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ORIGINAL ARTICLE OPEN ACCESS

In Vitro Fermentation Characteristics of Seven Commonly Used Dairy Roughages With Relatively High and Low Nutritive Values

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Received: 28 January 2025 | **Revised:** 14 May 2025 | **Accepted:** 10 June 2025

Funding: This work was supported by Agrifutures Australia (PRJ-012649).

Keywords: chemical composition | forages | methane | nitrogen | rumen fermentation

ABSTRACT

Roughage constitutes a fundamental component of dairy cow diets; it promotes rumen health and supports optimal animal productivity. This study applied an ANKOM gas production system to assess the in vitro fermentation characteristics of seven widely used dairy roughages (barley hay, corn silage, lucerne hay, oaten hay, ryegrass hay, timothy hay and wheaten hay) classified into relatively high and low nutritive value groups. Roughage samples representing high and low nutritive values were selected based on the upper and lower quartiles of the feed quality database. The results showed that high-nutritive value oaten hay exhibited higher dry matter (DM) digestibility (0.50 vs. 0.24–0.43 g/g DM, $p < 0.05$), total gas production (87.5 vs. 15.3–81.0 mL/g DM) and total volatile fatty acid (63.2 vs. 39.3–62.5 mM) than other roughages. High-nutritive value barley hay and oaten hay both exhibited higher methane production (7.58 and 7.77 mL/g DM, respectively) compared to other roughages (0.44–4.76 mL/g DM). Similarly, high-nutritive value barley hay (40.23 mg/100 mL) and lucerne hay (40.76 mg/100 mL) exhibited higher ammonia nitrogen (12.16–31.99 mg/mL) than other roughages. High-nutritive value oaten and barley hay promoted superior fermentation performance but also led to greater methane emissions, while barley and lucerne hay increased nitrogen release. These results underscore the need to balance productivity with environmental impacts when selecting roughages for dairy systems.

1 | Introduction

Roughage is a fundamental part of dairy cow diets. The fibrous structure of roughages stimulates saliva secretion and promotes rumen motility and buffering capacity, thereby enhancing rumen function and supporting sustainable dairy production (Jiang et al. 2017; Weiss et al. 2017). Different roughages exhibit diverse chemical compositions, nutritive values and rumen fermentation characteristics (Getachew et al. 2004). The ruminal fermentability and end-products of rumen digestion are largely affected by roughage nutritive values (also known as forage quality). For given roughage type, those who contains more fermentable substrates (e.g., starch, protein) tend to exhibit a greater fermentability and result in more gas production (GP), and potentially higher milk yields in dairy cows (Zhang et al. 2016). Therefore, selecting appropriate roughages is crucial to ensure a balanced diet that meets both nutritional and production goals. Roughage selection is often based on its availability, price, nutritive value and dairy production objectives. Although numerous studies have analysed on the nutritive value and fermentation characteristics through both in vitro and in vivo studies of some dairy roughages (Dewhurst et al. 2003; Cheng et al. 2011), few have systematically compared a broad range of commonly used dairy roughages within a unified experimental framework. Notably, oaten hay has been increasingly used in Asian dairy production, yet fewer than 10 refereed publications can be found to demonstrate how it compares with other roughages for milk production (Li et al. 2022). To address this gap, the present study aimed to evaluate and compare the in vitro fermentation profile of seven commonly used dairy roughages, with high- and low-nutritive values. Identifying the most efficient roughage sources based on fermentative characteristics and nutrient utilisation, which is key to supporting both productivity and environmental sustainability in modern dairy systems.

2 | Materials and Methods

2.1 | Experimental Design

All procedures related to animal handling and rumen fluid collection procedures were approved by the Animal Ethics Committee (Approval ID: 23333) of The University of Melbourne, Victoria, Australia. Three independent in vitro fermentation runs were conducted with a randomised complete block design to evaluate the fermentation characteristics and GP of seven different roughage types, each classified into two nutritive levels (high vs. low). Each treatment (seven roughage types × two levels of nutritive values = 14 treatments) was replicated three times within each run, resulting in a total of nine replications per treatment across the three runs. To ensure biological replication, each experimental run was carried out on different days using freshly collected rumen fluid from donor heifers, which had not been stored between runs.

