

Detoxifying signal grass: Ensiling *Brachiaria decumbens* improves rumen fermentation and growth performance in sheep

Nurul Adilah Abu Bakar¹, Eric Lim Teik Chung^{1,2*}, Nurul Aishah Ahmad Fauzi¹, Nazri Nayan^{1,2}, Mohd Farhan Hanif Reduan³, Awis Qurni Sazili^{2,4}, Faez Firdaus Abdullah Jesse⁵, and Ruangyote Pilajun⁶

¹Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

²Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

³Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, Pengkalen Chepa 16100, Kota Bharu, Kelantan, Malaysia

⁴Halal Products Research Institute, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

⁵Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

⁶Faculty of Agriculture, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand

ARTICLE HISTORY

Received : 5 January 2026

Accepted : 24 February 2026

Online : 30 September 2026

KEYWORDS

body weight gain,
Dorper cross sheep,
ensiled *B. decumbens*,
rumen fermentation,
rumen gas production,
steroidal saponins

✉ * CORRESPONDING AUTHOR

Dr. Eric Lim Teik Chung
Institute of Tropical Agriculture and
Food Security, Universiti Putra
Malaysia, 43400 UPM Serdang,
Selangor, Malaysia
Email: ericlim@upm.edu.my

ABSTRACT

Fresh *Brachiaria decumbens* contains high concentrations of steroidal saponins, which impair rumen degradability and depress growth performance in sheep. This study evaluated whether ensiling *B. decumbens* improves rumen fermentation and animal performance. The work combined *in vitro* rumen fermentation analysis with an *in vivo* growth trial lasting 98 days. Fresh *Pennisetum purpureum* was used as a control forage to reflect typical smallholder feeding practice, while *B. decumbens* was offered either fresh or ensiled to assess the effect of saponin reduction. Eighteen healthy six-month-old male Dorper cross sheep were dewormed, acclimatized, and randomly allocated to three dietary treatments, control (*P. purpureum*), fresh *B. decumbens*, or ensiled *B. decumbens*, with six animals per group. Forages were harvested at seven weeks of growth and offered twice daily at 3% of body weight on a dry matter basis. Rumen fluid collected after acclimatization was used to determine gas production, ruminal pH, *in vitro* organic matter digestibility, ammonia nitrogen, and volatile fatty acid profiles. Feed intake was recorded daily, and body weight was measured weekly. Data were analyzed using one-way ANOVA. Fresh *B. decumbens* resulted in significantly lower cumulative gas production over 72 hours. Although fermentation kinetics, ruminal pH, and digestibility did not differ among treatments, fresh *B. decumbens* markedly reduced ammonia nitrogen, total volatile fatty acids, acetate, and propionate concentrations. In contrast, ensiled *B. decumbens* produced higher fermentation end-products than the control. Sheep fed ensiled *B. decumbens* achieved the highest final body weight, weight gain, average daily gain, and feed efficiency. Overall, ensiling effectively mitigated the negative effects of saponins, enhanced rumen fermentation, and improved growth performance, supporting the use of ensiled *B. decumbens* as a viable basal forage for sheep.

© 2026 UMK Publisher. All rights reserved.

1. INTRODUCTION

More than 80% of the improved tropical pastures currently available are comprised of *Brachiaria decumbens* (Muniandy et al., 2021a). Commonly known as signal grass, *B. decumbens* is widely recognized for its value as a forage crop, owing to both its nutritional quality and ecological adaptability. This tropical species is noted for its vigorous growth, high dry matter yield, and resilience across diverse environmental conditions, making it well-suited to various grazing systems. Research has shown that *B. decumbens* has a relatively high crude protein content, which is essential for meeting the nutritional requirements of livestock. Specifically, its average crude protein concentration can reach up to 107.1

g/kg, surpassing that of several other grass species grown under similar conditions (Lima et al., 2013). This elevated protein level contributes to improved growth rates and overall performance in ruminants when *B. decumbens* is used as a primary forage source (Jaapar et al., 2022). In addition to its nutritional value, the grass demonstrates substantial yield potential under various cultivation practices. It performs particularly well in mixed cropping systems with legumes, where it has been shown to exhibit enhanced nutrient composition and forage quality.

Despite these benefits, *B. decumbens* poses certain risks due to the presence of steroidal saponins, particularly protodioscin. These compounds have been shown to exert toxic effects on rumen microbes, potentially impairing rumen

fermentation processes and reducing overall growth performance in ruminants (Lima et al., 2013; Jaapar et al., 2023). Saponins poisoning from *B. decumbens* has been associated with marked declines in ruminant growth performance, manifesting as weight loss, anorexia, and reduced feed intake. These clinical signs suggest that saponins may disrupt nutrient digestion and absorption, primarily by impairing the function of gastrointestinal microbes, particularly those in the rumen. Numerous studies have investigated the effects of saponins on ruminal fermentation and digestibility. Notably, saponins have been found to inhibit key fibrolytic bacteria such as *Fibrobacter* and *Ruminococcus* species, which are essential for the breakdown of plant cell walls. Research has shown that saponins can reduce both the activity and population of these cellulolytic microbes, thereby limiting the rumen's ability to efficiently degrade fibrous feedstuffs (Muniandy et al., 2021b). This decline in cellulolytic activity ultimately lowers forage digestibility, reducing nutrient availability and negatively impacting animal productivity.

Chung et al. (2018) highlighted various strategies developed to mitigate the negative effects of anti-nutritional compounds, such as saponins. Lima et al. (2015) found that protodioscin levels in *B. decumbens* silage decreased by 94% within the first four days and continued to decline over the next 20 days. Further supporting evidence from Pinos-Rodríguez et al. (2008) showed that saponin degradation in lucerne silage and *Agave salmiana* reached between 55% and 90% over 33 days. During ensiling, aerobic respiration depletes carbohydrates, followed by anaerobic fermentation. According to Lima et al. (2015), the decline in protodioscin to sugar loss during hay wilting and early fermentation is driven by plant respiration and microbial growth. Since steroidal saponins consist of sugar moieties linked to sapogenins, enzymatic activity during fermentation may break these glycosidic bonds, leading to saponins degradation (Das et al., 2012).

Although *B. decumbens* is a high-quality tropical forage, its steroidal saponins can impair rumen function and animal performance, and animal evidence on whether ensiling reduces these effects is still lacking. Prior work on *B. decumbens* has mainly focused on toxicity or *in vitro* observations, including reports that ensiling reduces protodioscin, but it has not shown in animals how detoxification links to fermentation changes and growth outcomes. Therefore, this study was designed to investigate both the *in vitro* and *in vivo* effects of feeding ensiled *B. decumbens* to male Dorper cross sheep over a 98-day feeding period. Specifically, it aimed to evaluate its impact on rumen gas production, fermentation parameters, and overall growth performance. This study addresses that gap by comparing

fresh and ensiled *B. decumbens* against a control forage and directly connecting saponin reduction, fermentation responses, and a 98-day sheep growth trial. This study tested the hypothesis that ensiling *B. decumbens* reduces steroidal saponins and improves rumen fermentation kinetics, leading to higher feed intake and growth performance. Outcomes were expected to be comparable to, or exceed, fresh *Pennisetum purpureum*.

2. MATERIALS AND METHODS

2.1. Forage planting and harvesting

This study involved two forage species specifically *P. purpureum* and *B. decumbens*. Both pastures were cultivated prior to the animal trial at Farm 15, Research Farm, Department of Animal Science, Universiti Putra Malaysia, located at 2°59'15.1"N, 101°44'08.3"E, with an altitude of 52 meters above sea level. The *P. purpureum* and fresh *B. decumbens* were harvested at seven weeks of growth prior to ensiling process and maintained at this growth stage throughout the feeding trial. Based on previous findings (Fauzi et al., 2025), week 7 was selected as the optimal harvesting time to balance biomass yield and nutrient content for both grass species. To ensure consistent regrowth and maintain forage quality during the study, a single application of 15:15:15 NPK green fertilizer was applied. Quantitative saponin analyses were conducted weekly (from Week 1 to Week 10) for both fresh *P. purpureum* and *B. decumbens* using the spectrophotometric method developed by Le Bot et al. (2022), as illustrated in Figure 1.

