE

Evaluation of enteric methane production in dairy cows fed *Acacia mearnsii*

Lindokuhle C. Mhlongo^{1,2} , Ignatius V. Nsahlai² 

¹Department of Animal Science, University of the Free State, Bloemfontein, South Africa

²Department of Animal and Poultry Sciences, University of KwaZulu–Natal, Scottsville, South Africa

ABSTRACT

Objectives: We investigated the effect of different incorporations of *Acacia mearnsii* forage (AM) in maize silage or *A. mearnsii* tannin extract (AME) in pellets on dairy rumen CH₄.

Materials and Methods: Using a completely randomized design per experiment, 24 crossbred Holstein–Friesian and Jersey dairy cows per experiment were divided into groups ($n = 6$ cows per experiment). Dairy cows were fed pellets with 0% (OPEL), 0.75% (0.75PEL), 1.5% (1.5PEL), or 3.0% (3PEL) of AME (Experiment 1). Furthermore, dairy cows were fed 0% (OAM), 5% (5AM), 15% (15AM), or 25% (25AM) of AM in maize silage (Experiment 2). Data sampling period (21 days) of ruminal CH₄ and nitrogen (N₂), carbon dioxide (CO₂), and hydrogen (H₂) gases (% vol) was conducted after the adaptation period (14 days) for each experiment.

Results: Enteric CH₄ was not affected by AME inclusion, but AM inclusions affected CH₄, except for CH₄ (% vol per cow per day). The inclusions of 25AM decreased CH₄ per nutrient intake (kg/day), such as dry matter (DM), organic matter (OM), crude protein, acid detergent fiber (ADF), and neutral detergent fiber (NDF). In addition, there was a linear and quadratic AM inclusion effect on CH₄ per intake of nutrients, including DM, NDF, ADF, and OM.

Conclusion: Enteric CH₄ was not affected by AME but was decreased by AM in dairy cows.

ARTICLE HISTORY

Received March 08, 2024

Revised January 31, 2025

Accepted February 08, 2025

Published September 22, 2025

KEYWORDS

Acacia mearnsii; CH₄; greenhouse gas; rumen–headspace gases; tannins



© The authors. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 License (<http://creativecommons.org/licenses/by/4.0>)

Introduction

Methane (CH₄) gas is produced during the digestion of fiber, a component of carbohydrate fermented in the rumen by rumen microbes [1]. This gas results from pyruvate breakdown into acetate during fiber digestion, where more hydrogen (H₂) molecules are produced for methanogens to utilize to produce CH₄ [2]. This gas contributes to global warming, and a large amount of agricultural CH₄ emissions comes from ruminant production [3]. In addition, the production of CH₄ also typically wastes feed since ruminants lose 2% of their energy intake from producing this gas [4]. Therefore, to decrease ruminant production costs and safeguard the environment, ruminant CH₄ emissions must be minimized using natural strategies.

Due to their capacity to reduce ruminant CH₄ generation, condensed tannins (CTs) have received scientific attention as a solution for this challenge [5,6]. Certain feeds naturally contain CTs, which bind nutrients to reduce CH₄ in ruminants and are a potential solution for this challenge. CT-rich feeds lower CH₄ by decreasing acetate, the

acetate–propionate ratio, methanogens, and the population of protozoa, which all have an indirect role in the synthesis of CH₄ [7]. Despite this, some studies have produced contradictory results, indicating that CT sources do not affect CH₄ emission [8].

Acacia mearnsii is a tannin source that can be used as an *A. mearnsii* tannin extract (AME) or an *A. mearnsii* forage (AM) to depress CH₄ emissions in cows. However, *A. mearnsii* has mostly been utilized to depress CH₄ in ruminants in the form of AME to lower CH₄ generation in ruminants [3]. There have been reports of AME failure in lowering CH₄ in ruminants [9], which necessitates the assessment of AM or AME inclusions on CH₄. Most of the literature available on the use of AM or AME to depress CH₄ in ruminants relies on *in vitro* studies, a poor predictor of *in vivo* CH₄. There is a need to expand the assessment of AM or AME under on-farm conditions to improve the understanding of CH₄ response *in vivo*.

