J. Dairy Sci. 107:10932–10944
https://doi.org/10.3168/jds.2024-25274

Hydroponic fodders as alternative feeds for ruminants to reduce ruminal methane emissions: An in vitro study

Yang Li,[1] Rong Peng,[1] Carmen Kunz,[1] Kai Wang,[1] Melissa Terranova,[2] Yixin Zhang,[1] Monika Macsai,[3] Emmanuel Frossard,[3] and Mutian Niu[1]*
[1]Animal Nutrition, Institute of Agricultural Sciences, Department of Environmental Systems Science, ETH Zürich, 8092 Zürich, Switzerland
[2]AgroVet-Strickhof, ETH Zürich, 8315 Lindau, Switzerland
[3]Plant Nutrition, Institute of Agricultural Sciences, Department of Environmental Systems Science, ETH Zürich, 8092 Zürich, Switzerland

ABSTRACT

Malate, a precursor in the ruminal propionate production pathway, competes with methanogenesis for metabolic hydrogen, offering a way to reduce ruminal methane (CH_4) production in ruminants. However, cost considerations hinder widespread use of malate in ruminant diets. An alternative approach involves use of transient malate levels generated during seed germination via the glyoxylate cycle. This study investigated the methane-mitigating potential of malate-containing hydroponic fodder. Fodder samples with peak malate concentrations from alfalfa, forage pea, Italian ryegrass, rye, soybean, triticale, and wheat during germination were subjected to in vitro rumen fermentation using the Hohenheim gas test. The basal diet of in vitro fermentation comprised 40% grass silage, 40% maize silage, 15% hay, and 5% concentrate on a DM basis, with nutritional characteristics including 42.1% NDF, 25.0% ADF, 14.0% starch, 12.7% CP, and 3.5% ether extract, on a DM basis. Experimental treatments were fodder inclusion involving replacing 20% of the basal diet (20R) and, additionally, 100% replacement of the silages with alfalfa d 10 and rye d 9 (SR), the 2 high-malate fodders. Reductions in CH_4 production were observed with soybean (20R, 6.7% reduction), alfalfa (20R, 6.6% reduction), and increased with rye (20R, 6.3% increase). In the setup replacing silages with high-malate fodders (SR), alfalfa decreased CH_4 production (17.7%) but increased ammonia (174%), whereas rye increased CH_4 production (35.8%). Organic matter digestibility increased with SR rye (12.6%). Marginal effects of dietary variables were analyzed in a generalized additive model. A negative relationship between dietary malate content and CH_4 production was observed, whereas dietary NDF and starch content were positively correlated with CH_4 production. In conclusion, malate within the hydroponic fodder could potentially reduce CH_4 emissions in ruminants. However, achieving sufficient efficacy requires high malate content. Additionally, use of hydroponic fodder may increase the risk of nitrogen emissions. Animal studies are required for further investigation.

Key words: malate, rumen fermentation, enteric methane

INTRODUCTION

Greenhouse gases are the primary driver of climate change, contributing to increases in the frequency and severity of extreme weather events such as heatwaves, floods, and wildfires worldwide (NASEM, 2016), thereby impacting global agricultural productivity and sustainability (Wehner et al., 2017). Moreover, the world will need to accommodate an additional 2 billion people, predominantly in Africa, a region already grappling with severe drought (FAO, 2021). Thus, to ensure global food security, it is imperative to mitigate GHG emissions with sustainable agricultural practices.

Methane (CH_4) is a potent GHG, with a global warming potential 28 times higher than that of carbon dioxide (CO_2) over 100 years (IPCC, 2014). Despite its potency, CH_4 has a relatively short atmospheric half-life of 8.6 years (Muller and Muller, 2017), making the mitigation of CH_4 emissions particularly effective in reducing the near-term impact of climate change. Approximately 17% of anthropogenic CH_4 emissions stem from enteric fermentation of ruminant livestock (Knapp et al., 2014). Ruminant livestock play a crucial role in converting human-inedible biomass into high-quality protein and fat. They rely on the complex microbiome within the rumen to ferment feed into VFA such as acetate, propionate, and butyrate, which serve as energy sources for the animals. However, this fermentation process also generates hydrogen (H_2) as a by-product, creating an ecological niche for methanogens.

Rumen methanogens derive energy exclusively through methanogenesis, the process of reducing CO_2 or

Received June 8, 2024.
Accepted August 26, 2024.
*Corresponding author: mutian.niu@usys.ethz.ch

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

methylated compounds to CH_4 by utilizing H_2 as a source of reducing potential (Janssen, 2010). They represent the primary source of enteric CH_4 emissions. Efforts to inhibit enteric CH_4 without adversely affecting the productivity of livestock have generated significant attention. For example, antibiotic ionophores such as monensin can increase ruminal propionate molar proportions and decrease CH_4 production by inhibiting gram-positive bacteria and protozoa, which reduces H_2 production and substrate availability to methanogens (Callaway and Martin, 1997; Rezaei Ahvanooei et al., 2024). However, due to concerns about antimicrobial resistance emergence (Russell and Houlihan, 2003), monensin usage as growth promoter on livestock has been banned in the EU since 2006. Along the lines of H_2 manipulation, another promising approach involves diverting H_2 away from methanogenesis through malate supplementation. Malate is an organic acid and an intermediate substrate in the propionate production pathway (Ungerfeld and Forster, 2011; Figure 1), where it incorporates H_2 and competes with methanogenesis for metabolic H_2 (Hook et al., 2010). In vitro studies have demonstrated that the supplementation with 8 and 12 m*M* malate reduced CH_4 production by 15% and 27%, respectively (Martin and Streeter, 1995). Additionally, supplementation with 7.5% DM pure malate reduced CH_4 production by 16% in beef cattle (Foley et al., 2009). A meta-analysis by Ungerfeld and Forster (2011) indicated a stoichiometry of −0.13 mol CH_4 per mole of malate used in batch culture. However, the use of malate in ruminant diets is significantly constrained by its cost, due to the high inclusion levels required.