2.2 | Proximate Nutritive Values Analysis

A total of 33 samples of barley hay, oaten hay, wheaten hay, ryegrass hay, lucerne hay, timothy hay and corn silage were collected between 2021 and 2022. Samples were analysed by using near-infrared reflectance spectroscopy (NIRS) at the NSW Department of Primary Industries Feed Test Laboratory (Wagga Wagga, Australia), which is certified under ISO 17025 and NATA accredited. The following parameters were analysed: dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), water-soluble carbohydrates (WSCs), metabolisable energy (ME), fat, organic matter (OM), dry matter disappearance (DMD), 48-h neutral detergent fibre digestibility (NDFD 48 h) and ash content. From these 33 samples, 14 representative samples—one with high- and one with low-nutritive value per roughage type—were selected for the fermentation experiment. These 14 samples were classified as either 'high-nutritive value' or 'low-nutritive value' based on forage quality parameters. Classification was determined by whether the values of NDF, ADF, WSC, ME, CP and (if available) starch fell within the upper (high) or lower (low) quartile of the forage database reported by NASEM (2021) database. As lucerne hay (named as alfalfa hay) and timothy hay were not included in the NASEM database, values from Feedipedia were used instead, in line with published studies. It should be noted that detailed records on harvest year and storage conditions were not consistently available for all samples and the duration and method of storage varied across sources. This diversity in sample background may contribute to within-species variation in nutritive profiles. However, the classification into high- and low-nutritive value groups was solely based on objective, compositional indices to ensure consistency and minimise subjective bias. The comparative nutritive value of selected roughages is provided in Table 1.

2.3 | In Vitro Rumen Fermentation Preparation

All roughage samples were oven-dried for 24 h at 60°C, ground in a cyclone mill and passed through a 1 mm sieve. One gram of each dried sample was weighed into Ankom F57 fibre filter bags (Ankom F57; ANKOM Corp., Fairport, NY, USA) and sealed with an impulse heat sealer. The initial dry weight was recorded for subsequent calculation of in vitro dry matter disappearance (IVDMD).

Rumen fluid was collected from five healthy heifers aged 15–24 months, maintained on a high-roughage diet based on perennial ryegrass (*Lolium perenne* L.) grazing. The selection of donor animals consuming predominantly fibrous roughage was intended to ensure that the microbial composition of the inoculum reflected rumen conditions typical of cattle receiving a roughage-based diet. The rumen fluid was collected through oral stomach tubing using a gastric rumen sampler (Anscitech Co. Ltd., Wuhan, China). To ensure sample representativeness, rumen fluid was collected from multiple ruminal locations using a 1.5 L syringe connected to an oral stomach tubing apparatus, following the method described by Muizelaar et al. (2020). This procedure was repeated several times per animal to obtain a sufficient volume for the fermentation procedure. Immediately after collection, the rumen fluid was transferred into two 2 L glass bottles pre-warmed to 39°C to maintain microbial viability. Then, the rumen fluid was transported to the laboratory and placed in the pre-warmed incubator set at 39°C. Before inoculation, rumen fluid was filtered through three layers of cheesecloth under continuous CO_2 flushing to ensure anaerobic conditions.

Each 310 mL incubation bottle was loaded with 1 g of the prepared substrate. To initiate fermentation, 75 mL of pre-warmed Kansas State buffer (pH 6.8, Marten et al. 1980) and 25 mL of filtered rumen fluid were added to each bottle, leaving approximately 210 mL of headspace. Bottles were flushed with carbon dioxide to maintain anaerobic conditions and then sealed with ANKOM GP modules (Ankom Technology, Macedon, NY, USA). The assembled units were placed into a temperature-controlled water bath set at 39 °C for fermentation, where they were incubated for 48 h under anaerobic conditions.

2.4 | Gas Production and Fermentation Characteristics

Cumulative GP over a 48-h period was monitored using the ANKOM Gas Production System (ANKOM Corp., Fairport, NY, USA), based on the protocol by Alvarez-Hess et al. (2019). This automated system continuously measured internal pressure in each bottle, with readings taken every 5 min and transmitted via

TABLE 1 | Dry matter content and chemical composition of hay and silage used in the in vitro experiment.