Research on the AM or AME inclusions under on-farm conditions can also improve the comprehension of how tannin sources affect CH₄. The emission of CH₄ depends on

Contact Lindokuhle C. Mhlongo ✉ chrisokuhle@gmail.com 🏢 Department of Animal Science, University of the Free State, Bloemfontein, South Africa.

How to cite this article: Mhlongo LC, Nsahlai IV. Evaluation of enteric methane production in dairy cows fed *Acacia mearnsii*. J Adv Vet Anim Res 2025; 12(3):1030–1036.

the dietary components of the basal diet fed to ruminants. Investigations into the inclusion of AM or AME in different basal diets on CH₄ are needed to identify AM or AME inclusions that depress CH₄ for a given type of basal diet. We investigated the effect of different incorporations of AM in maize silage or AME in pellets on dairy rumen CH₄. We hypothesized that increasing AM or AME incorporation would decrease enteric CH₄ in dairy cows.

Materials and Methods

Ethical approval

The Animal Research Ethics Committees of KwaZulu-Natal Department of Agriculture and Rural Development [ref: 12/11/1/15 (2387JD)] and University of KwaZulu-Natal (ref: AREC/00003470/2021) approved this research.

Study site

The study site for this research was a commercial dairy farm, Springfontein dairy farm located in Kokstad, Kwa-Zulu-Natal province, South Africa (coordinates, 270°W, 30°15'52"S, 29°10'15"E S and altitude, 1450 m).

Acacia mearnsii-enriched pellets

A total of 24 cows were divided into four treatment groups (*n* = 6 dairy cows per treatment group) by a complete randomization design. The treatment groups were control 0% (0PEL), 0.75% (0.75PEL), 1.5% (1.5PEL), or 3.0% (3PEL) addition of powdered *Acacia mearnsii* (AME) in a pelleted mixed feed (Tables 1 and 2). Before the 21-day data collection period, cows were adapted to treatments for 14 days [10]. Using animal spray paint for identification, cows

in each treatment group were tagged with the diets they were assigned. The intakes of 0PEL, 0.75PEL, 1.5PEL, and 3PEL were the difference between feed left and feed fed (as is). During milking, the study site's herd was given a 15% commercial concentrate of crude protein (CP) (AFGRI, Pietermaritzburg, South Africa), which was used as 0PEL. The manufacturer withheld information regarding the ingredient composition of 0PEL. Tannin purity of AME was 68% and was determined using the butanol–hydrochloric acid method (Mimosa, Pietermaritzburg, South Africa).

Crossbred Holstein–Friesian and Jersey cows being mechanically milked [60-point rotational milking machine (E100, De Laval, Pinetown, South Africa)] and with 199.5 ± 5.12 days in milk (mean ± SD) were used in this study. On the farm, there were no precise lactation numbers available for each cow. Every morning and afternoon milking session, each cow received 2 kg (as-fed) of their allocated pellets using the milking unit feeders.

Dairy cows were fed *ad libitum* soybean and maize silages in a fenced camp overnight. During the day, dairy cows grazed perennial ryegrass and white clover pasture between morning (5:00 am) and afternoon (14:00) milking sessions. Before and after milking, cows were screened for mastitis.

Acacia mearnsii-enriched maize silage

Using complete randomization, 24 crossbred Jersey and Holstein–Friesian cows of 211.46 ± 16.41 days in milk (days in milk ± SD) were assigned to 0% (0AM), 5% (5AM), 15% (15AM), and 25% (25AM) (as-fed) AM in maize silage diets (Table 3), whereby each treatment was assigned six cows. Since the farm lacked precise statistics regarding the

Table 1. Composition of experimental pellets.

Items (kg)	3PEL	1.5PEL	0.75PEL
Crushed maize	498	498	498
Hominy chop	275	275	275
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
Monocalcium phosphate 21	5	5	5
Mineral and vitamin premix	5	5	5
Condensed tannin extract powder	44	22	11

$$\dagger \text{ Tannin inclusion} = \frac{\text{tannin extract (kg)} \times 68 \% \text{ tannin extract purity}}{988 \text{ kg of pellets}}$$

herd's lactation numbers, parity was not used in the study design. Before the 21-day data collection period, cows were adapted to treatments for 14 days [10]. Using animal spray paint for identification, cows in each treatment group were tagged with the diets they were assigned. The intakes of 0AM, 5AM, 15AM, and 25AM were measured weekly from the difference between feed left and feed given (as is).