This study delves into a natural source of malate: hydroponic fodder seedlings, which offer a sustainable feed option for smallholding farmers or during drought conditions (Gebremedhin, 2015; Shit, 2019; Kidane and Dagnachew, 2022). Notably, it was discovered that 9-d-old alfalfa (cv Alfagraze) seedlings can contain as much as 7.5% DM of malate, with this concentration gradually decreasing as the plant matures (Callaway et al., 1997). This unusually high malate concentration stems from the glyoxylate cycle during germination, occurring

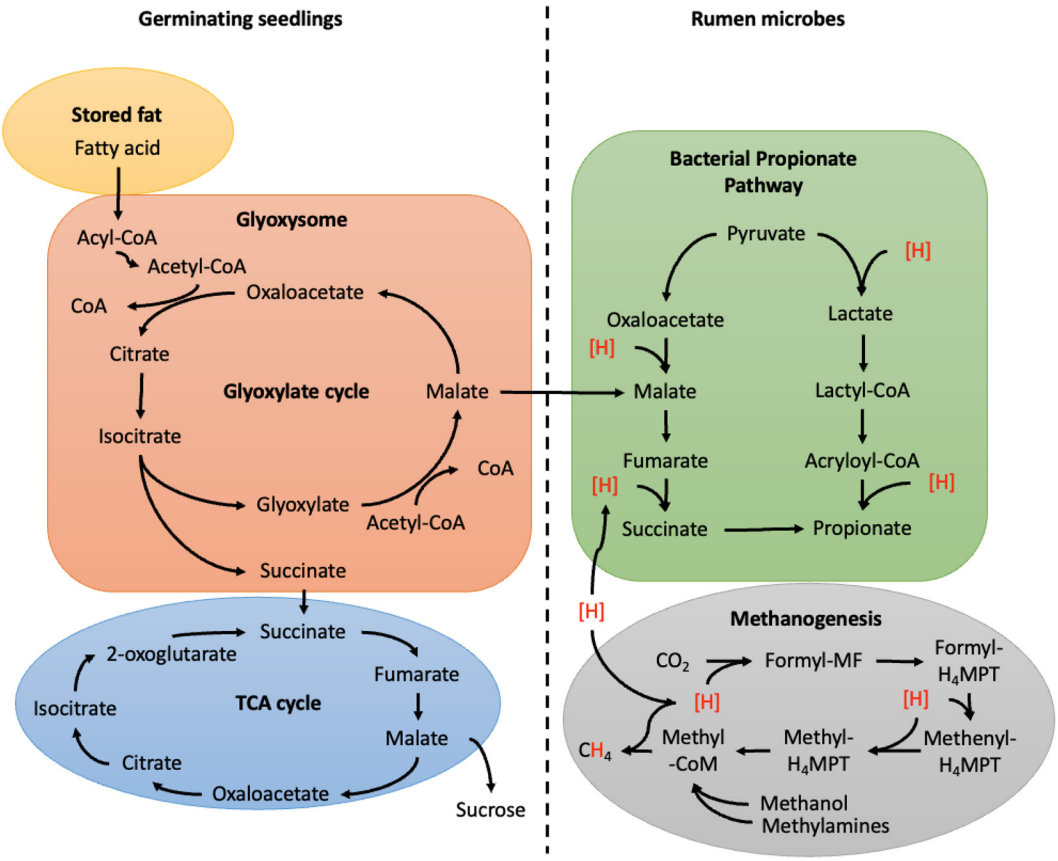

Figure 1. The role of malate from the glyoxylate cycle in propionate production and competition with methanogenesis. Known pathways for glyoxylate cycle in germinating seedlings, propionate production in rumen bacterial fermentation, and simplified methanogenesis pathway in rumen methanogens. [H] = metabolic hydrogen; MF = methanofuran; H_4MPT = tetrahydromethanopterin; CoM = coenzyme M; TCA = tricarboxylic acid. The molar number of [H] was not balanced.

within transient glyoxysomes that catalyze the mobilization of stored fat in seeds into sugar (Graham, 2008). Malate emerges as an intermediate product of this cycle, explaining the fluctuation in concentration during seed germination and growth. However, there remains a lack of detailed characterization regarding the profile of organic acids as the seeds germinate. We hypothesized that malate-containing hydroponic fodders have potential as an alternative feed source for enteric CH_4 mitigation. The objectives of this study were (1) to explore changes in malate and other organic acid concentrations in seedlings during germination, selecting various species commonly used for grass and legume fodder to investigate the potential for elevated transient malate concentrations during germination; and (2) to assess the effects of malate supplied via hydroponic fodders on ruminal CH_4 production through in vitro rumen fermentation.

MATERIALS AND METHODS

Hydroponic Germination

Seven seeds of commonly used grass and legume fodder were obtained from the Union des Fédérations Agricoles AG (Herzogenbuchsee, Switzerland), including forage pea, wheat (cv Poncione), Italian ryegrass (cv Morunga, 4n), alfalfa (cv Cannelle), rye (winter rye, cv Serafino), triticale (winter triticale, cv Triangoli), and soybean (cv Galice). These seeds and peas underwent a 10-d germination process in a controlled growth chamber with natural light, maintaining temperatures ranging from a minimum of 12.9°C to an average of 20.7°C, with a maximum of 23.4°C, and 60% relative humidity. The seeds and peas were cultivated on multiple 18-cm × 22-cm trays, with each tray containing 10 g of alfalfa seeds; or 30 g of wheat, Italian ryegrass, rye, and triticale seeds; or 40 g of forage pea and soybeans. Before germination, seeds were soaked in distilled water and covered with black and white polyamide film, with the white side facing outward to minimize evaporation. Subsequently, the seedlings were watered twice daily at 0800 and 1700 h. Three trays of alfalfa and one tray of other seedlings were harvested daily following the morning watering and immediately frozen.