Samples	DM (g/kg fresh weight)	NDF (g/kg DM)	ADF (g/kg DM)	CP (g/kg DM)	WSC (g/kg DM)	ME (MJ/Kg DM)	NDFD 48h (g/kg of NDF)
Barley hays (H)	924	567	318	56	209	9.2	630
Barley hays (L)	930	722	410	72	49	7.7	530
Corn silages (H)	383	375	196	70	178	10.9	350
Corn silages (L)	397	507	286	38	124	9.6	320
Lucerne hays (H)	925	418	278	211	<40	8.6	400
Lucerne hays (L)	929	470	325	151	<40	7.5	340
Oaten hays (H)	881	470	289	65	250	10.2	640
Oaten hays (L)	927	578	324	90	143	9.3	540
Ryegrass hays (H)	920	482	264	87	228	11.3	680
Ryegrass hays (L)	932	690	372	79	<40	6.5	490
Timothy hays (H)	907	572	357	47	302	11	390
Timothy hays (L)	936	593	339	87	84	8.4	580
Wheaten hays (H)	895	586	308	89	175	8.9	560
Wheaten hays (L)	947	642	387	71	147	7.8	480

Abbreviations: ADF: acid detergent fibre on dry matter basis; CP: crude protein on dry matter basis; DM: dry matter in fresh hay or silage samples; H: relative high-nutritive value; L: relative low-nutritive value; ME: metabolisable energy on dry matter basis; NDF: neutral detergent fibre on dry matter basis; NDFD 48h: neutral detergent fibre digestibility at 48h on NDF basis; WSC: water-soluble carbohydrates on dry matter basis.

radio frequency for digital logging. When internal pressure exceeded 6.89kPa above atmospheric level, a valve opened briefly (1s) to release gas, thereby preventing pressure buildup and ensuring gas did not diffuse back into the fermentation medium (Cattani et al. 2014).

To account for background GP, each run included three blank bottles containing only rumen fluid and buffer. At the end of the incubation period, the bottles were removed from the water bath and placed on ice to terminate fermentation. Gas samples were extracted from the ANKOM module's vent using a 50mL syringe fitted with a 23-gauge needle and injected into pre-evacuated Exetainer vials (12mL, Labco Ltd., Buckinghamshire, UK). Methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O) concentrations were analysed via gas chromatography, and the production values were corrected using the blank measurements.

After 48h of incubation, IVDMD was measured according to the method of Alvarez-Hess et al. (2019). The ANKOM F57 filter bags were removed from the bottles, rinsed with distilled water and oven-dried at 60°C for 48h. The difference between initial and final bag weights was used to determine the IVDMD. A 50mL subsample of rumen fluid was collected from each ANKOM GP bottle for pH measurement and subsequent analyses of ammonia nitrogen (NH_3–N) and volatile fatty acids (VFAs) at the NSW Department of Primary Industries Feed Test Laboratory (Wagga Wagga, Australia). The samples were cooled and the pH was measured by using a calibrated pH metre. A 4.8mL rumen fluid subsample was transferred into a 15mL tube containing 0.2mL undiluted hydrochloric acid; the samples were temporarily stored at −18°C for later NH_3–N analysis. The NH_3–N concentration was determined by a flow injection analysis method based on nitroprusside-salicylate colour development chemistry. Another 10mL rumen fluid subsample was measured and transferred into 15mL tubes with no additional preservatives. The samples were temporarily stored at −18°C until analysis. VFA concentrations were determined by capillary gas chromatography using an Agilent 7890B system (Agilent Technologies, Santa Clara, CA, USA). To ensure accurate quantification, background values measured from blank control bottles containing only buffer and rumen fluid (without substrate) were subtracted from total GP, VFA and NH_3–N results of each treatment.

2.5 | Statistical Analysis

Statistical analyses were performed using GenStat 19th edition (VSN International Ltd., Hemel Hempstead, UK). The data were analysed by using a two-way ANOVA with a factorial arrangement of 7 roughage types × 2 nutritive values. Each run formed a block and the experimental treatments were specified as combinations of roughages and nutritive value. Least squares means were compared using Fisher's LSD at a significance level of $p \leq 0.05$. The statistical model used for ANOVA is represented as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + R_k + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the response variable, μ is the overall mean, α_i is the fixed effect of roughage type (i = 1…7), β_j is the fixed effect of nutritive value level (j = 1, 2), R_k is the random effect of block (run,

$k = 1, 2, 3$), $(\alpha\beta)_{ij}$ is the fixed interaction effect between roughage type and nutritive value level and $\mathcal{E}_{ijk}$ is the residual error.

3 | Results

3.1 | GP and Fermentation Characteristics

Significant differences ($p < 0.01$) in GP were observed among the roughage types after 48 h of in vitro fermentation (Table 2). In general, the high-nutritive value roughage exhibited a significantly higher GP per gram of DM than low-nutritive value roughage ($p < 0.01$). However, the GP of low-nutritive value corn silage was numerically higher than its high-nutritive value counterpart, although the difference was not statistically significant ($p > 0.05$).