Weighed fresh corn silage gathered from the pit each day was combined with various AM inclusions, transferred into labelled bags, mixed, and fed to treatment groups daily. AM (twigs and leaves) was ground with a wood chipper (Tomcat Wood Chipper, Worcester, South Africa) and dehydrated by sunlight under shelter (7 days) pending use.

Following afternoon milking (14:00 pm), cows were fed treatments (2 kg, as fed for 2 hours) in lockable, open-air wooden individual pens. Every pen had a gate constructed with barbed wire and iron standards and fresh water (20 l) in plastic containers. Each pen was constructed with slatted poles (51 cm spacing), into which plastic feeders were affixed so that each cow could fit their head to get feed.

Dairy cows were fed lucerne and veld hay (*ad libitum*) in the overnight camp. During the day, dairy cows grazed white clover and perennial ryegrass. Dairy cows were given a custom feed concentrate (15% CP; AFGRI, Pietermaritzburg, South Africa) during milking and

were teat-dipped every quarter for mastitis pre- and post-milking.

Methane determination

Every week, after milking in the morning, cows were gently immobilized in the headlock gate. Hair was clipped, and the left paralumbar fossa area was cleaned (F10). The ruminal gases were collected at the *paralumbar fossa* region of the cows by injecting a needle (14 G needle and 60 ml syringe with a three-way stopper). Sampled gases (CH₄, N₂, CO₂, and H₂, % vol) were injected into the portable multiple gas analyzer (SKZ-1050D, China) for headspace gas (% vol) determination.

Feed sampling and proximate analysis

Feed was sampled weekly by diagonally sampling each pasture paddock and sampling the top, middle, and bottom sections of each feed bale. Thereafter, feed samples were separately refrigerated in labeled feed bags while pending analysis.

Feed samples were oven-dried (60°C for 72 hours), ground (2 mm sieve), packed in labeled zip-lock bags, and sent to the Cedara Analytical Laboratory (Hilton, South Africa) for proximate analysis using AOAC methods [11]. The nitrogen analyzer (Leco Truspec, LECO, Pretoria,

Table 2. Chemical composition of the basal feeds and experimental pellets.

Items (gm/kg)	DM	Ash	EE	ADF	NDF	CP
Maize silage	964.4	39.0	28.5	220.0	396.0	65.0
Perennial ryegrass + White clover	941.0	150.0	18.5	222.0	356.0	217.0
#PEL	916.3	64.0	26.5	48.9	163.8	145.3
OPEL	980.0	88.5	22.5	110.1	280.7	150.0
Soya silage	573.0	193.0	–	281.0	345.0	11.5

ADF, acid detergent fiber; CP, crude protein; DM, dry matter; NDF, neutral detergent fiber*; #Chemical composition of experimental pellets without inclusion of tannins ; OPEL, custom concentrate pellets which were used as the control.

Table 3. Chemical composition of experimental feeds.

Feeds	Chemical composition (gm/kg)							
	DM	Ash	EE	ADF	NDF	CP	ADIN	CT
Lucerne hay	967.3	108	28.7	304.2	406.4	119.6	9.3	–
AM	879.7	44.7	31.8	658.3	753.3	157	16.5	31
0AM	964.6	56.5	25.8	339.8	607.4	81	6.6	–
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	–
5AM	966.8	201	20.1	289.6	476.8	67.2	–	1.56
15AM	969.2	49.4	24.4	37.73	615.1	75.4	–	4.65
25AM	964	46	29.3	454.7	641.3	97.9	–	7.75

0AM, 0% AM inclusion in corn silage; 5AM, 5% AM inclusion in CS; ADF, acid detergent fiber; ADIN, acid detergent insoluble nitrogen; CP, crude protein, CT, condensed tannins; DM, dry matter; EE; ether extract; NDF; neutral detergent fiber.