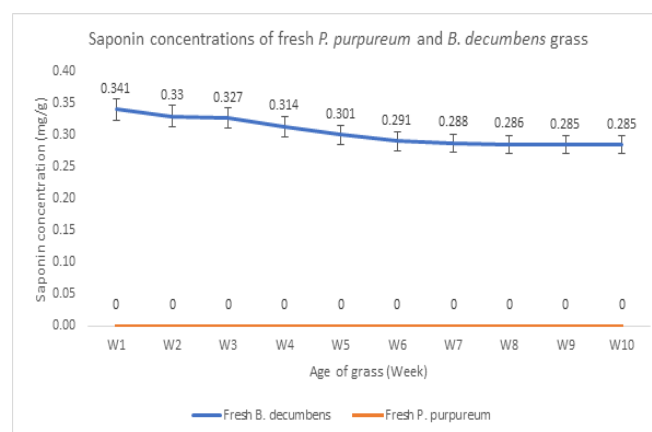


Figure 1: Weekly variation in saponin concentration (mg/g) in fresh *P. purpureum* and *B. decumbens* from Week 1 to Week 10.

2.2. Ensiling process

Fresh *B. decumbens* grass was harvested at seven weeks of age using a forage harvester and allowed to wilt for four hours. Following wilting, the grass was chopped into lengths of approximately 3-4 inches using an electric chopper. The chopped forage was then packed into high-density

polyethylene (HDPE) plastic bags, with molasses added at 1% of the total weight to facilitate fermentation. The silage bags were compacted, vacuum-sealed, and left to ferment for a period of four weeks prior to being used in the feeding trial. During the ensiling period, samples were collected at weekly intervals for ten weeks to conduct quantitative saponin analysis. Notably, no detectable levels of saponin were observed after four weeks of fermentation (Figure 2), indicating that the ensiling process effectively eliminated saponin content in *B. decumbens*. As a result, the silage was considered safe and nutritionally suitable and was subsequently used as the primary feed in the 98-day sheep feeding trial, following conventional silage preparation protocols.

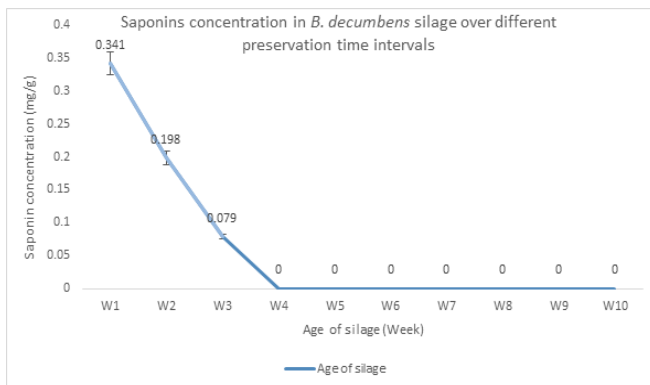


Figure 2: Changes in saponin concentration (mg/g) in *B. decumbens* during a 10-week ensiling period.

2.3. Animal model

All experimental procedures were conducted in accordance with ethical standards and received prior approval from the Institutional Animal Care and Use Committee of Universiti Putra Malaysia (UPM/IACUC/AUP-R003/2023).

A total of 18 non-castrated male Dorper cross sheep, aged six months and sourced from the same farm, were used in this experiment. Upon arrival, each sheep's initial body weight was individually recorded, with an average weight of approximately 20 kg. The sheep were ranked by initial body weight and then randomly assigned to the three treatments using a stratified randomization method to ensure comparable average weights across groups at the start of the experiment. Each sheep was housed individually in a metabolic cage (1.3 × 0.7 × 2.2 meters) located in a shaded, well-ventilated facility. Animals had free access to clean drinking water and mineral salts throughout the trial. Before the feeding trial, all sheep underwent a two-week acclimatization period during which they received *P. purpureum* twice daily and were orally administered an antiparasitic treatment (Iverin 1%; Range Pharma Pvt. Ltd., Malaysia). Feeding commenced after the acclimatization phase, following a dietary regimen consisting of a 70:30 roughage-to-concentrate ratio. Feed was provided

at 3% of each animal's body weight on a dry matter basis to ensure adequate intake. Feeding was conducted twice daily, at 0900 and 1700 hours, in accordance with procedures described by Muniandy et al. (2021a).

2.4. Experimental design

At the end of the acclimatization period, rumen fluid was collected from each sheep to analyze gas production, pH, *in vitro* organic matter digestibility (IVOMD), ammonia nitrogen (NH₂-N), and volatile fatty acids (VFA). During the 98-day feeding trial, all 18 sheep were randomly assigned to one of three dietary treatments namely (T1) *P. purpureum* (control), (T2) fresh *B. decumbens*, and (T3) ensiled *B. decumbens*, with six sheep per treatment group. Each animal was housed in an individual metabolic cage to allow for accurate monitoring. Both *P. purpureum* and *B. decumbens* used in the study were harvested at seven weeks of growth, with *B. decumbens* utilized either fresh or after undergoing the ensiling process. Table 1 presents the nutritional composition (on a DM basis) of fresh *P. purpureum*, fresh *B. decumbens*, and ensiled *B. decumbens*. Daily feed intake and refusals were recorded to monitor consumption, while body weight measurements were taken weekly to evaluate growth performance over the course of the trial.

Table 1: Nutritional composition (on a DM basis) of fresh *P. purpureum*, fresh *B. decumbens*, and ensiled *B. decumbens*.

Composition	T1	T2	T3	p-value
Dry matter (%)	25.11 ± 0.53	24.61 ± 0.23	24.87 ± 0.30	0.411
Organic matter (%)	90.22 ± 1.89	89.78 ± 1.51	90.71 ± 1.19	0.567
Crude protein (%)	16.23 ± 0.33	15.71 ± 0.38	16.36 ± 0.81	0.078
Ether extract (%)	2.31 ± 0.11	1.95 ± 0.33	2.11 ± 0.45	0.892
Crude fiber (%)	30.65 ± 0.16	28.22 ± 0.26	27.15 ± 0.11	0.132
Neutral detergent fiber (%)	62.78 ± 0.88	60.91 ± 0.27	60.10 ± 0.41	0.257
Acid detergent fiber (%)	41.11 ± 0.25	38.91 ± 0.34	37.83 ± 0.76	0.121
Acid detergent lignin (%)	5.67 ± 0.22	4.87 ± 0.12	4.88 ± 0.31	0.069
Gross energy (MJ/kg DM)	17.87 ± 0.31	17.21 ± 0.20	17.88 ± 0.27	0.089

Note: All values were expressed as mean ± SE. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens*.

It is important to note that *P. purpureum* was provided in its fresh form, reflecting common practice among smallholder farmers in the study region, whereas *B. decumbens* was ensiled to reduce its saponin content and improve palatability. This design was chosen to allow a practical comparison between the typical feeding of fresh *P. purpureum* and ensiled *B. decumbens*, thereby reflecting real-world on-farm conditions.

2.5. Rumen fluid collection

A soft stomach tube was carefully inserted through a PVC guide placed over the sheep's tongue to prevent biting or accidental ingestion of the tube during rumen fluid collection. Approximately 200 mL of rumen fluid was extracted via stomach tubing and immediately transferred into a pre-warmed vacuum thermal flask to maintain anaerobic conditions. To preserve the rumen's natural environment, the flask was kept sealed and insulated throughout the process. In the laboratory, the collected rumen fluid was filtered through four layers of cheesecloth into a conical flask submerged in a 39 °C water bath to maintain physiological temperature. To ensure an anaerobic environment during subsequent analyses, the filtrate was mixed with phosphate-bicarbonate buffer at a 1:2 ratio while continuously flushed with carbon dioxide (CO₂). This preparation preserved microbial activity and ensured stable conditions for *in vitro* fermentation assays.