Significant variation ($p < 0.01$) was observed in IVDMD among various roughages. High-nutritive value oaten hay exhibited the highest IVDMD values, while low-nutritive value ryegrass hay recorded the lowest. In general, high-nutritive value roughages consistently showed higher IVDMD values than their low-nutritive value counterparts.

Substantial variation in NH_3–N concentrations was observed across the different roughage treatments. The highest NH_3–N concentrations were recorded in high-nutritive value lucerne hay (40.76 mg/100 mL) and barley hay (40.23 mg/100 mL). In contrast, the lowest NH_3-N concentrations were observed in low-nutritive value barley hay (15.71 mg/100 mL), high-nutritive value ryegrass hay (17.33 mg/100 mL), low-nutritive value ryegrass hay (15.12 mg/100 mL), high-nutritive value timothy hay (10.97 mg/100 mL), low-nutritive value timothy hay (18.90 mg/100 mL) and low-nutritive value wheaten hay (12.16 mg/100 mL). A strong positive correlation ($R^2 = 0.75$; $p < 0.001$; $n = 126$) was found between IVDMD and GP across all treatments, showing that higher digestibility is associated with greater GP.

3.2 | Methane Production

High-nutritive value barley hay exhibited the highest volume of CH_4 gas and CH_4 density among all 14 treatments (Table 3).

High-nutritive value barley hay exhibited the highest CH_4 yield per gram of IVDMD (17.63 mL/g IVDMD) in all roughages, followed by high-nutritive value oaten hay (15.55 mL/g IVDMD). However, both roughages had a significantly higher ranking in overall CH_4 production than other roughages. Across all roughage types, high-nutritive value samples generally produced more CH_4 than their low-nutritive counterparts. A moderately strong positive correlation ($R^2 = 0.58$; $p < 0.001$; $n = 126$) was observed between IVDMD and CH_4 production across the 14 treatments. The relationship is illustrated in Figure 1.

3.3 | Volatile Fatty Acid Production

After 48-h of fermentation, high-nutritive value barley hay (62.5 mM) and high-nutritive value oaten hay (63.2 mM) exhibited significantly higher total VFA production across all

TABLE 2 | Forty-eight hours fermentation characteristics of seven different roughages with two quality levels.

Samples	IVDMD (g/g DM)	pH	Total gas production (mL/g DM)	NH_3–N (mg/100 mL)
Barley hays (H)	0.43^{b}	6.22^{ab}	81.0^{a}	40.2^{a}
Barley hays (L)	0.28^{g}	6.47^{fg}	17.1^{f}	15.7^{efg}
Corn silages (H)	0.35^{de}	6.26^{bcd}	53.8^{cd}	32.0^{b}
Corn silages (L)	0.41^{bc}	6.24^{ab}	58.1^{bcd}	20.6^{cde}
Lucerne hays (H)	0.41^{bc}	6.37^{def}	62.0^{bc}	40.8^{a}
Lucerne hays (L)	0.35^{de}	6.44^{efg}	42.4^{e}	27.4^{bc}
Oaten hays (H)	0.50^{a}	6.13^{a}	87.5^{a}	27.1^{bc}
Oaten hays (L)	0.38^{cd}	6.35^{cde}	51.6^{d}	19.7^{cdef}
Ryegrass hays (H)	0.43^{b}	6.20^{ab}	66.4^{b}	17.3^{defg}
Ryegrass hays (L)	0.24^{h}	6.49^{g}	15.3^{f}	15.1^{efg}
Timothy hays (H)	0.32^{ef}	6.24^{abc}	54.2^{cd}	11.0^{g}
Timothy hays (L)	0.33^{ef}	6.32^{bcd}	37.6^{e}	18.9^{defg}
Wheaten hays (H)	0.41^{bc}	6.27^{bcd}	61.2^{bc}	25.1^{bcd}
Wheaten hays (L)	0.30^{fg}	6.36^{def}	34.7^{e}	12.2^{fg}
SEM	0.007	0.014	2.03	1.09
p	< 0.001	< 0.001	< 0.001	< 0.001

Abbreviations: DM: dry matter; H: relative high-nutritive value; IVDMD: in vitro dry matter disappearance; L: relative low-nutritive value; NH_3–N: ammonia nitrogen; SEM, standard error of the mean. Different superscript letters (a–g) within a row indicate significant differences between treatment means ($p < 0.05$; LSD test).