South Africa) was used to quantify nitrogen content, and the nitrogen content ($\text{ID } 968.06 \times 6.25$) equaled the CP content estimate (Leco FP200, LECO). Dry matter (DM) in an air-forced oven and ash content resulted from combustion in a muffle furnace. Furthermore, ANKOM 200 fiber analyzers (ANKOM Technology, Macedon, New York) were used for neutral detergent fiber (NDF) and acid detergent fiber (ADF) determination [12]. The 810 Fat Analyzer determined ether extract content (Soxhlet Buchi, Flawil, Switzerland). The AM samples contained in a medium-sized zip-lock bag were sent to the MIMOSA extract company (NTE House, Redlands Estate, Pietermaritzburg, South Africa) for tannin content determination [13].

Statistical analyses and calculations

The DM intake was determined by multiplying the feed intake of treatments (as-fed) by their corresponding DM content. CH_4 per nutrient intake of nutrients was determined by multiplying the intake of each of the above-mentioned nutrients by its corresponding CH_4 volume measured (% vol). The data were analyzed using statistical analysis software (SAS, 9.4), general linear model procedure using the following model: $Y_i = \mu + a_i + e_i$, where Y_i is the response variable, a_i is the treatment incorporation effect, and e_i is the error term. The Tukey test was used to separate the means. The differences among the least square means were tested by Tukey's test for statistical significance of $p < 0.05$ by diets on the dependent variables. Furthermore, linear and quadratic effects were ascertained using the orthogonal contrast statement.

Results

The effect of AME incorporation in pellets on the gases and CH_4 in dairy cows is demonstrated in Table 4. The effect of AME incorporation in pellets on CH_4 and other gases was absent ($p > 0.05$). Furthermore, there was the absence of the linear and quadratic trend effects of AME incorporations on CH_4 and other gases and CH_4 ($p > 0.05$).

The effect of AM incorporation in maize silage diets on CH_4 in dairy cows is demonstrated in Table 5. The effect of AM incorporation in maize silage and their linear and quadratic trend effects on CH_4 %, CO_2 , and N_2 (% vol) were absent ($p > 0.05$). H_2 had 0% vol content in the rumen headspace. Different AM inclusions affected CH_4 per kg intake of DM, CP, OM, NDF, and ADF ($p < 0.05$). Linear and quadratic trend effects of AM incorporation on CH_4 per kg intake of DM, CP, OM, NDF, and ADF were observed ($p < 0.05$). However, CH_4 per kg nutrient intake of DM, CP, OM, NDF, and ADF were similar at 5%–15% AM incorporations but decreased at the 25% AM incorporations compared to the control.

Discussion

Effect of AME inclusions on methane emissions in dairy cows

AMEs are used to limit CH_4 in livestock, but their use is preliminary due to their inconsistency in CH_4 emissions. The inconsistency of AME inclusions necessitates their investigation to identify inclusions that limit CH_4 . Our results showed that including AME in pellets of up to 3% does not affect CH_4 production or nutrient intake. Our hypothesis was rejected, as it assumed that the incremental inclusions of *A. mearnsii* tannins in pellets decrease CH_4 emissions.

Demonstration of previous results shows that 0%, 0.75%, 1.5%, and 2.25% inclusions of AME do not affect CH_4 emissions [14]. This is consistent with other tannin extract types, such as mimosa and quebracho, that do not affect CH_4 emissions and nutrient intake at inclusion rates of up to 1% [15] or 2% [16] in the diet. This suggests that the inclusions of AME used in the current study were insufficient to control CH_4 emissions. Supplementation of field grass with 0.12% and 0.18% tannin decreased CH_4 emissions and increased DM intake with suppression of CP digestibility [17,18]. Another finding was that tannin extract decreased CH_4 with a decrease in nutrient intake and digestibility [19]. Therefore, AME inclusions used in the present study may have been due to failure to elicit nutrient digestibility suppression as well as their intakes.

In some instances, AME inclusions of up to 20% decrease CH_4 with a decrease in gas production [20]. It is possible that the decrease in CH_4 was not realized due to the failure to suppress gas production, which contains substrates for CH_4 production, CO_2 , and H_2 [1]. However, inclusions of 0%, 0.5%, 1%, and 1.5% AME decreased CH_4 emissions with suppression of rumen microbes [21]. Decrement of methanogenesis relies on the depression of nutrient intake and digestibility. Our study was limited to grouping cows according to their milk emission yield, a factor with a positive relationship with CH_4 production. Therefore, further studies should investigate the effect of AME in cows of different milk yields.