2.6. *In vitro* gas production

In vitro gas production was conducted following the procedure described by Menke & Steingass (1988). Approximately 0.200 ± 0.001 g of dry matter (DM) from samples ground to pass through a 1.0 mm sieve was accurately weighed and placed into 100 mL calibrated glass syringes fitted with pistons and secured with rubber clips at the tips. Standard samples, consisting of roughly 0.200 ± 0.001 g DM of standard grass hay, were included alongside blank syringes containing only buffered rumen fluid to serve as calibration controls and to correct for baseline gas production during substrate fermentation. The experiment was designed as a randomized complete block design (RCBD) and conducted over two separate runs on different days. Gas production was recorded at intervals of 2, 4, 8, 12, 24, 48, and 72 hours throughout the 72-hour incubation period. Cumulative gas production data were analyzed using the NEWAY computer program, which fits the data to the Ørskov and McDonald (1979) model to estimate fermentation kinetics:

$$y = a + b(1 - e^{-ct})$$

Where:

a = the amount of gas produced from the immediately soluble fraction (mL);

b = the amount of gas produced from the insoluble fraction (mL);

c = the constant of the gas production rate for the insoluble fraction (mL/h);

a+b = the amount of potential gas production (mL);

t = the incubation time (h); and

y = the amount of gas produced at time "t".

2.7. Rumen fermentation characteristics

Following the incubation period, the same rumen

fluid was collected for analysis of pH, volatile fatty acids (VFA), and ammonia nitrogen (NH₃-N). The pH of the rumen fluid was measured immediately after 48 hours of incubation using a Mettler-Toledo pH meter to ensure accuracy and prevent changes due to exposure or temperature fluctuations.

Rumen fluid samples were analyzed following the procedure described by Filipek & Dvorak (2009). Initially, 25% metaphosphoric acid (w/v) was added to the rumen fluid in a 4:1 (v/v) ratio to precipitate proteins and stabilize volatile fatty acids (VFA). The mixture was then centrifuged at 3000 × g for 10 minutes. After centrifugation, the clear supernatant was filtered, and 0.5 mL was transferred to a clean vial. An internal standard, 4-methyl-N-valeric acid (Sigma, St. Louis, MO), was added to each sample to aid in quantification. For peak identification, an external standard solution was prepared containing 20 mM acetic acid and 10 mM of propionic acid, butyric acid, and 4-methyl-valeric acid, all sourced from Sigma-Aldrich (St. Louis, MO). VFA separation was performed using a Quadrex 007 Series bonded phase fused silica capillary column (15 m length, 0.250 mm internal diameter, 0.25 µm film thickness) installed in a 6890 N Network GC System gas chromatograph (Agilent Technologies) equipped with a flame ionization detector (FID). The column oven temperature was set at 200°C, while both the injector and detector were maintained at 230°C. Nitrogen was used as the carrier gas at a flow rate of 60 mL/min. VFA concentrations were identified and quantified based on comparison with internal and external standards, using a single-point calibration approach.

The determination of ammonia nitrogen (NH₃-N) concentration was conducted following the method described by Parsons et al. (2013). Five reagents such as phenol solution, sodium nitroprusside solution, alkaline reagent solution, sodium hypochlorite solution, and an oxidizing solution were prepared for the analysis. The alkaline reagent was composed of 0.25 M sodium hydroxide, 0.775 M sodium citrate, and one part of 5.25% (v/v) sodium hypochlorite. These reagents facilitated the colorimetric reaction necessary for quantifying NH₃-N in the rumen fluid samples.

After incubation, the full contents of each glass syringe, including those from the treatment and blank groups, were transferred into pre-weighed sintered glass crucibles. Any residual rumen fluid was thoroughly rinsed out using distilled water. The crucibles were then placed in a drying oven at 105°C until a constant weight was achieved. The *in vitro* dry matter digestibility (IVDMD) was determined by calculating the difference between the sample weight before and after incubation. Organic matter digestibility (OMD) of the substrate was subsequently calculated using the equation proposed by Menke et al. (1979):

$$\text{OMD (\%)} = 14.88 + 0.889\text{GP} + 0.45\text{CP} + 0.0651\text{AC}$$

Where:

GP = net gas produced at 24 h (mL/200 mg) of incubation

CP = percentage of crude protein

AC = percentage of ash of the substrate.

2.8. Growth performance

Each sheep was individually weighed using a digital scale on Day 0, with subsequent weekly weight measurements taken prior to the morning feeding. Daily feed refusals and the dry matter (DM) content of the offered diet were recorded for each animal to calculate daily dry matter intake (DMI). To determine average daily gain (ADG), body weight gain (BWG) was tracked weekly throughout the duration of the trial until the experiment's conclusion. Besides, the feed conversion ratio (FCR) was determined based on the DMI and BWG.

2.9. Statistical analysis

All data were analyzed using R Software. One-way ANOVA was employed to determine differences among treatment groups, followed by Tukey's Honest Significant Difference (HSD) post-hoc test to compare group means where significant differences were detected. *In vitro* data from the two-run design included run as a block term. Gas production time-series inference relied on the fitted kinetics rather than separate uncorrected tests at each hour. Weekly body weight data were evaluated with a repeated-measures framework using animal as the subject factor and week as the repeated factor, with diet, week, and diet × week effects reported. Exact p-values are reported, and effect sizes with 95% confidence intervals are provided for primary outcomes.

3. RESULT AND DISCUSSION

3.1 *In vitro* gas production

Table 2 presents the *in vitro* gas production profiles of fresh *P. purpureum* (T1), fresh *B. decumbens* (T2), and ensiled *B. decumbens* (T3) over a 72-hour incubation period. All treatments followed a typical logarithmic-linear gas production pattern, with significant differences ($p < 0.001$) observed among treatments beginning at the 4th hour of incubation (Figure 3).

Initially, T3 exhibited lower gas production, similar to T2, up to the 12th hour when compared to the control group (T1), which consistently produced the highest gas volumes. Specifically, T3 recorded 22.61%, 18.47%, and 8.79% less gas at 4, 8, and 12 hours, respectively, compared to T1. However, from the 24th hour onward, gas production in T3 increased significantly and became statistically comparable ($p > 0.05$) to that of the control group, suggesting improved fermentation over time. In contrast, T2 consistently produced

the lowest gas volumes across all incubation intervals, with significant reductions of 25.16%, 37.77%, 12.00%, 10.71%, 9.00%, and 10.81% at 4, 8, 12, 24, 48, and 72 hours, respectively, relative to T1 ($p < 0.001$).

Table 2: *In vitro* gas production of fresh *P. purpureum*, fresh and ensiled *B. decumbens* throughout 72 hours of incubation.