TABLE 3 | Forty-eight hours in vitro methane production of seven different roughages with two quality levels.

Samples	CH_4 Production: CH_4 mL/g DM	CH_4 Production: CH_4 mL/g IVDMD	CH_4 Production: CH_4 mL/MJ ME	CH_4 Intensity: Methane/gas Production %
Barley hays (H)	7.58[a]	17.6[a]	0.82	9.35[a]
Barley hays (L)	0.68[f]	2.46[h]	0.09	3.97[f]
Corn silages (H)	3.56[c]	10.5[cd]	0.33	6.62[bc]
Corn silages (L)	3.32[c]	8.16[e]	0.35	5.71[cde]
Lucerne hays (H)	4.76[b]	11.8[c]	0.55	7.68[b]
Lucerne hays (L)	2.56[d]	7.57[ef]	0.34	6.05[cd]
Oaten hays (H)	7.77[a]	15.6[b]	0.76	8.89[a]
Oaten hays (L)	3.12[cd]	8.30[e]	0.34	6.04[cd]
Ryegrass hays (H)	4.83[b]	11.3[cd]	0.43	7.27[b]
Ryegrass hays (L)	0.44[f]	1.99[h]	0.07	2.84[g]
Timothy hays (H)	3.21[c]	10.0[d]	0.29	5.91[cd]
Timothy hays (L)	1.81[e]	5.64[g]	0.22	4.81[ef]
Wheaten hays (H)	4.59[b]	11.2[cd]	0.52	7.51[b]
Wheaten hays (L)	1.91[e]	6.48[fg]	0.24	5.49[de]
SEM	0.197	0.413	—	0.180
p	<0.001	<0.001	—	<0.001

Abbreviations: DM: dry matter; H: relative high-nutritive value; IVDMD: in vitro dry matter disappearance; L: relative low-nutritive value; ME: metabolisable energy; SEM: standard error of the mean. Different superscript letters (a–h) within a row indicate significant differences between treatment means ($p<0.05$; LSD test).

treatments. In contrast, the low-nutritive value ryegrass hay (39.3 mM) demonstrated the lowest VFA concentration. The low-nutritive value roughages generally led to reduced VFA production across all roughage types. High-nutritive value samples of each roughage exhibited lower acetate-to-propionate (A:P) ratios than their low-nutritive counterparts. The lowest A:P ratio (1.84) was observed in low-nutritive value corn silage (Table 4). High-nutritive value barley hay produced the highest butyric

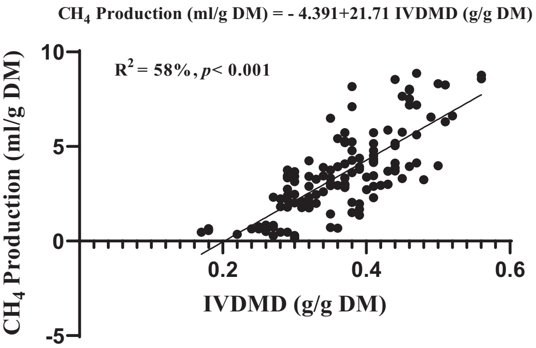

FIGURE 1 | The regression between the in vitro dry matter disappearance (IVDMD; g/g DM) and methane (CH_4) production (mL/g DM).

acid concentration (7.71 mM), whereas low-nutritive value lucerne hay yielded the lowest (4.62 mM).

4 | Discussion

Although several studies have assessed the fermentation characteristics of roughages used in ruminant production systems, most have focused on either comparing different forage types or evaluating variation within a single type based on nutritive value (Doane et al. 1997; Getachew et al. 2004). Limited studies have investigated the interaction between roughage types and nutritive value on fermentation characteristics. The present study addressed this gap by examining seven commonly used roughages, each evaluated at two nutritive value levels.

4.1 | IVDMD and GP

The IVDMD serves a quantitative indication of feed fermentability and available nutrients to animals in the in vitro conditions, which reflects the ruminal dry matter disappearance (Ávila et al. 2011). It estimates the proportion of feed degraded and potentially converted into energy and nutrients necessary for animal metabolism, growth and production (Homem Junior et al. 2017). Overall, high-nutrient availability in high-nutritive value roughages, as they had relatively higher IVDMD values compared to low-nutritive value parts. This is primarily attributed to the higher digestible fractions in high-nutritive value roughages. Apart from CP contents, a negative relationship was observed between roughage quality and fibre content (i.e., NDF and ADF). High-nutritive value roughages consistently showed lower values of NDF and ADF when compared with their low-nutritive value counterparts across different roughage pairs. Furthermore, high-nutritive value roughages exhibited higher concentration of WSC. Additionally, the higher 48 h NDFD in higher quality roughages contributed to more digestible fibre than low-quality roughage during the 48 h in vitro fermentation.