Effect of AM inclusion on methane emissions in dairy cows

Reducing CH_4 emissions in dairy cows using nonconventional feeds such as *Acacia mearnsii* forage (AM) potentiates a solution to controlling climate change. Our study showed that increased inclusions of AM in maize silage decreased CH_4 emissions as a proportion of DM, OM, CP, ADF, and NDF intakes. The decline of CH_4 gas as a proportion of the above-mentioned nutrients correlated with decreased nutrient intake. Therefore, the 25% inclusion of AM in maize silage depresses CH_4 gas as a proportion of nutrient intake, but with nutrient intake dependence.

Table 4. The effect of dietary inclusions of *Acacia mearnsii* tannin extract in pellets on enteric gases in dairy cows (experiment 1).

Items	OPEL	0.75PEL	1.5PEL	3PEL	SEM	Significance		
						GLM	Linear	Quadratic
Nutrient intake (kg/day, DM)								
DMI	3.67	3.63	3.67	3.67	0.02	0.413	0.66	0.33
OMI	3.43	3.4	3.43	3.43	0.02	0.413	0.66	0.33
CPI	0.53	0.53	0.53	0.53	0.003	0.413	0.66	0.33
NDFI	0.60	0.59	0.60	0.60	0.003	0.413	0.66	0.33
ADFI	0.18	0.18	0.18	0.18	0.0008	0.413	0.66	0.33
CH ₄ (% vol) and CH ₄ per nutrient intake (kg/day)								
CH ₄	19.76	19.95	19.83	20.38	0.42	0.7229	0.3574	0.6729
CH ₄ /DMI	0.72	0.72	0.73	0.75	0.03	0.5362	0.2231	0.4458
CH ₄ /OMI	0.68	0.68	0.68	0.70	0.01	0.5670	0.2451	0.4532
CH ₄ /CPI	0.11	0.11	0.11	0.11	0.0027	0.4670	0.2221	0.3588
CH ₄ /NDFI	0.12	0.12	0.12	0.12	0.12	0.7600	0.7352	0.4521
CH ₄ /ADFI	0.04	0.04	0.04	0.04	0.0019	0.8559	0.6990	1.000
Other gases (% vol)								
H ₂	0	0	0	0	–	–	–	–
N ₂	30.73	29.33	30.74	29.23	0.78	0.3529	0.3896	0.9448
CO ₂	47.08	48.95	46.11	48.52	0.98	0.1836	0.7407	0.7858

Significant difference, $p < 0.05$; CP, crude protein; DM, dry matter; GLM, general linear model; NDF, neutral detergent; OM, organic matter; SEM, standard error of means.

Table 5. Effect of different dietary inclusions of *Acacia mearnsii* forage in pellets on enteric gases in dairy cows (experiment 2).

Items	OAM	5AM	15AM	25AM	SEM	Significances		
						GLM	Linear	Quadratic
Nutrient intake (kg/day)								
DMI	4.90 ^a	4.47 ^a	4.36 ^a	3.13 ^b	0.15	<0.0001	<0.0001	0.0078
CPI	0.40 ^a	0.39 ^a	0.41 ^a	0.30 ^b	0.01	<0.0001	<0.0001	0.0006
OMI	4.62 ^a	4.28 ^a	4.11 ^a	2.93 ^b	0.14	<0.0001	<0.0001	0.0032
ADFI	1.65 ^a	1.62 ^a	1.68 ^a	1.22 ^b	0.06	<0.0001	<0.0001	0.0008
NDFI	2.95 ^a	2.80 ^a	2.74 ^a	1.99 ^b	0.09	<0.0001	<0.0001	0.0014
CH ₄ (% vol) and CH ₄ per nutrient intake (kg/day)								
CH ₄	23.07	23.98	23.02	22.07	1.00	0.5423	0.7620	0.4140
CH ₄ /DMI	1.13 ^a	1.07 ^a	1.00 ^a	0.68 ^b	0.06	<0.0001	0.0001	0.0470
CH ₄ /CPI	0.09 ^a	0.09 ^a	0.09 ^a	0.07 ^b	0.01	0.0002	0.0256	0.0200
CH ₄ /OMI	1.07 ^a	1.02 ^a	0.94 ^a	0.64 ^b	0.05	<0.0001	0.0001	0.0358
CH ₄ /ADFI	0.38 ^a	0.39 ^a	0.39 ^a	0.27 ^b	0.02	<0.0001	0.0161	0.0161
CH ₄ /NDFI	0.68 ^a	0.67 ^a	0.63 ^a	0.44 ^b	0.04	<0.0001	0.0009	0.0287
Other enteric gases (% vol)								
H ₂	0	0	0	0	—	—	—	—
N ₂	32.80	32.92	36.37	35.59	1.65	0.2510	0.1501	0.8325
CO ₂	39.81	37.86	36.12	36.35	1.58	0.2986	0.0793	0.4445