Parameter	T1	T2	T3	p-value
<u>Incubation time (h)</u>				
2	10.67 ± 0.35	10.33 ± 0.22	10.50 ± 0.28	0.780
4	15.08 ± 0.44 ^a	11.17 ± 0.35 ^b	11.67 ± 0.30 ^b	<0.001
8	21.50 ± 0.20 ^a	14.67 ± 0.18 ^c	17.53 ± 0.29 ^b	<0.001
12	26.50 ± 0.31 ^a	23.50 ± 0.28 ^b	24.17 ± 0.20 ^b	<0.001
24	29.50 ± 0.17 ^a	26.50 ± 0.22 ^c	28.00 ± 0.31 ^a	<0.001
48	31.00 ± 0.45 ^a	28.33 ± 0.37 ^b	30.67 ± 0.28 ^a	<0.001
72	31.50 ± 0.20 ^a	28.27 ± 0.25 ^b	31.21 ± 0.30 ^a	<0.001
<u>Gas production kinetics</u>				
a (mL)	12.15 ± 2.87	11.21 ± 1.99	11.89 ± 2.10	0.056
b (mL)	21.71 ± 8.21	20.63 ± 5.52	23.80 ± 6.75	0.169
c (mL/h)	0.72 ± 0.59	0.65 ± 0.38	0.67 ± 0.22	0.098
a+b	33.86 ± 13.32	31.84 ± 11.76	35.69 ± 10.69	0.078
IVOMD (%)	60.34 ± 0.20	57.55 ± 0.28	58.75 ± 0.31	0.063

Note: All values were expressed as mean ± SE; a, b, c values with superscript within row are significantly different at $p < 0.05$. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens*. a: volume of gas produced from immediate soluble fraction, b: volume of gas produced from insoluble fraction, c: gas production rate constant from insoluble fraction, a+b: potential extent of gas production, IVOMD: *in vitro* organic matter digestibility.

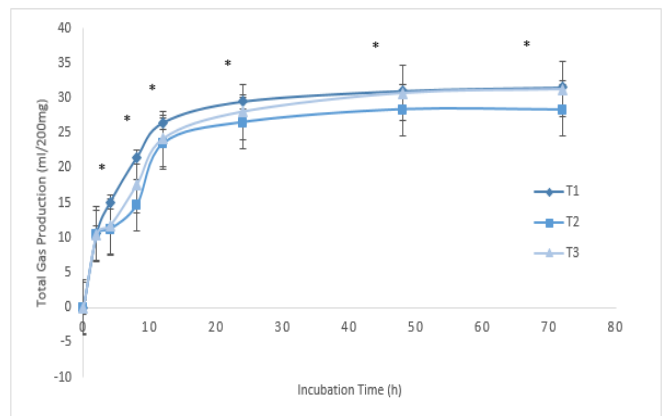


Figure 3: Cumulative gas production kinetics of fresh *P. purpureum*, fresh *B. decumbens*, and ensiled *B. decumbens* throughout 72 hours of incubation. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens*. (*) displayed among treatments indicates significant difference at $p < 0.05$. Asterisk (*) indicates statistical significance between treatments.

No significant differences ($p > 0.05$) were found among treatments in terms of gas fermentation kinetics or *in vitro* organic matter digestibility (IVOMD). Nonetheless, T2 showed the lowest numerical values for fermentation kinetics parameters (a, b, c, a + b) and IVOMD throughout the

incubation period. These findings suggest that the ensiling process improves the fermentability of *B. decumbens*, making T3 comparable to *P. purpureum* (T1) in terms of rumen gas production, particularly after the initial stages of fermentation.

Monitoring total gas production helps estimate ruminal degradability because gas output reflects the rate and extent of microbial fermentation during anaerobic carbohydrate breakdown, producing mainly CH₄ and also CO₂, H₂, and N₂ (Ungerfeld, 2020). Plant secondary metabolites often shift gas production in a dose-dependent way, with lower doses supporting fermentation and higher doses suppressing methanogenesis and total gas, and the direction of response also depends on plant source and the basal diet (Rira et al., 2015; Widiawati & Puastuti, 2016; Ángeles-Mayorga et al., 2022).

In this study, ensiled *B. decumbens* (T3) produced higher total gas than fresh *B. decumbens* (T2), which indicates more active fermentation and better substrate use by rumen microbes (Palangi & Lackner, 2022). Higher fermentation efficiency improves energy supply for animal function and output (Mizrahi et al., 2021). The T3 response aligns with known effects of ensiling, where lactic acid bacteria convert water-soluble carbohydrates (WSC) into organic acids, which can improve forage quality by loosening fiber structure and reducing anti-nutritional factors, including saponins (Astuti et al., 2000). Fiber composition also supports this pattern. Lower NDF often permits faster microbial access and fermentation, while high-NDF feeds show different fermentation dynamics (Maccarana et al., 2016). Reduced NDF can ease substrate limits for cellulolytic bacteria and improve degradation efficiency, consistent with the lower NDF reported for T3 (Cabral et al., 2020). Molasses addition during ensiling also supplies fermentable carbohydrate and supports acidification, which together favor fermentation.

T2 showed lower gas despite using the same forage species, pointing to an inhibitory factor in the fresh material. Steroidal saponins can depress fermentation by disrupting protozoal and bacterial cell membranes through sterol binding, increasing permeability and causing cell damage (Zaynab et al., 2021). Prior work reports similar saponin-linked suppression of rumen microbes and gas production (Wang et al., 2012; Jayanegara et al., 2014; Guyader et al., 2017; Widyarini et al., 2021; Jaapar et al., 2022; Qu et al., 2023; Yanza et al., 2024). This mechanism matches the consistently lower gas observed for T2 across the 72-hour incubation.

Overall, ensiled *B. decumbens* (T3) increased gas production relative to fresh *B. decumbens* (T2), supporting improved digestibility and fermentability. The contrast suggests that ensiling reduced the negative fermentation impact linked to saponins in the fresh forage, while

maintaining performance close to the *P. purpureum* control (T1). These results support using gas production as a practical indicator of fermentation efficiency and feed suitability in ruminant diets.

3.2 Rumen fermentation characteristics

Table 3 illustrates the effects of feeding *P. purpureum* (T1), fresh *B. decumbens* (T2), and ensiled *B. decumbens* (T3) on rumen fermentation characteristics following a 72-hour *in vitro* incubation.

Table 3: Rumen fermentation characteristics effects of feeding fresh *P. purpureum*, fresh *B. decumbens*, and ensiled *B. decumbens* following 72 hours of incubation.

Parameter	T1	T2	T3	p-value
pH	6.27 ± 0.23	6.28 ± 0.29	6.25 ± 0.19	0.856
NH ₃ -N (nm)	0.487 ± 0.14 ^a	0.395 ± 0.11 ^b	0.476 ± 0.15 ^a	<0.001
Total VFA (mM)	136.04 ± 8.23 ^a	121.08 ± 6.31 ^b	138.14 ± 7.21 ^a	<0.001
Acetic acid (A)	92.52 ± 5.15 ^a	79.92 ± 4.20 ^b	93.47 ± 5.25 ^a	<0.001
Propionic acid (P)	30.43 ± 4.17 ^a	25.11 ± 3.23 ^b	30.49 ± 3.21 ^a	<0.001
Iso-butyric acid	1.34 ± 0.11	1.31 ± 0.31	1.32 ± 0.22	0.149
Butyric acid	8.71 ± 0.22	8.72 ± 0.12	8.68 ± 0.11	0.875
Valeric acid	1.98 ± 0.14	1.93 ± 0.11	2.01 ± 0.14	0.381
Iso-valeric acid	1.01 ± 0.13	1.12 ± 0.14	1.07 ± 0.21	0.559
A:P	3.04 ± 0.22	3.09 ± 0.21	3.07 ± 0.31	0.125

Note: All values were expressed as mean ± SE; ^{a, b} values with superscript within row are significantly different at p<0.05. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens*. NH₃-N: Ammonia nitrogen, VFA: Volatile fatty acids, A:P: Acetic to propionic ratio.

No significant differences ($p > 0.05$) were observed in ruminal pH across the three dietary treatments. However, ammonia nitrogen (NH₃-N) concentration in T2 was significantly lower than in T1 and T3, with reductions of 3.90% and 1.67%, respectively. While individual volatile fatty acids (VFAs) showed no significant variation among treatments, total VFA, acetic acid, and propionic acid concentrations differed significantly ($p < 0.001$). Specifically, total VFA in T2 was reduced by 11.00% and 11.71% compared to T1 and T3, respectively. Conversely, T3 exhibited slightly higher concentrations of total VFA, acetic acid, and propionic acid relative to T1, with increases of 0.80%, 1.02%, and 0.20%, respectively. Despite these differences, the acetic to propionic acid (A:P) ratio remained statistically unchanged ($p > 0.05$) across all treatments.