Organic Acid Analysis

Frozen fodder samples underwent freeze-drying (Christ Gamma 1-16 LSC, Adolf Kuhner AG, Basel, Switzerland) and were ground to a particle size of 1 mm (Retsch ZM 200, Schieritz & Hauenstein AG, Arlesheim, Switzerland). The content of organic acids, including malate, fumarate, citrate, quinate, and succinate, was determined following the method outlined by South (1996). Each sample, comprising approximately 300 mg of DM, underwent initial extraction by homogenization with 5 mL of 0.1 *M* sulfuric acid, followed by agitation for 30 min and subsequent centrifugation at 16,000 × *g* for 5 min at room temperature (Eppendorf 5418, Eppendorf AG, Hamburg, Germany). The resulting supernatant was filtered through a 0.45-µm syringe filter. For each organic acid, a standard from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany) was used to generate an external standard curve. Subsequently, 100 µL of the sample was injected into an HPLC (model Chromaster, equipped with a UV/VIS-detector, Merck-Hitachi, Hitachi Ltd., Tokyo, Japan) and detected at 210 nm. The mobile phase, consisting of 0.10 *M* sulfuric acid, was maintained isocratically at a flow rate of 0.5 mL/min through a Column Aminex HPX-87H (300 mm × 7.8 mm; Bio-Rad, Hercules, CA).

In Vitro Fermentation

A 24-h in vitro incubation of the hydroponic fodders was conducted using the Hohenheim gas test method as described by Menke and Steingass (1988). Rumen fluids for in vitro incubations were collected from 3 lactating Original Brown-Swiss rumen-cannulated cows housed at AgroVet-Strickhof (Lindau, Switzerland), according to the approved license ZH115/2022 of the Cantonal Veterinary Office in Zürich, Switzerland. The cows were fed a TMR comprising grass silage, corn silage, ensiled sugar beet pulp, concentrates (AgroVet Thalheim Mix 2020, Getreidesammelstelle und Futtermühle, Thalheim, Switzerland), hay, and minerals at proportions of 54.4%, 14.5%, 14.5%, 11.7%, 4.7%, 2.3%, and 0.9% DM basis, respectively. Rumen fluid from each cow was collected before morning feeding and treated as a biological replicate. The rumen fluid was immediately stored in a prewarmed thermos bottle and filtered through 4 layers of gauze (1-mm pore size) to remove solid particles before use.

The basal diet used for the in vitro incubation consisted of 40% grass silage, 40% maize silage, 15% hay, and 5% concentrates. The chemical compositions of the basal diet were as follows, on a DM basis: 42.1% NDF, 25.0% ADF, 14.0% starch, 12.7% CP, 3.5% ether extract (**EE**). Each of the 7 hydroponic fodder was included at 20% DM replacement of the basal diet. Additionally, the hydroponic fodders with the highest malate content (alfalfa and rye) were also tested with 100% silage replacement (**SR**; 80% hydroponic fodder, 15% hay, 5% concentrates). Purified malate (Sigma, St. Louis, MO) was used as a positive control at a dosage of 12 m*M*, as demonstrated to inhibit CH_4 production (Martin and Streeter, 1995). Each treatment was conducted in 3 technical replicates within each

biological replicate, resulting in a total of 9 replicates. The group assignment was not masked. Before incubation, the pH and ammonia (NH_3) concentration of all rumen fluids were measured, ranging from 6.55 to 6.80 and 2.09 to 14.6 mmol/L, respectively. A buffer was prepared according to Menke and Steingass (1988) and continuously sparged with carbon dioxide (CO_2). Rumen fluids were added to the prewarmed buffer (39°C) in a ratio of 1:2 (rumen fluid:buffer). Each incubation run used rumen fluid from a single cow. Scaled glass syringes with 2 outlets (Soliva and Hess, 2007) were prepared, each containing 200 mg of basal diet alone. Each syringe was assigned by unrestricted randomization. A total of 30 mL of buffered rumen fluid was then added to each glass syringe. Each syringe was incubated for 24 h in a rotating forced-air incubator at a constant temperature of 39°C (Binder Ltd., Tuttlingen, Germany), with each treatment incubated in triplicates following the protocol described by Soliva and Hess (2007). Each experimental run also included 3 blanks without any feeds. After the 24-h incubation, the total gas volume produced was recorded, and gas profile was measured for each syringe. The buffered rumen fluids were then collected from the syringes for subsequent analyses. The pH and NH_3 were measured within 5 min of incubation termination. The sample for VFA measurement was centrifuged at 3,200 × *g* for 5 min at room temperature and stored in microcentrifuge tubes at −20°C until HPLC.

Chemical and Gas Production Analyses

Analyses of DM and OM contents in basal diet were conducted using an automated thermogravimetric analyzer (TGA 701, Leco Corporation, St. Joseph, MI) following the methods outlined in AOAC International (1997) index no. 942.05 and by Thiex et al. (2012). Specifically, OM was calculated as DM − ash. The chemical compositions of fodder samples and the basal diet were determined according to AOAC International (1997) guidelines. The NDF and ADF contents were assessed using a Fibertec System M 1020 Hot Extractor and a 1021 Cold Extractor (Tecator, Högamäs, Sweden), following the protocol of Van Soest et al. (1991). These values were expressed without residual ash. The EE contents were determined using ether by Soxhlet extractor (Extraction System B-811, Büchi, Flawil, Switzerland; AOAC International, 1997, index no. 963.15). Nitrogen (N) contents were measured with a C/N analyzer (TruMac CN, Leco Corporation, St. Joseph, MI; AOAC International, 1997, index no. 968.06), and CP contents were calculated as 6.25 × N. Starch was extracted as described by Smith and Zeeman (2006) and subsequently quantified using a spectrophotometer at 340 nm (UV-6300PC, double-beam spectral photometer, VWR International GmbH, Dietikon, Switzerland). As some fodder, such as alfalfa, are known to contain saponin, which has CH_4-mitigation properties, saponin was quantified. The quantification of total saponins followed the method outlined by Le et al. (2018), with samples undergoing 3 ethanol extractions using an ultrasonic bath for 10 min each. Subsequently, they were subjected to vanillin-sulfuric acid treatment in a water bath at 60°C for 15 min, followed by a cooling period of 5 min. The solution was then measured at 560 nm using a UV-Vis spectrophotometer (UV-6300PC, double beam, VWR International GmbH, Dietikon, Switzerland). Total saponin content was expressed as milligrams of escin equivalent per gram of feed. All samples were analyzed in duplicates.