Significant difference, $p < 0.05$; ADF, acid detergent fiber; CP, crude protein; DM, dry matter; GLM, general linear model; NDF, neutral detergent fiber; OM, organic matter; SEM, standard error of means.

We tested the hypothesis that the increment of AM in maize silage would decrease CH₄ production. This partially agreed with our hypothesis because CH₄ gas declined with inclusion of AM, but was equally refuted as the enteric CH₄ as a nutrient intake proportion was not affected by AM incorporation in maize silage.

Similarly, previous studies showed that up to 20% AM inclusion decreases CH₄ gas production [22]. Furthermore, AM tannins decrease CH₄ emissions in association with nutrient intake [10]. Conversely, lipid-encapsulated AM decreased CH₄ without negatively affecting nutrient intake [23]. Depression of CH₄ in association with that of nutrient intake attributes a lack of encapsulation of AM in the present study [24]. The lack of tannin encapsulation can cause tannins to limit nutrient intake through their astringent taste. Thus, AM tannins may have depressed substrate availability for CH₄-producing microbes to produce CH₄.

CH₄ production depends on nutrient digestibility. Alternatively, our results for AM demonstrate that AM inclusions decreased nutrient digestibility. AM leaf meal decreases CH₄ gas and DM degradability in the rumen [25]. DM degradability depression by AM lowers protozoa populations, which contribute to CH₄ emissions [25].

Our study was limited by not using the exact AM inclusions and basal diet that were previously tested *in vitro* [26]. This would have quantified how certain inclusions of AM tannins tested *in vitro* affect CH₄ emissions *in vivo*. This limitation necessitates the need for future studies to investigate AM inclusions that have been previously investigated for their effect on digestibility and rumen microbial populations to ascertain the AM inclusions associated with the depression of CH₄ without performance decrease [27].

Conclusion

Feeding dairy cows varying AME inclusions in pellets did not affect CH₄ production. However, feeding dairy cows varying incorporations of AM decreased CH₄ per intake of DM, CP, OM, NDF, and ADF with linear and quadratic trend effects. Different inclusions of AME need to be assessed for cows of different CH₄ emission levels. Furthermore, *in vivo* studies are necessary to confirm these findings using milk yield and previously tested inclusions of AME under *in vitro* conditions.

List of abbreviations

ADF, acid detergent fiber; AM, *Acacia mearnsii* forage; AME, *Acacia mearnsii* tannin extract; CH₄, methane gas; CO₂, carbon dioxide gas; CP, crude protein; CT, condensed tannins; DM, dry matter content; H₂, hydrogen; N₂, nitrogen gas; NDF, neutral detergent fiber; OM, organic matter; PEL, pelleted concentrate; SAS, statistical analysis software.

Acknowledgements

The authors would like to thank assistants from the University of KwaZulu-Natal and from the study site for assistance during data collection. Authors would also like to thank the Interbursary from the Council for Scientific and Industrial Research (no grant number), the Health and Welfare Sector Education and Training Authority post-graduate bursary (no grant number), and the Nestlé YES program for funding (no grant number).

Conflicts of interest

There is no conflict of interest.

Authors' contributions

Study concept and design: LCM and IVN. Acquisition of data: LCM. Analysis and interpretation of data: LCM. Drafting of the manuscript: LCM and IVN. Critical revision of the manuscript for important intellectual content: LCM and IVN. Statistical analysis: LCM. Administrative, technical, and material support: IVN and LCM. Study supervision: IVN.