Overall, rumen fermentation parameters were affected by the type of forage provided. The significant reductions in NH₃-N, total VFA, acetic acid, and propionic acid in sheep fed fresh *B. decumbens* (T2) suggests impaired

rumen fermentation, likely due to the presence of saponins. In contrast, the improved fermentation profile observed in T3 indicates that ensiling *B. decumbens* effectively mitigates these negative effects, making it a safer and nutritionally comparable alternative to the control forage, *P. purpureum*.

Rumen fermentation measures help judge whether a diet supports healthy digestion and efficient feed use in sheep and other ruminants. Common markers include ruminal pH, ammonia nitrogen (NH₃-N), total volatile fatty acids (VFA), and the main VFAs, acetic, propionic, and butyric acids (Qiu et al., 2021; Wang et al., 2023). Plant secondary metabolites do not always harm fermentation, yet they can shift energy supply to the animal, so diet formulation should consider both compound type and dose (Orzuna-Orzuna et al., 2021; Menci et al., 2021; Besharati et al., 2022).

Ensiled *B. decumbens* (T3) maintained ruminal pH within the normal range for sheep (6.4 to 6.8) (Jasmin et al., 2011). NH₃-N did not differ between T3 and the control (T1), which supports normal rumen nitrogen turnover under a *B. decumbens* silage basal diet. T3 also increased total VFA, acetic acid, and propionic acid versus fresh *B. decumbens* (T2), even though both diets used the same forage species, which points to higher fermentability after ensiling. Ensiling can increase readily fermentable substrates by converting complex carbohydrates into water-soluble carbohydrates (WSC), including sugars such as glucose, galactose, sucrose, cellobiose, and maltose, with profiles shaped by ensiling conditions and additives (Zhang et al., 2021). Wilting before ensiling also raises WSC, with higher values reported after 2 to 4 hours of wilting (Zhang et al., 2021). Higher sugar availability supports microbial activity and VFA yield. This matters because acetic acid forms a large share of total VFA and supplies a major fraction of VFA-derived energy (Lopes & Ahring, 2023), while propionic acid drives gluconeogenesis and offers higher metabolic efficiency than acetate or butyrate (Chen et al., 2020). Molasses addition during ensiling also increases fermentable carbohydrate supply and supports higher dry matter and non-fiber carbohydrate, which aligns with faster fermentation and higher VFA output (Hristov & Ropp, 2003; Melo et al., 2016). Taken together, improved sugar supply after ensiling, alongside removal of steroidal saponins in T3, fits the higher VFA profile observed.

By contrast, fresh *B. decumbens* (T2) reduced NH₃-N, which signals weaker nitrogen availability for microbial protein synthesis. Higher NH₃-N supports microbial protein formation, while low NH₃-N can constrain microbial growth and reduce conversion of non-protein nitrogen into microbial protein (Dewhurst & Newbold, 2021). Steroidal saponins offer a plausible mechanism because they inhibit protein synthesis by binding ribosomes and disrupting translation (Patra &

Saxena, 2009), and prior studies report lower NH₃-N with high saponin exposure (Śliwiński et al., 2004; Singh et al., 2017). T2 also showed lower total VFA, acetic acid, and propionic acid than T1 and T3, which indicates suppressed fermentation. Saponin-driven microbial lysis and shifts in acetate-to-propionate balance have been reported under similar conditions (Das et al., 2012). Lower acetate and propionate also fit reduced activity of key acetate and propionate producers, including *Prevotella brevis*, *Prevotella ruminicola*, *Acetivomaculum ruminis*, *Eubacterium limosum*, *Ruminococcus productus*, and *Propionibacterium acidipropionici* (Wang et al., 2012; Zhao et al., 2015; Zou et al., 2019; Zhang et al., 2021). Protozoa such as *Isotricha*, *Dasytricha*, *Diplodinium*, and *Entodinium* also contribute to acetate formation, and saponins disrupt protozoa by forming complexes with membrane cholesterol that lead to membrane damage and cell death (Wina et al., 2005). Reports also describe broad inhibitory effects on rumen protozoa, bacteria, fungi, and archaea (Patra & Saxena, 2009), inhibition of cellulolytic pure cultures by *Yucca* saponins (Wang et al., 2000), and protozoa reduction with tea saponins (Liu et al., 2019; Qu et al., 2023). These lines of evidence match the weaker NH₃-N and VFA profiles under T2.

Overall, ensiled *B. decumbens* improved rumen fermentation, shown by stable pH, adequate NH₃-N, and higher VFA production. Fresh *B. decumbens* showed the opposite pattern, consistent with steroidal saponin interference in microbial activity and end-product formation. Ensiling therefore supports safer use of *B. decumbens* by improving fermentability while removing the fermentation penalties linked to steroidal saponins.

3.3 Growth performance

Based on the results presented in Table 4, there were statistically significant differences ($p < 0.001$) in final body weight (FBW), body weight gain (BWG), average daily gain (ADG), and feed conversion ratio (FCR) among sheep fed the different dietary treatments.

Table 4: Growth performance of sheep feeding on fresh *P. purpureum*, fresh *B. decumbens*, and ensiled *B. decumbens* after 98-days trial.

Parameter	T1	T2	T3	P-value
DMI (g/d)	790.10 ± 23.22 ^a	736.69 ± 24.45 ^c	767.17 ± 33.72 ^b	<0.001
IBW (kg)	23.00 ± 1.13	23.00 ± 1.26	23.00 ± 1.25	0.379
FBW (kg)	31.63 ± 0.73 ^a	30.85 ± 0.80 ^b	31.85 ± 0.88 ^a	<0.001
BWG (kg)	8.63 ± 0.46 ^b	7.88 ± 0.33 ^c	8.85 ± 0.47 ^a	<0.001
ADG (g)	88.01 ± 5.79 ^b	80.41 ± 6.36 ^c	90.31 ± 5.80 ^a	<0.001
FCR	8.98 ± 0.19 ^b	9.26 ± 0.21 ^c	8.51 ± 0.21 ^a	<0.001

Note: All values were expressed as mean ± SE; ^{a,b,c} values with superscript within row are significantly different at $p < 0.05$. Treatment 1: Fresh *P. purpureum* (control group), Treatment 2: Fresh *B. decumbens*, Treatment 3: Ensiled *B. decumbens*. DMI: Dry matter intake; IBW: Initial body weight; FBW: Final body weight; BWG: Body weight gain; ADG: Average daily gain; FCR: Feed conversion ratio.

Sheep in the T3 group (ensiled *B. decumbens*) exhibited the highest growth performance, with BWG and ADG increasing by 2.52% and 2.59%, respectively, leading to a lower FCR compared to the control group (T1) indicating a significant improvement ($p < 0.001$). In contrast, sheep in the T2 group (fresh *B. decumbens*) demonstrated the lowest performance across most growth parameters. Specifically, T2 recorded lower dry matter intake (DMI) by 7.00%, FBW by 2.41%, BWG by 9.09%, and ADG by 9.01% with the highest FCR compared to the control group.

Overall, sheep fed the ensiled *B. decumbens* diet (T3) achieved the best growth performance, followed by the control group (T1), while those fed fresh *B. decumbens* (T2) showed the poorest outcomes. These findings suggest that the ensiling process effectively reduces saponin content in *B. decumbens* while preserving its nutritional value, thereby enhancing growth performance in sheep.