Total GP reflects fermentative activity of rumen microbes, where complex microbial communities break down roughages for further digestion and adaptation (Ávila et al. 2011; Hua et al. 2022). In this study, IVDMD accounted for a major portion of total GP (Total GP (mL/g DM) = 254.1 × IVDMD (g DM) − 41.79, $R^2=0.75$; $p<0.001$; $n=126$). This is consistent with the principle that high levels of plant cell wall content

TABLE 4 | Forty-eight hours in vitro volatile fatty acid production of seven different roughages with two quality levels.

Samples	Total VFA mM	VFA mM			
		Acetic (A)	Propionic (P)	Butyric	A:P ratio
Barley hays (H)	62.5^{ab}	37.74^{a}	14.26^{b}	7.71^{a}	2.66^{cede}
Barley hays (L)	40.8^{h}	26.79^{gh}	8.62^{g}	4.10^{h}	3.14^{ab}
Corn silages (H)	57.8^{cd}	32.71^{de}	14.73^{b}	7.87^{a}	2.25^{f}
Corn silages (L)	57.1^{cd}	30.29^{ef}	16.52^{a}	7.70^{a}	1.84^{g}
Lucerne hays (H)	57.6^{cd}	36.68^{ab}	12.86^{d}	5.44^{cde}	2.88^{bc}
Lucerne hays (L)	50.6^{fg}	34.09^{bcd}	10.03^{ef}	4.62^{fgh}	3.44^{a}
Oaten hays (H)	63.2^{a}	36.55^{abc}	16.58^{a}	7.46^{a}	2.22^{f}
Oaten hays (L)	52.2^{ef}	32.66^{de}	12.41^{d}	5.25^{cdef}	2.65^{cde}
Ryegrass hays (H)	58.4^{bc}	34.95^{abcd}	15.06^{b}	6.37^{b}	2.34^{ef}
Ryegrass hays (L)	39.3^{h}	24.79^{h}	8.72^{fg}	4.43^{gh}	2.90^{bc}
Timothy hays (H)	53.7^{def}	33.27^{de}	13.30^{cd}	5.69^{cd}	2.52^{def}
Timothy hays (L)	46.4^{g}	29.41^{fg}	10.04^{ef}	5.23^{def}	3.09^{ab}
Wheaten hays (H)	55.0^{cde}	33.55^{cd}	13.25^{cd}	5.90^{bc}	2.55^{cdef}
Wheaten hays (L)	46.3^{g}	29.48^{fg}	10.57^{e}	4.91^{efg}	2.79^{bcd}
SEM	0.751	0.432	0.263	0.128	0.049
p	<0.001	<0.001	<0.001	<0.001	<0.001

Abbreviations: H: high-nutritive value; L: low-nutritive value; SEM: standard error of the mean; VFA: volatile fatty acid. Different superscript letters (a–h) within a row indicate significant differences between treatment means ($p<0.05$; LSD test).

(e.g., NDF) limit microbial accessibility can lead to slower microbial breakdown and lower GP during the fermentation (Oba and Allen 1999), while high WSC and ME indicate feeds with more digestible components that can be easily fermented and result in higher GP due to increase of microbial activity (Amanzougarene and Fondevila 2020). Consequently, GP can be used as an indicator of feed fermentability and digestibility. While IVDMD and GP provide valuable in vitro estimates of feed fermentability, their direct relevance to in vivo performance requires caution. In actual ruminant systems, factors such as feed intake, passage rate and microbial protein synthesis efficiency also influence nutrient utilisation and production outcomes. Higher IVDMD is generally associated with increased energy availability, which can potentially support greater milk yield and growth rate, but further in vivo studies are needed to validate this inference. Such within-species differences in digestibility and GP may originate from cultivar selection, environmental growth conditions and harvest timing (AEXCO 2016; Moran 2005). Even within the same forage species, these factors can significantly alter NDF digestibility and WSC content, thereby influencing microbial degradation rates (Hoffman et al. 2003; Ma et al. 2022). Hence, agronomic and post-harvest management practices play a critical role in shaping the fermentative potential of each roughage.