The pH and NH_3 concentration of incubated buffered rumen fluids were directly measured using a Metrohm pH meter model 632 with a glass electrode (6.0204.100) and model 713 with electrode (6.0506.100), respectively (Metrohm, Herisau, Switzerland). Fermentation gas samples were analyzed for CH_4, H_2, and CO_2 concentrations using a gas chromatograph (6890N, Agilent Technologies, Wilmington, DE) equipped with a thermal conductivity detector, following the method described by Soliva and Hess (2007). Concentrations of VFA in the incubation fluids were analyzed using HPLC following the method outlined by Ehrlich et al. (1981).

Calculations and Statistical Analysis

To obtain the net gas production, the total gas production from blanks was subtracted from the total gas production of all incubation units.

The in vitro OM digestibility (**IVOMD**) and ME were calculated according to Menke and Steingass (1988) using the following equations:

$$\text{IVOMD (\%)} = 14.88 + 0.889 \times \text{total gas production (mL 200/mg DM)} + 0.448 \times \text{CP (g/kg DM)} + 0.0651 \times \text{ash (g/kg DM)}; \quad [1]$$

$$\text{ME (MJ/kg DM)} = 0.46 + 0.1181 \times \text{total gas production (mL/200 mg DM)} + 0.0088 \times \text{CP (g/kg DM)} + 0.0247 \times \text{EE (g/kg DM)} + 0.0036 \times \text{N free extract (g/kg DM)}. \quad [2]$$

Data were analyzed via a mixed-effect model using the *lmer* procedure (Bates et al., 2015) using R statistical language (R Core Team, 2022; version 4.2.1). The model is shown below:

$$Y_{ijk} = \mu + T_i + C_j + e_{ijk},$$

where Y_{ijk} is the variable of interest, μ is the overall mean, T_i the treatment effect of hydroponic fodder supplementa-

tion (i = 1 to 11), C_j is the random effect of donor cow, and e_{ijk} is the residual error (Sun et al., 2024). In all analyses, data points with studentized residuals outside of ±3 were considered outliers and were removed from the analysis. No more than 8 entries were removed in all analysis combined. Multiple comparisons were performed using Tukey's post hoc test. Significance and tendency were declared at $P < 0.05$ and $0.05 < P < 0.10$, respectively.

To check whether NDF content is solely responsible for changes in CH_4 production, CH_4 production was normalized to 200 mg of NDF. Because the contents of dietary NDF, EE, and starch, which may influence CH_4 production, varied across treatments, we estimated the marginal responses of CH_4 production using the generalized additive model (**GAM**). This data-driven non-parametric method also incorporated the random effect of the cow into the model, as per Andersen (2009). The effect of dietary variables, including EE (%), malate (m*M*), NDF (%) and starch (%), on CH_4 production per 200 mg of DM was assessed by GAM. The 12 m*M* malate group and the rye d-9 SR group were excluded from the model fitting as outliers due to their unusually high malate content (12.0 m*M*) and starch content (58.6%), respectively. Smoothing terms and variable effects of the fitted GAM were estimated and visualized using mgcv and mgcViz packages in R (Wood and Wood, 2015; Fasiolo et al., 2020). The performance of GAM was evaluated using adjusted R^2 value.

RESULTS

Hydroponic Fodder Parameters

The weight of fodders at harvest and the freeze-dried weight are summarized in Supplemental Table S1 (see Notes). The chronological content of malate is presented in Figure 2a and Supplemental Table S2 (see Notes). Similarly, the chronological contents of citrate, fumarate, quinate, and succinate are outlined in Supplemental Tables S3–S6 (see Notes). Sampling time points with the highest malate contents of each hydroponic fodder were chosen to be tested in vitro: alfalfa (d 6, d 10), wheat (d 7), triticale (d 10), rye (d 9), Italian ryegrass (d 9), forage pea (d 10), and soybean (d 10). As 2 peaks were observed for the malate content in alfalfa, 2 time points were chosen. The high malate content of Italian ryegrass at d 0 was suspected to be contaminated by the prior sample, d-10 rye, despite the sieve and centrifuge mill being cleaned by compressed air between each sample. Italian ryegrass d 0 was therefore excluded. The nutritional parameters of the selected hydroponic fodders are summarized in Supplemental Table S7 (see Notes), and the saponin contents of hydroponic fodders are included in Supplemental Table S8 (see Notes).

Ruminal Gas Production and Fermentation

The nutritional parameters of the incubated feeds are summarized in Table 1, and Table 2 provides a summary of the in vitro fermentation characteristics. The 12 m*M* malate positive control decreased CH_4 production (mL/200 mg DM) by 29.4% ($P < 0.05$). It also decreased CH_4 production per NDF (mL/200 mg NDF) by 15.4% ($P < 0.05$). This CH_4 reduction was accompanied by decreased total gas H_2, CO_2 production, IVOMD, pH, and butyrate molar proportions ($P < 0.05$), and an increased NH_3 concentration ($P < 0.05$; Table 2).

The CH_4 production (mL/200 mg DM) decreased for the alfalfa d-10 replacing 20% of the basal diet (**20R**), alfalfa d-10 SR, and soybean 20R by 6.6%, 17.6%, and 6.7% ($P < 0.05$), respectively (Figure 3). The declines in CH_4 production for alfalfa d-10 20R, alfalfa d-10 SR, and soybean 20R were accompanied by concomitant decreases in total gas production, H_2 production, and CO_2 production ($P < 0.05$), but also a simultaneous increase in NH_3 concentration ($P < 0.05$; Table 2). Surprisingly, rye 20R and rye SR increased the CH_4 production by 6.26% and 35.8% ($P < 0.05$), respectively (Figure 3), with a concomitant increase observed in total gas, CO_2 and H_2 production, ME, and IVOMD from rye SR ($P < 0.05$; Table 2). Although the difference in pH was significant between alfalfa d-10 20R, alfalfa d-10 SR, and rye SR compared with the control, the sizes of the differences were minute and likely lack biological significance.