Growth performance measures help farmers judge whether a feeding strategy pays off. Body weight gain (BWG), dry matter intake (DMI), average daily gain (ADG), and feed conversion ratio (FCR) show how well sheep use nutrients and convert feed into live weight (Zampiga et al., 2021; Shoukry et al., 2023). These indicators guide diet decisions and support better feeding management (Bowen et al., 2006).

B. decumbens has strong nutritive value, with high dry matter (DM) and crude protein (CP), and CP often above 10%, which meets minimum protein needs for ruminants (Silva et al., 2016; Chung et al., 2018; Castro et al., 2018). In this study, sheep fed ensiled *B. decumbens* (T3) achieved higher BWG and ADG than those fed fresh *B. decumbens* (T2), even though both diets used the same forage. This pattern supports an ensiling benefit linked to improved fiber use and removal of harmful steroidal saponins, which together promote better rumen function and growth. Improved digestibility often translates into better FCR and higher final body weight, consistent with prior findings (Ayele et al., 2017). Rumen conditions such as stable pH, adequate ammonia nitrogen ($\text{NH}_3\text{-N}$), and higher volatile fatty acids (VFA) support microbial fermentation and nutrient synthesis. Rumen microbes supply a large share of the animal's protein and energy needs, which underpins muscle gain and overall performance (Lin et al., 2023).

Fresh *B. decumbens* (T2) showed the lowest DMI, which fits known saponin effects on both rumen function and feeding behavior. Saponins disrupt rumen microbes and have also been linked to slower digesta passage, which can shift fermentation dynamics and reduce nutrient-use efficiency (Wina et al., 2005; Patra & Saxena, 2009). They also add a bitter, astringent taste that reduces voluntary intake. A recent report observed lower DMI when sheep received high

levels of *B. decumbens*, which supports this explanation (Jaapar et al., 2023).

Overall, ensiling reduced the feeding and performance penalties linked to steroidal saponins in fresh *B. decumbens*. The higher final weights under T3 suggest better nutrient use and stronger farm-level returns, supporting ensiling as a practical preservation and detoxification option for farmers who rely on *B. decumbens* as an accessible forage.

4. CONCLUSION

In conclusion, this study demonstrates that ensiling *B. decumbens* effectively eliminates harmful steroidal saponins, improves rumen fermentation, enhances nutrient digestibility, and increases growth performance in sheep compared to its fresh form. A major strength of this work is the combined *in vitro* and *in vivo* evaluation over an extended feeding period, providing comprehensive insights into the biological effects of ensiled *B. decumbens* under practical feeding conditions. The findings of this study are expected to provide practical insights for improving the use of *B. decumbens* as a safe and nutritionally viable feed resource. In the long term, this research may guide forage management practices and promote more sustainable feeding strategies for ruminant production systems.

One limitation is the lack of an ensiled *P. purpureum* treatment, so the analysis does not separate species effects from a general ensiling effect. Nonetheless, the main hypothesis remains supported because the study tests ensiling within *B. decumbens* and benchmarks the ensiled forage against the fresh *P. purpureum* diet used in practice. Future research should address these limitations by including multiple forage types in both fresh and ensiled forms, incorporating precise intake monitoring, and exploring rumen microbial dynamics to better understand the mechanisms driving the observed improvements. These efforts will enhance the applicability of ensiling as a practical strategy for improving the nutritional value and safety of tropical forages in ruminant production systems.

ACKNOWLEDGEMENT

The project was funded by the Geran Putra - Geran Putra Berimpak (GP-GPB), Universiti Putra Malaysia (Grant no: 9713700).

REFERENCES

- Ángeles-Mayorga, Y., Cen-Cen, E. R., Crosby-Galván, M. M., Ramírez-Briebesca, J. E., Candelaria-Martínez, B., Sánchez-Villarreal, A., Ramírez-Mella, M. (2022). Foliage of tropical trees and shrubs and their secondary metabolites modify *in vitro* ruminal fermentation, methane and gas production without a tight correlation with the microbiota. *Animals*, 12(19), 2628. <https://doi.org/10.3390/ani12192628>