4.2 | Methane Production

High-nutritive value roughage had higher total CH_4 production per gram of DM or IVDMD as well as CH_4 density than low-nutritive value roughages. CH_4 density, defined as the volume of methane produced per unit of digestible substrate (mL CH_4/g IVDMD), serves as an indicator of the intensity of methanogenesis relative to the amount of digestible feed. This result was in line with previous research findings (Lee et al. 2011). This suggested that high-nutritive value roughages had relatively lower feed conversion efficiency than low-nutritive value ones. This is mainly because the high-nutritive value roughages are rich in easily fermentable carbohydrates, such as sugars and starches, which can lead to increased microbial fermentation activity in the rumen, particularly, those involved in methanogenesis (Li et al. 2021).

Among the different roughage types, high-nutritive value corn silage exhibited comparatively lower CH_4 emissions (3.56 mL) when compared to the average value of high-nutritive value hays (5.46 mL). This is likely due to silage fermentation, which reduces NFC content and leads to lower ruminal pH (Lee et al. 2011). The decrease in pH levels and propionate formation resulted in the inhibition of methanogen activity, thereby reducing methane production. This inhibition was further influenced by the decreased levels of acetate and butyrate and the subsequent increase in propionate production (Cieslak et al. 2013; Kumar et al. 2013). Cobellis et al. (2016) provided evidence demonstrating that the modulation of ruminal fermentation towards increased propionate levels can effectively suppress hydrogen-producing microbes. Moreover, a shift in VFA production from acetate towards propionate may reduce hydrogen availability for methanogens, as propionate formation acts as a competitive hydrogen sink. This shift is generally considered favourable for lowering methane emissions (Ungerfeld 2015).

However, it is noteworthy that high-nutritive value barley hay and oaten hay are still producing more methane than corn silage, even with lower pH and higher propionate generated. Rapid fermentation of WSC in the rumen may offset the methane-suppressing effects, allowing hydrogen-producing microbes to remain active (Kataria 2016). Notably, a relatively high variation in methane density was observed among different roughage types. This variability may originally come from differences in the fermentable carbohydrate profile, fibre digestibility, microbial community structure and fermentation kinetics among substrates (Ávila et al. 2011; Greening et al. 2019; Ma et al. 2022). High-quality roughages often promote rapid fermentation and hydrogen production and they potentially drive higher CH_4 emissions. In contrast, slower-fermenting low-quality roughages may show lower short-term CH_4 output but potentially accumulate more over extended digestion periods (Hess et al. 2006). This underscores the complexity of predicting methane emissions solely based on digestibility parameters and highlights the need for caution when extrapolating in vitro methane yield data to in vivo systems.

4.3 | Ammonia Nitrogen

Ammonia nitrogen (NH_3–N) concentration in the rumen reflects the balance between dietary protein supply and energy availability in the rumen; it can also reflect the microbial protein synthesis efficiency, environmental nitrogen emissions and overall animal health and productivity (Zhang, Shahzad, et al. 2022). Unlike the GP and IVDMD, the high-nutritive value roughages were not consistently higher in producing NH_3–N in the rumen than low-nutritive value roughages. Variation in NH_3–N levels may result from the combined effects of CP content and fermentable energy availability. The lack of consistent trends in NH_3–N levels across nutritive value levels of the same forage type further highlights intra-species variability. Variations in CP solubility, protein-fibre binding and synchrony with fermentable energy within the same species can markedly affect ruminal nitrogen utilisation (Zhang, Shahzad, et al. 2022). Efficient ruminal nitrogen utilisation depends not only on CP concentration but also on the synchronisation of nitrogen and energy supply. When fermentable carbohydrates are insufficient, ammonia cannot be effectively incorporated into microbial protein, leading to elevated NH_3–N levels and nitrogen loss via urea excretion (Sinclair et al. 2009). This highlights the importance of synchronising dietary energy and nitrogen availability for efficient microbial utilisation and reduced nitrogen losses.

4.4 | VFA

VFA are key end-products of rumen microbial fermentation that supply energy for maintenance and production (Mahboubi et al. 2022). Acetate, propionate and butyrate are key VFAs produced in the rumen that are related to tissue and milk fat synthesis, energy supply and lactose production, as well as energy supply and gut development (Baldwin and Connor 2017). A:P ratio in the rumen is an important indicator of the overall fermentation, energy metabolism and production efficiency in ruminants (Wang et al. 2023). A lower A:P ratio indicates a shift towards propionate-dominated fermentation, which is associated with increased energy availability and potentially increase energy efficiency and animal performance (Amir Mahboubi et al. 2022; Wang et al. 2023).