The ruminal VFA profile of fermentation was summarized in molar proportions (Table 3) and concentrations (Supplemental Table S9; see Notes). The total VFA concentration was increased by 11.7% for rye SR. The molar proportions and concentrations for acetate, propionate, and isobutyrate remain unchanged. The changes in VFA absolute percentage molar proportions are indicated as **%pt**. The butyrate molar showed a tendency to reduce by 1.34%pt (0.93 m*M*) for alfalfa d-10 SR ($P < 0.05$) and increased by 1.49%pt (1.03 m*M*) and 1.71%pt (1.94 m*M*) for forage pea 20R and rye SR ($P < 0.05$), respectively. The valerate molar proportions increased by 0.496%pt (0.304 m*M*) and 0.506%pt (0.337 m*M*) for alfalfa d-10 SR and triticale 20R ($P < 0.05$), respectively. The isovalerate molar proportion increased by 0.858%pt (0.276 m*M*) for alfalfa d-10 SR ($P < 0.05$).

Modeling the Effect of Dietary Malate

The GAM model was used to assess the response of the CH_4 production per 200 mg of DM (adjusted R^2: 0.857) along a range of dietary variables including EE, malate, NDF, and starch contents in the in vitro fermentation (Figure 4). The model explained 86.7% of deviance. Within the bounds of the available data in this study, the increase

in malate content exhibited a near linear, negative effect on ruminal CH_4 production ($P < 0.05$). Additionally, the increases in dietary NDF and starch content both led to an increase in CH_4 production ($P < 0.05$), with the effect from starch being more nonlinear. The EE content was found to have little effect ($P = 0.46$). It is important to note that, although inclusion of rye SR in the GAM model produced similar result (Supplemental Figure S1; see Notes), its removal was necessary to maintain the stability of the model.

DISCUSSION

Link Between Glyoxylate Cycle Metabolites and Propionate Production Pathway

Among the selected hydroponic fodders, only 10-d-old alfalfa and soybean seedlings reduced ruminal CH_4 production while also reducing total gas production (Table 2; Figure 3). In contrast, feeding 9-d-old rye seedlings increased ruminal CH_4 production.

Some intermediate products of the glyoxylate pathway in the seedlings fueling the propionate production pathway (Figure 1) may partly explain these results. Malate and fumarate could mitigate ruminal CH_4 production by competing with methanogens for metabolic H_2 (Hook et al., 2010), as H_2 is incorporated downstream in the propionate production pathway. Although succinate is also a part of the propionate production pathway, it does not incorporate H_2 (Ungerfeld and Forster, 2011). Therefore, the transiently high succinate contents of alfalfa, soybean, and forage pea (Supplemental Table S6) were unlikely to contribute to the observed CH_4 mitigation effects. However, as an intermediate of the glyoxylate cycle, succinate may indicate its transient activity during germination, suggesting that forage pea may indeed benefit from longer growth than the 10 d studied here. The analyses showed that the fumarate content was negligible in the hydroponic fodders (Supplemental Table S3) and was therefore unlikely to have influenced the results. However, the malate profiles showed that the alfalfa seeds had a significantly higher baseline malate

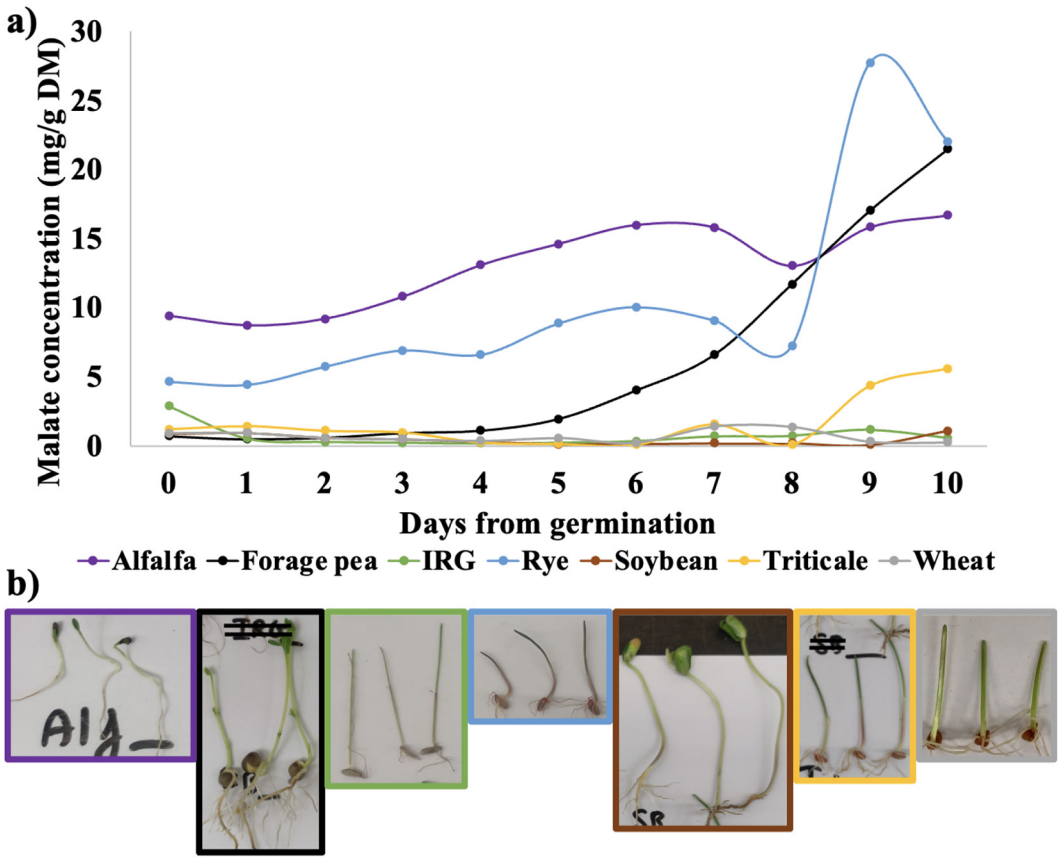

Figure 2. The malate content of hydroponic fodder during germination (d 0–10). (a) Chronological malate content (mg/g DM) of hydroponic fodders. (b) Morphology of hydroponic fodders with highest malate content: alfalfa d 10, forage pea d 10, wheat d 7, triticale d 10, rye d 9, Italian ryegrass (IRG) d 9, and soybean d 10.