- Astuti, M., Meliala, A., Dalais, F. S., Wahlqvist, M. L. (2000). Tempe, a nutritious and healthy food from Indonesia. *Asia Pacific Journal of Clinical Nutrition*, 9(4), 322–325. <https://doi.org/10.1046/j.1440-6047.2000.00176.x>
- Ayele, S., Urge, M., Animut, G., Yusuf, M. (2017). Feed intake, digestibility, growth performance and blood profiles of three Ethiopian fat tail hair sheep fed hay supplemented with two levels of concentrate supplement. *Open Journal of Animal Sciences*, 7(2), 149–167. <https://doi.org/10.4236/ojas.2017.72013>
- Besharati, M., Maggolino, A., Palangi, V., Kaya, A., Jabbar, M., Eseceli, H., De Palo, P., Lorenzo, J. M. (2022). Tannin in ruminant nutrition: Review. *Molecules*, 27(23), 8273. <https://doi.org/10.3390/molecules27238273>
- Bowen, M. K., Ryan, M. P., Jordan, D. J., Beretta, V., Kirby, R. M., Stockman, C., McIntyre, B. L., Rowe, J. B. (2006). Improving sheep feedlot management. *International Journal of Sheep and Wool Science*, 54(2), 27–34.
- Cabral, A. M. D., Carvalho, F. F. R., Santos, G. C. L., Ferreira, J. C., Silva, M. J. M. S., Santos, G. R. A., Belo Júnior, G. S., Melo, N. D. (2020). Use of sugar cane to feed lactating dairy goats. *Arquivos Brasileiros de Medicina Veterinária e Zootecnia*, 72(6), 2297–2307. <https://doi.org/10.1590/1678-4162-12022>
- Castro, M. B., Gracindo, C. V., Landi, M. F. A., Cabral Filho, S. L. S., Resende Filho, N. J., Lima, E. M. M., Riet-Correa, F. (2018). Sheep adaptation management and investigation of inherited resistance to prevent *Brachyaria* spp. poisoning. *Small Ruminant Research*, 158, 42–47. <https://doi.org/10.1016/j.smallrumres.2017.12.001>
- Chen, J., Harstad, O. M., McAllister, T., Dörsch, P., Holo, H. (2020). Propionic acid bacteria enhance ruminal feed degradation and reduce methane production *in vitro*. *Acta Agriculturae Scandinavica, Section A – Animal Science*, 69(3), 169–175. <https://doi.org/10.1080/09064702.2020.1737215>
- Chung, E. L. T., Predith, M., Nobilly, F., Samsudin, A. A., Jesse, F. F. A., Loh, T. C. (2018). Can treatment of *Brachyaria decumbens* (signal grass) improve its utilisation in the diet in small ruminants? A review. *Tropical Animal Health and Production*, 50(8), 1727–1732. <https://doi.org/10.1007/s11250-018-1641-4>
- Das, T. K., Banerjee, D., Chakraborty, D., Pakhira, M. C., Shrivastava, B., Kuhad, R. C. (2012). Saponin: Role in animal system. *Veterinary World*, 5(4), 248–254. <https://doi.org/10.5455/vetworld.2012.248-254>
- Dewhurst, R. J., Newbold, J. R. (2022). Effect of ammonia concentration on rumen microbial protein production *in vitro*. *British Journal of Nutrition*, 127(6), 847–849. <https://doi.org/10.1017/S000711452100458X>
- Fauzi, N. A. A., Chung, E. L. T., Abu Bakar, N. A. A., Kamalludin, M. H., Reduan, M. F. H., Jesse, F. F. A., Dunshea, F. R. (2025). Blood indices of sheep fed with ensiled signal grass (*Brachyaria decumbens*). *New Zealand Journal of Agricultural Research*, 1–18. <https://doi.org/10.1080/00288233.2025.2475944>
- Guyader, J., Eugène, M., Doreau, M., Morgavi, D. P., Gérard, C., Martin, C. (2017). Tea saponin reduced methanogenesis *in vitro* but increased methane yield in lactating dairy cows. *Journal of Dairy Science*, 100(3), 1845–1855. <https://doi.org/10.3168/jds.2016.11644>
- Hristov, A. N., Ropp, J. K. (2003). Effect of dietary carbohydrate composition and availability on utilization of ruminal ammonia nitrogen for milk protein synthesis in dairy cows. *Journal of Dairy Science*, 86(7), 2416–2427. [https://doi.org/10.3168/jds.S0022-0302\(03\)73836-3](https://doi.org/10.3168/jds.S0022-0302(03)73836-3)
- Jaapar, M. S., Chung, E. L. T., Nayan, N., Kamalludin, M. H., Saminathan, M., Muniandy, K. V., Hamdan, M. H. M., Jusoh, S., Jesse, F. F. A. (2022). Effects of different levels of *Brachyaria decumbens* diets on *in vitro* gas production and ruminal fermentation. *Journal of Animal Health and Production*, 10(2), 245–251. <https://doi.org/10.17582/journal.jahp/2022/10.2.245.251>
- Jaapar, M. S., Chung, E. L. T., Nayan, N., Muniandy, K. V., Hamdan, M. H. M., Jusoh, S., Jesse, F. F. A. (2023). Digestibility, growth performance, body measurement and hormone of sheep fed with different levels of *Brachyaria decumbens* diets. *Tropical Life Sciences Research*, 34(1), 67–83. <https://doi.org/10.21315/tlsr2023.34.1.5>
- Jasmin, B. H., Boston, R. C., Modesto, R. B., Schaer, T. P. (2011). Perioperative ruminal pH changes in domestic sheep (*Ovis aries*) housed in a biomedical research setting. *Journal of the American Association for Laboratory Animal Science*, 50(1), 27–32. <https://doi.org/10.1037/j.iamlabs.50.1.27>
- Jayanegara, A., Wina, E., Takahashi, J. (2014). Meta-analysis on methane mitigating properties of saponin-rich sources in the rumen: Influence of addition levels and plant sources. *Asian-Australasian Journal of Animal Sciences*, 27(10), 1426–1435. <https://doi.org/10.5713/ajas.2014.14086>
- Ku-Vera, J. C., Jiménez-Ocampo, R., Valencia-Salazar, S. S., Montoya-Flores, M. D., Molina-Botero, I. C., Arango, J., Gómez-Bravo, C. A., Aguilar-Pérez, C. F., Solorio-Sánchez, F. J. (2020). Role of secondary plant metabolites on enteric methane mitigation in ruminants. *Frontiers in Veterinary Science*, 7, 584. <https://doi.org/10.3389/fvets.2020.00584>
- Le Bot, M., Thibault, J., Pottier, Q., Boisard, S., Guillet, D. (2022). An accurate, cost-effective and simple colorimetric method for the quantification of total triterpenoid and steroidal saponins from plant materials. *Food Chemistry*, 383, 132597. <https://doi.org/10.1016/j.foodchem.2022.132597>
- Lima, F. G. D., Lee, S. T., Pfister, J. A., Miyagi, E. S., Costa, G. L., Silva, R. D. D., Fioravanti, M. C. S. (2015). The effect of ensiling and haymaking on the concentrations of steroidal saponin in two *Brachyaria* grass species. *Ciência Rural*, 45(5), 858–863. <https://doi.org/10.1590/0103-8478cr20141305>
- Lima, F. G. D., Ribeiro, C. S., Andrade, D. D. F., Costa, G. L., Pires, H. C. M., Guimarães, V. Y., Haraguchi, M., Fioravanti, M. C. S. (2012). Effects of *Brachyaria brizantha* hay containing a steroidal saponin in lambs. *International Journal of Poisonous Plant Research*, 2(1), 20–26. <https://doi.org/10.1590/0103-8478cr20141305>
- Lin, W., Song, H., Shen, J., Wang, J., Yang, Y., Yang, Y., Cao, J., Xue, L., Fang, Z., Xiao, T., Lin, R. (2023). Functional role of skeletal muscle-derived interleukin-6 and its effects on lipid metabolism. *Frontiers in Physiology*, 14, 1110926. <https://doi.org/10.3389/fphys.2023.1110926>
- Liu, Y., Ma, T., Chen, D., Zhang, N., Si, B., Deng, K., Tu, Y., Diao, Q. (2019). Effects of tea saponin supplementation on nutrient digestibility, methanogenesis, and ruminal microbial flora in Dorper crossbred ewe. *Animals*, 9(1), 29. <https://doi.org/10.3390/ani9010029>
- Stefanini Lopes, R., Ahring, B. (2023). Enhancing acetic acid production in *in vitro* rumen cultures by addition of a homoacetogenic consortia from a kangaroo: Unravelling the impact of inhibition of methanogens and effect of almond biochar on rumen fermentations. *Fermentation*, 9(10), 885. <https://doi.org/10.3390/fermentation9100885>
- Maccarana, L., Cattani, M., Tagliapietra, F., Bailoni, L., Schiavon, S. (2016). Influence of main dietary chemical constituents on the *in vitro* gas and methane production in diets for dairy cows. *Journal of Animal Science and Biotechnology*, 7(1), 54. <https://doi.org/10.1186/s40104-016-0109-5>
- Melo, M. J. A. F., Backes, A. A., Fagundes, J. L., Melo, M. T., Silva, G. P. (2016). Características fermentativas e composição química da silagem de capim Tanzânia com aditivos. *Boletim de Indústria Animal*, 73(3), 189–197. <https://doi.org/10.17523/bia.v73n3p189>
- Menci, R., Coppa, M., Torrent, A., Natalello, A., Valenti, B., Luciano, G., Priolo, A., Niderkorn, V. (2021). Effects of two tannin extracts at different doses in interaction with a green or dry forage substrate on *in vitro* rumen fermentation and biohydrogenation. *Animal Feed Science and Technology*, 278, 114977.
- Menke, K. H., Steingass, H. (1988). Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Development*, 28, 7–55.
- Menke, K. H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. *The Journal of Agricultural Science*, 93(1), 217–222. <https://doi.org/10.1017/S0021859600086305>
- Mizrahi, I., Wallace, R. J., Morais, S. (2021). The rumen microbiome: Balancing food security and environmental impacts. *Nature Reviews Microbiology*, 19(9), 553–566. <https://doi.org/10.1038/s41579-021-00543-6>
- Muniandy, K. V., Chung, E. L. T., Jaapar, M. S., Hamdan, M. H. M., Reduan, M. F. H., Salleh, A., Jesse, F. F. A. (2021b). The influence of feeding low and high level of *Brachyaria decumbens* diets on the haematology, serum biochemistry, and acute phase proteins of sheep. *Tropical Animal Health and Production*, 53(3), 372. <https://doi.org/10.1007/s11250-021-02820-1>
- Muniandy, K. V., Chung, E. L. T., Reduan, M. F. H., Paul, B. T., Jaapar, M. S., Hamdan, M. H. M., Jesse, F. F. A. (2021a). Clinico-pathological responses of sheep to graded levels of *Brachyaria decumbens* diets. *Journal of Advanced Veterinary Research*, 11(3), 167–173.
- Ørskov, E. R., McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *The Journal of Agricultural Science*, 92(2), 499–503. <https://doi.org/10.1017/S0021859600063048>
- Orzuna-Orzuna, J. F., Dorantes-Iturbide, G., Lara-Bueno, A., Mendoza-Martínez, G. D., Miranda-Romero, L. A., Hernández-García, P. A. (2021). Effects of dietary tannins' supplementation on growth performance, rumen fermentation, and enteric methane emissions in beef cattle: A meta-analysis. *Sustainability*, 13(13), 7410. <https://doi.org/10.3390/su13137410>