References

- [1] Mhlongo L, Mseleku C, Tenza T, Nsahlai I. Factors influencing dietary tannin inclusion in dairy diets: a review. *J Appl Vet Sci* 2024; 9(1):73–84; <https://doi.org/10.21608/javs.2023.242317.1282>
- [2] Patra AK, Puchala R. Methane mitigation in ruminants with structural analogues and other chemical compounds targeting archaeal methanogenesis pathways. *Biotechnol Adv* 2023; 69:108268; <https://doi.org/10.1016/j.biotechadv.2023.108268>
- [3] Silva TH, Magnani E, De Andrade JGP, Amâncio BR, Meurer GW, Reolon HG, et al. Evaluation of increasing levels of condensed tannin extracted from *Acacia mearnsii* on performance, carcass traits, meat quality, methane emission, and health of finishing Nellore bulls. *Anim Feed Sci Technol* 2024; 315:116046; <https://doi.org/10.1016/j.anifeedsci.2024.116046>
- [4] Piñeiro-Vázquez A, Canul-Solís J, Alayón-Gamboa J, Chay-Canul A, Ayala-Burgos A, Aguilar-Pérez C, et al. Potential of condensed tannins for the reduction of emissions of enteric methane and their effect on ruminant productivity. *Arch Med Vet* 2015; 47(3):263–72; <https://doi.org/10.4067/S0301>
- [5] Adejoro FA, Hassen A, Akanmu AM, Morgavi DP. Replacing urea with nitrate as a non-protein nitrogen source increases lambs' growth and reduces methane production, whereas *Acacia* tannin has no effect. *Anim Feed Sci Technol* 2020; 259:114360; <https://doi.org/10.1016/j.anifeedsci.2019.114360>
- [6] Grainger C, Clarke T, Auldist MJ, Beauchemin KA, Mcginn SM, Waghorn GC, et al. Potential use of *Acacia mearnsii* condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. *Can J Anim Sci* 2009; 89(2):241–51; <https://doi.org/10.4141/CJAS08110>
- [7] Moate PJ, Jacobs JL, Hixson JL, Deighton MH, Hannah MC, Morris GL, et al. Effects of feeding either red or white grape marc on milk production and methane emissions from early-lactation dairy cows. *Animals* 2020; 10(6):976; <https://doi.org/10.3390/ani10060976>