Table 1. Nutritional parameters of feed incubated in vitro[1]

Feed composition (%)	Control	12 m*M* malate	Alfalfa d 6	Alfalfa d 10	Alfalfa d-10 SR	Forage pea d 10	IRG d 9	Rye d 9	Rye d-9 SR	Soybean d 10	Triticale d 10	Wheat d 7
DM	93.6	95.0	93.4	93.7	92.8	93.6	93.7	93.7	92.8	93.9	93.7	93.4
EE	3.47	2.78	4.28	3.75	4.55	3.04	3.02	2.99	1.55	5.25	3.20	3.08
RF	20.9	16.7	19.8	19.6	19.6	18.6	19.3	17.5	10.9	18.0	17.4	17.5
NDF	42.1	33.7	38.9	39.4	30.9	40.2	38.6	40.6	35.6	36.6	37.9	36.8
ADF	25.0	20.0	23.8	24.3	21.9	22.4	22.5	20.8	8.03	22.2	20.7	20.7
CP	12.7	10.2	18.1	18.7	37.4	15.1	12.9	12.3	11.7	18.6	13.0	13.0
Ash	6.80	5.44	6.18	6.30	5.83	6.38	6.20	5.86	4.09	6.79	5.85	5.89
Starch	14.0	11.2	11.6	11.3	15.8	19.5	15.0	22.0	58.6	12.1	22.9	22.5
OM	92.8	94.2	92.0	92.2	87.0	92.0	92.3	92.6	88.7	91.9	92.6	92.4
Nfe	47.0	52.9	40.8	44.4	18.6	46.8	47.1	46.5	43.7	43.3	46.4	47.8
Malate[2] (m*M*)	0	12	0.147	0.156	0.624	0.200	0.0109	0.259	1.04	0.0101	0.0523	0.0129

[1]EE = ether extract; RF = raw fiber; Nfe = nitrogen-free extract; IRG = Italian ryegrass; SR = silage replacement. All groups, with exception of control, 12 m*M* malate. Alfalfa d-10 SR and rye d-9 SR are 20R.

[2]Final concentrations of malate in the 30-mL incubation fluid.

Table 2. Effects of hydroponic fodder on fermentation parameters and gas composition (n = 9)[1]

	LSM													
Hydroponic fodder	Control	12 m*M* malate	Alfalfa d 6	Alfalfa d 10	Alfalfa d-10 SR	Forage pea d 10	IRG d 9	Rye d 9	Rye d-9 SR	Soybean d 10	Triticale d 10	Wheat d 7	SE	*P*-value
pH	6.81^{a}	6.77^{b}	6.83^{a}	6.87^{c}	6.88^{c}	6.80^{a}	6.79^{a}	6.80^{a}	6.70^{d}	6.82^{a}	6.80^{a}	6.80^{a}	0.009	<0.01
NH_3 (m*M*)	13.1^{a}	16.1^{b}	16.5^{b}	17.3^{bd}	28.5^{c}	15.6^{b}	14.1^{a}	13.4^{a}	12.6^{a}	18.1^{d}	14.2^{a}	13.8^{a}	0.47	<0.01
IVOMD (%)	70.0^{ac}	59.1^{b}	69.1^{ac}	68.2^{a}	68.8^{ac}	68.9^{ac}	67.7^{a}	70.8^{c}	82.6^{d}	68.2^{a}	70.0^{ac}	70.5^{ac}	0.91	<0.01
ME (MJ/kg DM)	10.0^{a}	8.86^{b}	9.97^{a}	9.86^{a}	9.72^{a}	9.93^{a}	9.77^{a}	10.2^{a}	11.8^{e}	9.91^{a}	10.2^{a}	10.2^{a}	0.236	<0.01
Gas production (mL/200 mg DM)														
Total gas	50.8^{ae}	40.6^{c}	47.4^{b}	45.9^{b}	37.5^{d}	48.4^{ab}	48.3^{ab}	52.4^{e}	67.2^{f}	45.3^{b}	51.1^{ae}	51.7^{ae}	1.02	<0.01
H_2	0.00898^{a}	0.00695^{be}	0.00752^{be}	0.00792^{be}	0.00491^{c}	0.00865^{ab}	0.00843^{ab}	0.00881^{ab}	0.0132^{d}	0.00730^{e}	0.00938^{a}	0.00927^{a}	0.000236	<0.01
CH_4	7.12^{a}	5.04^{b}	6.90^{ac}	6.65^{c}	5.86^{d}	7.13^{a}	6.87^{ac}	7.58^{e}	9.66^{f}	6.64^{c}	7.46^{ae}	7.47^{ae}	0.323	<0.01
CO_2	39.6^{af}	32.9^{b}	37.3^{ac}	36.2^{c}	29.3^{d}	38.2^{ac}	38.3^{ac}	41.5^{af}	52.9^{e}	35.9^{c}	40.3^{af}	40.5^{af}	1.36	<0.01
CH_4 production (mL/200 mg NDF)	33.8^{a}	28.6^{b}	35.6^{ac}	33.8^{a}	38.0^{c}	35.4^{ac}	35.6^{ac}	37.4^{c}	54.2^{d}	36.4^{c}	39.4^{e}	40.6^{e}	1.72	<0.01

[a–f]Different superscripts within a row indicate a significant ($P < 0.05$) difference.

[1]All values are displayed as 3 significant figures; SE are displayed in 1 additional decimal place. IVOMD = in vitro OM digestibility; IRG = Italian ryegrass. All groups, with exception of control, 12 m*M* malate. Alfalfa d-10 SR and rye d-9 SR are 20R. SR = silage replacement.