- Palangi, V., Lackner, M. (2022). Management of enteric methane emissions in ruminants using feed additives: A review. *Animals*, 12(24), 3452. <https://doi.org/10.3390/ani12243452>
- Parsons, T. R., Maita, Y., Lalli, C. M. (1984). A manual of chemical and biological methods for seawater analysis. Pergamon Press.
- Patra, A. K., Saxena, J. (2010). A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. *Phytochemistry*, 71(11–12), 1198–1222. <https://doi.org/10.1016/j.phytochem.2010.05.010>
- Pinos-Rodríguez, J. M., Gonzalez-Muñoz, S., Badillo, B., García-López, J. C., Aguirre-Rivera, J. R., Infante, S. (2008). Chemical composition and ruminal *in vitro* degradation of fresh or silage of Agave salmiana Otto ex. Salm-Dyck. *Journal of Applied Animal Research*, 33(1), 45–48. <https://doi.org/10.1080/09712119.2008.9706894>
- Qiu, Q., Gao, C., Su, H., Cao, B. (2021). Rumen fermentation characteristics require more time to stabilize when diet shifts. *Animals*, 11(8), 2192. <https://doi.org/10.3390/ani11082192>
- Qu, X., Raza, S. H. A., Zhao, Y., Deng, J., Ma, J., Wang, J., Alkhorayef, N., Alkhalil, S. S., Pant, S. D., Lei, H., Zan, L. (2023). Effect of tea saponins on rumen microbiota and rumen function in Qinchuan beef cattle. *Microorganisms*, 11(2), 374. <https://doi.org/10.3390/microorganisms11020374>
- Rira, M., Chentli, A., Boufenera, S., Bousseboua, H. (2015). Effects of plants containing secondary metabolites on ruminal methanogenesis of sheep *in vitro*. *Energy Procedia*, 74, 15–24. <https://doi.org/10.1016/j.egypro.2015.07.513>
- Shoukry, M. M., El-Nameary, Y. A. A. E., Salman, F. M., El-Sayed, W. M. (2023). Improving the productive performance of growing lambs using prebiotic and probiotic as growth promoters. *Tropical Animal Health and Production*, 55(6), 375. <https://doi.org/10.1007/s11250-023-03752-8>
- Silva, R., Silva, R. M., Silva, C., Maria, M. (2016). Forage yield and nutritive value of naturally growing *Brachiaria decumbens* as undergrowth to an aroeira tree stand in a silvopasture system. *African Journal of Agricultural Research*, 11(40), 3922–3928. <https://doi.org/10.5897/ajar2016.11529>
- Singh, B., Singh, J. P., Singh, N., Kaur, A. (2017). Saponins in pulses and their health promoting activities: A review. *Food Chemistry*, 233, 540–549. <https://doi.org/10.1016/j.foodchem.2017.04.161>
- Śliwiński, B. J., Kreuzer, M., Sutter, F., Machmüller, A., Wettstein, H. R. (2004). Performance, body nitrogen conversion and nitrogen emission from manure of dairy cows fed diets supplemented with different plant extracts. *Journal of Animal and Feed Sciences*, 13(1), 73–91. <https://doi.org/10.22358/jafs/67390/2004>
- Ungerfeld, E. M. (2020). Metabolic hydrogen flows in rumen fermentation: Principles and possibilities of interventions. *Frontiers in Microbiology*, 11, 589. <https://doi.org/10.3389/fmicb.2020.00589>
- Wang, B., Ma, M. P., Diao, Q. Y., Tu, Y. (2019). Saponin-induced shifts in the rumen microbiome and metabolome of young cattle. *Frontiers in Microbiology*, 10, 356. <https://doi.org/10.3389/fmicb.2019.00356>
- Wang, X., Li, X., Zhao, C., Hu, P., Chen, H., Liu, Z., Liu, G., Wang, Z. (2012). Correlation between composition of the bacterial community and concentration of volatile fatty acids in the rumen during the transition period and ketosis in dairy cows. *Applied and Environmental Microbiology*, 78(7), 2386–2392. <https://doi.org/10.1128/AEM.07545-11>
- Wang, Y., Zhou, J., Wang, X., Yu, Q., Sun, Y., Li, Y., Zhang, Y., Shen, W., Wei, X. (2023). Rumen fermentation parameters prediction model for dairy cows using a stacking ensemble learning method. *Animals*, 13(4), 678. <https://doi.org/10.3390/ani13040678>
- Widiawati, Y., Puastuti, W. (2016). The effect of condensed tannin and saponin in reducing methane produced during rumen digestion of agricultural byproducts. *Proceedings of the International Seminar on Livestock Production and Veterinary Technology (LPVT 2016)*, 139–146. <https://doi.org/10.14334/proc.intsem.lpvt-2016-p.139-146>
- Widiyarini, S., Nagari, F. S., Hanim, C., Bachruddin, Muhlisin, Z., Yusiati, L. M. (2021). Effect of *Nigella sativa* L. as saponin sources on *in vitro* rumen fermentation, enzyme activity and nutrients digestibility. *Advances in Animal and Veterinary Sciences*, 9(12), 2247–2257. <https://doi.org/10.17582/journal.aavs/2021/9.12.2247.2257>
- Wina, E., Muetzel, S., Becker, K. (2005). The impact of saponins or saponin-containing plant materials on ruminant production: A review. *Journal of Agricultural and Food Chemistry*, 53(21), 8093–8105. <https://doi.org/10.1021/jf048053d>
- Yanza, Y. R., Irawan, A., Jayanegara, A., Ramadhani, F., Respati, A. N., Fitri, A., Hidayat, C., Niderkorn, V., Cieslak, A., Szumacher-Strabel, M., Hidayat, R., Tanuwiria, U. H. (2024). Saponin extracts utilization as dietary additive in ruminant nutrition: A meta-analysis of *in vivo* studies. *Animals*, 14(8), 1231. <https://doi.org/10.3390/ani14081231>
- Zampiga, M., Calini, F., Sirri, F. (2021). Importance of feed efficiency for sustainable intensification of chicken meat production: Implications and role for amino acids, feed enzymes and organic trace minerals. *World's Poultry Science Journal*, 77(3), 639–659. <https://doi.org/10.1080/00439339.2021.1959277>
- Zaynab, M., Sharif, Y., Abbas, S., Afzal, M. Z., Qasim, M., Khalofah, A., Ansari, M. J., Khan, K. A., Tao, L., Li, S. (2021). Saponin toxicity as key player in plant defense against pathogens. *Toxicon*, 193, 21–27. <https://doi.org/10.1016/j.toxicon.2021.01.009>
- Zhang, H., Cheng, X., Elsabagh, M., Lin, B., Wang, H. (2021). Effects of formic acid and corn flour supplementation of banana pseudostem silages on nutritional quality of silage, growth, digestion, rumen fermentation and cellulolytic bacterial community of Nubian black goats. *Journal of Integrative Agriculture*, 20(8), 2214–2226. [https://doi.org/10.1016/S2095-3119\(20\)63470-0](https://doi.org/10.1016/S2095-3119(20)63470-0)
- Zhao, L., Meng, Q., Ren, L., Liu, W., Zhang, X., Huo, Y., Zhou, Z. (2015). Effects of nitrate addition on rumen fermentation, bacterial biodiversity and abundance. *Asian-Australasian Journal of Animal Sciences*, 28(10), 1433–1441. <https://doi.org/10.5713/ajas.15.0091>
- Zou, H., Hu, R., Wang, Z., Shah, A. M., Zeng, S., Peng, Q., Xue, B., Wang, L., Zhang, X., Wang, X., Shi, J., Li, F., Zeng, L. (2019). Effects of nutritional deprivation and re-alimentation on the feed efficiency, blood biochemistry, and rumen microflora in yaks (*Bos grunniens*). *Animals*, 9(10), 807. <https://doi.org/10.3390/ani9100807>