When comparing high-nutritive value hays and low-nutritive value hays, high-nutritive value hays tended to produce relatively higher total VFA and propionic acid levels compared to their low-nutritive value counterparts, while they also showed variable acetic and butyric acid production. The lower A:P ratios in high-nutritive roughages were observed (2.49 vs. 2.84) compared with low-nutritive roughages. Therefore, these low A:P values primarily suggested a more propionate-favourable fermentation profile which caused lower ruminal pH (6.24 vs. 6.38) and led to higher overall energy efficiency. Among individual roughage types, corn silage (A:P ratio = 2.05) and oaten hay (A:P ratio = 2.43) seem to be more energy-efficient than other roughages. Acetate supports lipogenesis and is closely linked to milk fat synthesis, whereas propionate is a primary gluconeogenic precursor supporting lactose production and energy metabolism (Baldwin and Connor 2017). Thus, a lower A:P ratio may reflect a more energy-efficient fermentation pathway with implications for both productivity and methane mitigation. Our observations of VFA profile shifts within the same forage species across quality levels support the notion that even minor differences in composition can redirect fermentation pathways. This intra-species variability in fibre digestibility and carbohydrate availability may alter VFA proportions, energy yield and associated methane outcomes. Despite variations in A:P ratios resulting from different feed types and qualities, it has been acknowledged that these ratios are primarily influenced by the degradation rate of the feed and the composition of the rumen microbial community structure (Lin et al. 2020). Further investigation is warranted to elucidate the role of rumen microbiota in modulating fermentation efficiency across roughage types.

Overall, the observed differences across roughage types and quality levels illustrated the complex interaction among feed digestibility, fermentation profile and emission potential. Optimising roughage selection thus required an integrated evaluation of both nutritive value and environmental impact, especially under the growing demand for sustainable ruminant production systems.

5 | Conclusion

High-nutritive value roughages were correlated with increased IVDMD, GP, methane emissions as well as lower A:P ratio, suggesting a more intensive fermentation process. Among high-nutritive value roughages, both barley hay and oaten hay emerge as high in nutritive fermentation value. Notably, oaten hay exhibited a more favourable fermentation profile with its higher digestibility and relatively lower methane emissions per gram of IVDMD, along with moderated NH_3–N concentrations than barley hay. This may indicate that despite its strong fermentation characteristics of oaten hay, it may offer a more environmental favourable option to lower greenhouse gas emissions per unit of nutrient digested.

Among low-nutritive value roughages, oaten hay and corn silage stand out with relatively greater IVMDM and VFA production. These roughages also demonstrated a relatively balanced methane output and ruminal NH_3–N profile compared to other low-quality roughages. This highlights their potential to maintain efficient fermentation without releasing excessive greenhouse gas emissions. However, these in vitro findings should be validated in in vivo experiments to fully understand their implications in actual ruminant metabolism and environmental impact.

In conclusion, this study provides comparative insights into the fermentation performance of seven commonly used dairy roughages at two distinct nutritive levels. High-nutritive value oaten and barley hay promoted digestibility and VFA production, but they were associated with increased methane emissions. This result highlighted the importance of balancing nutritional benefits with environmental impacts. These findings provide valuable evidence for formulating roughage-based dairy rations that are both productive and sustainable.

Acknowledgements

We express our sincere gratitude to AgriFutures for the financial support provided for project [PRJ-012649]. Our thanks extend to Yi Ma, Hassan Khanaki and Hang Zhi for their partial contributions to the laboratory work, demonstrating an invaluable commitment to our research objectives. We are also grateful to Nathan Anderson, Farm Manager at the University of Melbourne and Louie Minoza, Coordinator of Dookie Dairy for their indispensable support of our fieldwork. Special acknowledgments are due to Pat Guerin and Yvette Williams from Australian export fodder and dairy industry for their insightful discussions. Furthermore, we would like to thank the University of Melbourne, Melbourne TrACEES Platform for providing the necessary testing services that were crucial to our project's success. Open access publishing facilitated by The University of Melbourne, as part of the Wiley - The University of Melbourne agreement via the Council of Australian University Librarians.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from AgriFutures Australia. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from the author(s) with the permission of AgriFutures Australia.

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