Table 3. Effects of hydroponic fodder on the VFA molar proportions in the incubated rumen fluid (n = 9)[1]

	LSM												
Hydroponic fodder	Control	12 m*M* malate	Alfalfa d 6	Alfalfa d 10	Alfalfa d-10 SR	Forage pea d 10	IRG d 9	Rye d 9	Rye d-9 SR	Soybean d 10	Triticale d 10	Wheat d 7	SE	*P*-value
Total VFA (m*M*)	58.3[a]	58.8[a]	55.2[b]	55.3[b]	56.4[a]	59.4[a]	55.7[b]	58.6[a]	65.7[c]	57.9[a]	58.3[a]	58.3[a]	0.76	<0.01
Acetate (%)	64.3	63.3	61.4	60.8	64.3	60.6	62.7	63.3	64.4	63.3	63.6	61.8	1.55	0.29
Propionate (%)	22.9	23.7	24.7	24.4	21.8	23.5	22.6	21.9	21.2	22.9	22.0	22.8	1.71	0.32
Butyrate (%)	11.2[a]	9.42[b]	11.3[a]	10.3[ab]	9.83[ab]	12.7[c]	11.8[a]	11.8[a]	12.9[c]	11.5[a]	11.8[a]	11.3[a]	0.430	<0.01
Isobutyrate (%)	0.925	1.03	1.03	1.59	0.850	1.14	1.11	0.894	0.731	0.808	0.959	1.17	0.2120	0.17
Valerate (%)	0.627[a]	0.678[ab]	0.802[ab]	0.820[ab]	1.12[b]	0.716[ab]	0.994[ab]	1.08[ab]	0.738[ab]	0.605[a]	1.13[b]	0.948[ab]	0.1700	<0.01
Isovalerate (%)	0.981[a]	0.837[a]	1.18[a]	1.48[ab]	1.87[ab]	1.43[ab]	0.937[a]	0.941[a]	1.21[a]	1.28[a]	1.27[a]	1.18[a]	0.2640	<0.01
A:P ratio	2.82	2.62	2.51	2.52	2.35	2.68	2.81	2.92	2.57	2.72	2.92	2.74	0.190	0.08

[a–c]Different superscripts within a row indicate a significant ($P < 0.05$) difference.

[1]All values are displayed as 3 significant figures; SE are displayed in 1 additional decimal place. IRG = Italian ryegrass; SR = silage replacement; A:P = acetate:propionate. All groups, with exception of control, 12 m*M* malate. Alfalfa d-10 SR and rye d-9 SR are 20R.

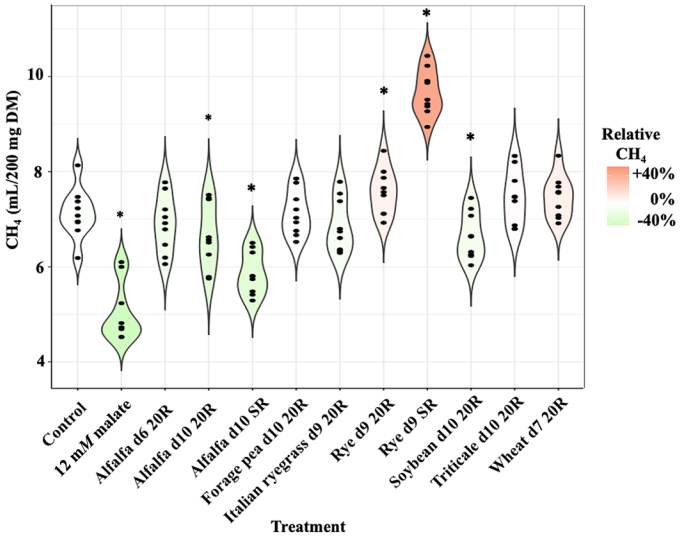

Figure 3. The CH_4-mitigating capability of hydroponic fodders. Violin plots of CH_4 produced from 200 mg of DM across different treatments. IRG = Italian ryegrass; 20R = 20% replacement; SR = silage replacement; colors indicate the changes in CH_4 production relative to the control. *P-value of the contrast between hydroponic fodder versus control <0.05. Control = baseline treatment with no replacement; 12 m*M* malate = fodder treated with 12 m*M* malate; alfalfa d6 20R = 20% replacement with alfalfa after 6-d hydroponic growth; alfalfa d10 20R = 20% replacement with alfalfa after 10-d hydroponic growth; alfalfa d10 SR = silage replacement with alfalfa after 10 d; forage pea d10 SR = silage replacement with forage pea after 10 d; Italian ryegrass d9 20R = 20% replacement with Italian ryegrass after 9 d; rye d9 20R = 20% replacement with rye after 9 d; rye d9 SR: silage replacement with rye after 9 d; soybean d10 20R = 20% replacement with soybean after 10 d; triticale d10 20R = 20% replacement with triticale after 10 d; wheat d7 20R = 20% replacement with wheat after 7 d. The CH_4 production was altered by −29.4%, −6.6%, −17.6%, +6.3%, +35.8%, and −6.7% for 12 m*M* malate, alfalfa d-10 20R, alfalfa d-10 SR, rye d-9 20R, rye d-9 SR, and soybean d-10 20R, respectively.

content compared with wheat, forage pea, and soybean, even before sprouting (Supplemental Table S2). According to the literature, the effectiveness of malate for CH_4 mitigation is directly proportional to the conversion of malate to propionate. For every mole of malate added, 0.48 mol is converted to propionate and 0.2 mol to acetate (Ungerfeld and Forster, 2011). Although the highest propionate molar proportions were observed with the 12 m*M* malate, the increment was not significant, suggesting that propionate may be insufficient to act as an indicator for malate efficacy in this study.