- [8] Montoya-Flores MD, Molina-Botero IC, Arango J, Romano-Muñoz JL, Solorio-Sánchez FJ, Aguilar-Pérez CF, et al. Effect of dried leaves of *Leucaena leucocephala* on rumen fermentation, rumen microbial population, and enteric methane production in cross-bred heifers. *Animals* 2020; 10(2):300; <https://doi.org/10.3390/ani10020300>
- [9] Akanmu AM, Hassen A, Adejoro FA. Gas production, digestibility and efficacy of stored or fresh plant extracts to reduce methane production on different substrates. *Animals* 2020; 10(1):146; <https://doi.org/10.3390/ani10010146>
- [10] Griffiths WM, Clark CEF, Clark DA, Waghorn GC. Supplementing lactating dairy cows fed high-quality pasture with black wattle (*Acacia mearnsii*) tannin. *Animal* 2013; 7(11):1789–95; <https://doi.org/10.1017/S1751731113001420>
- [11] AOAC. Official methods of analysis. 17th edition, Association of Official Analytical Chemists, Inc., Arlington, VA, 2000.
- [12] ANKOM Technology. Method for Determining Acid Detergent Fiber [Internet]. 2011. Available via <https://www.ankom.com/procedures.aspx> (Accessed 14 July, 2025)
- [13] Society of leather trades chemists (Cambridge). Official Methods of Analysis. 4th ed. Society of Leather Trade Chemists, Northampton, UK. 1965; p.433.
- [14] Tseu RJ, Junior FP, Carvalho RF, Sene GA, Peres AH, Tropaldi CB, et al. Gas Emission from waste of cows fed Monensin and *Acacia mearnsii* tannins. *Iran J Appl Anim Sci* 2021; 11(3):443–56; Available via http://ijas.iaurasht.ac.ir/article_684567.html
- [15] Ebert PJ, Bailey EA, Shreck AL, Jennings JS, Cole NA. Effect of condensed tannin extract supplementation on growth performance, nitrogen balance, gas emissions, and energetic losses of beef steers. *J Anim Sci* 2017; 95(3):1345–55; <https://doi.org/10.2527/jas.2016.0341>
- [16] Beauchemin KA, Mcginn SM, Martinez TF, Mcallister TA. Use of condensed tannin extract from Quebracho trees to reduce methane emissions from cattle. *J Anim Sci* 2007; 85(8):1990–6; <https://doi.org/10.2527/jas.2006-686>
- [17] Ramaiyulis R, Metri Y, Irda I, Kurnia D, Syukriani D. Effects of tannin-containing supplement on enteric methane emissions, total digestible nutrient, and average daily gain of local Indonesian beef cattle. *World Vet J* 2022; 12(4):358–62; <https://doi.org/10.54203/scil.2022.wvj45>
- [18] Brenda VD, Zain M, Agustin F. Strategy to reduce methane to increase feed efficiency in ruminants through adding essential oils as feed additives. *Int J Vet Sci* 2024; 13(2):195–201; <https://doi.org/10.47278/journal.ijvs/2023.087>
- [19] Carulla JE, Kreuzer M, Machmüller A, Hess HD. Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. *Aust J Agric Res* 2005; 56(9):961–70; <https://doi.org/10.1071/AR05022>
- [20] Hassanat F, Benchaar C. Assessment of the effect of condensed (*Acacia* and *Quebracho*) and hydrolysable (*Chestnut* and *Valonea*) tannins on rumen fermentation and methane production *in vitro*. *J Sci Food Agric* 2013; 93(2):332–9; <https://doi.org/10.1002/jsfa.5763>
- [21] Perna Junior F, Nogueira RGN, Carvalho RF, Cassiano ECO, Rodrigues PHM. Use of tannin extract as a strategy to reduce methane in Nellore and Holstein cattle and its effect on intake, digestibility, microbial efficiency and ruminal fermentation. *J Anim Physiol Anim Nutr* 2022; 107(1):89–102; <https://doi.org/10.1111/jpn.13702>
- [22] Vargas-Ortiz L, Andrade-Yucailla V, Barros-Rodríguez M, Lima-Orozco R, Macías-Rodríguez E, Contreras-Barros K, et al. Influence of *Acacia Mearnsii* fodder on rumen digestion and mitigation of greenhouse gas production. *Animals* 2022; 12(17):2250; <https://doi.org/10.3390/ani12172250>
- [23] Adejoro FA, Hassen A, Akanmu AM. Effect of lipid-encapsulated *Acacia* tannin extract on feed intake, nutrient digestibility and methane emission in sheep. *Animals* 2019; 9(11):863; <https://doi.org/10.3390/ani9110863>
- [24] Fitri A, Yanza YR, Jayanegara A, Ridwan R, Astuti WD, Sarwono KA, et al. Divergence effects between dietary *Acacia* and *Quebracho* tannin extracts on nutrient utilization, performance, and methane emission of ruminants: a meta-analysis. *Anim Sci J* 2022; 93(1):e13765; <https://doi.org/10.1111/asj.13765>
- [25] Acosta-Lozano N, Barros-Rodríguez M, Guishca-Cunhahay C, Andrade-Yucailla V, Contreras-Barros K, Sandoval-Castro C, et al. Potential effect of dietary supplementation of tannin-rich forage on mitigation of greenhouse gas production, defaunation and rumen function. *Vet Sci* 2023; 10(7):467; <https://doi.org/10.3390/vetsci10070467>
- [26] Vargas-Ortiz L, Chavez-Garcia D, Barros-Rodríguez M, Andrade-Yucailla V, Lima-Orozco R, Macías-Rodríguez E, et al. Rumen function and *in vitro* gas production of diets influenced by two levels of tannin-rich forage. *Fermentation* 2022; 8(11):607; <https://doi.org/10.3390/fermentation8110607>
- [27] Mhlongo LC, Kenyon P, Nsahlai IV. Effect of dietary inclusions of different types of *Acacia mearnsii* on milk performance and nutrient intake of dairy cows. *Vet Anim Sci* 2023; 21:100299; <https://doi.org/10.1016/j.vas.2023.100299>