Effects of Dietary Malate on Ruminal CH_4 Production and Fermentation

In addition to malate, several dietary factors could affect CH_4 production, including NDF, starch, EE, and saponin contents. Dietary NDF is a positive predictor for enteric CH_4 production, and EE is a negative predictor for CH_4 production (Niu et al., 2018). Moreover, starch

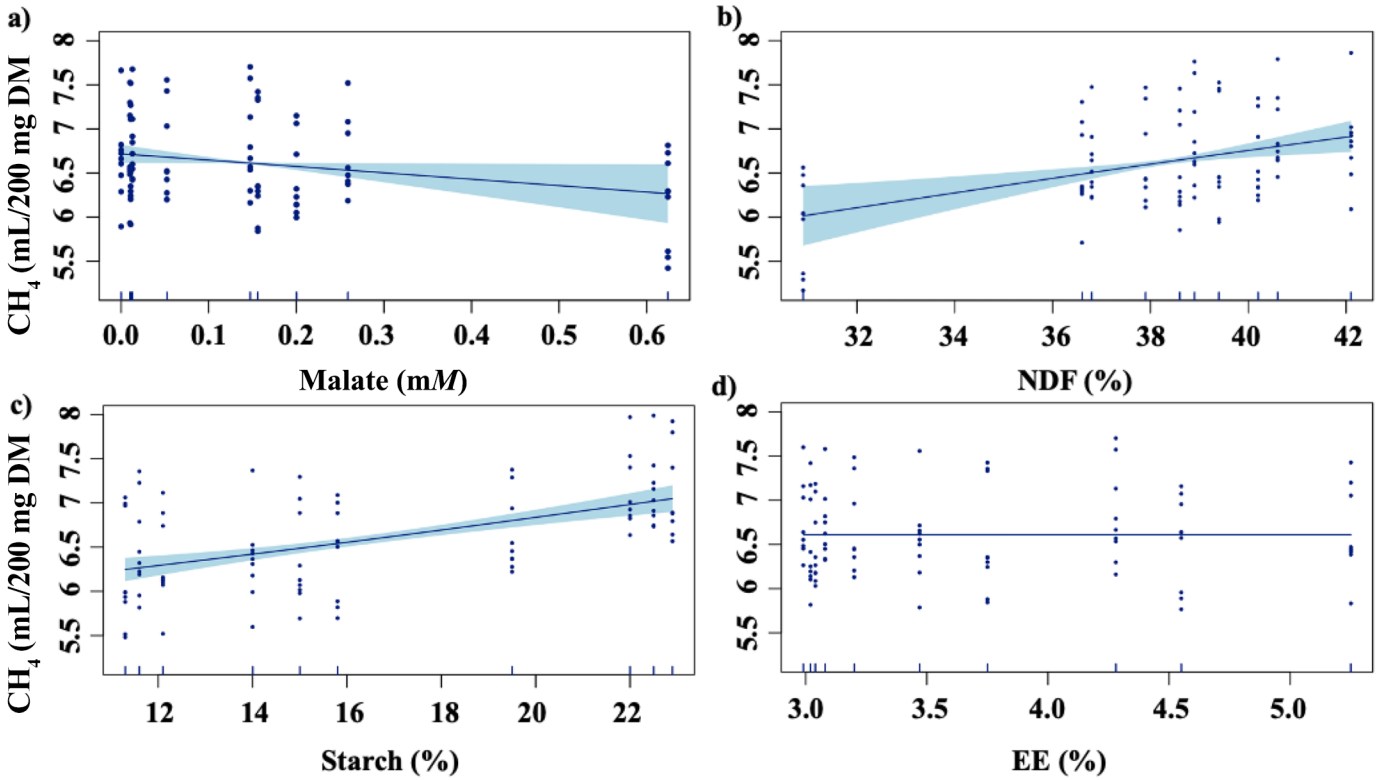

Figure 4. Response curves from the generalized additive model (GAM). The changes in CH_4 production from 200 mg of DM were best estimated by a combination of (a) malate ($P < 0.05$), (b) NDF ($P < 0.05$), and (c) starch ($P < 0.05$), with (d) ether extract (EE; $P = 0.46$) being not significant. The model deviance explained was 86.7%. The response patterns shown are partial effect splines from GAM, with shaded area indicating 95% credible intervals. The 12 m*M* malate group and the rye d-9 SR were excluded from this model as outliers in malate content and starch content. See Supplemental Figure S1 for the GAM model that includes the rye d-9 SR.

content has been found to be positively associated with ruminal CH_4 production in vitro (Jonker et al., 2016). We want to understand whether changes in CH_4 production are because of differences in a component called NDF in the feed. To compare fairly, we adjusted the CH_4 production based on the amount of NDF. Specifically, we looked at how much CH_4 was produced per 200 mg of NDF. Only the positive control group actually decreased CH_4 production under these conditions. This suggests that the NDF content in hydroponic fodders makes it tricky to interpret CH_4 production changes.

To address the confounding effects of the dietary parameters mentioned above on ruminal CH_4 production, the GAM framework was used to estimate the nonlinear response of CH_4 production to dietary malate, NDF, starch, and EE contents (Figure 4). The GAM elucidated that, within this study, malate content is negatively related to ruminal CH_4 production. This suggested that, despite the relatively low malate contents from hydroponic fodders in this study, they still exerted an effect to reduce ruminal CH_4 production, consistent with previous findings (Foley et al., 2009).

Both the NDF and starch contents were positively related to CH_4 production, thus confounding the CH_4-mitigating effect of malate. A most notable example of this confounding effect was the increased CH_4 production from rye 20R. Among the 20% replacement groups, rye 20R had the highest NDF content and the third highest starch content (Table 1), suggesting that its stimulatory effect on CH_4 production may be due to the combined effect of its high NDF and starch content, overshadowing any potential effect its malate content might produce. Among the 20% replacement of basal diet, soybean exhibited the lowest CH_4 production. Despite its low malate content, the GAM outputs suggested that the reduced CH_4 production of soybean was likely due to a combined effect of its low NDF and starch contents. Alfalfa d-10 20R had the lowest starch content among the 20% replacement treatments, likely working in concert with its malate content to reduce CH_4 production. Another fodder with high malate content was the forage pea 20R, which, similar to rye 20R, also had high NDF and starch contents, potentially negating the effect of malate.

Dietary fat, included in EE, is mostly not fermented by rumen microbes, yet unsaturated fatty acids may reduce ruminal CH_4 production through biohydrogenation (Jafari et al., 2016). However, in this study, the GAM indicated that EE has little effect on CH_4 production. This may be attributed to the samples not containing high proportions of unsaturated fatty acids. Saponins have the potential to mitigate enteric CH_4 production (Holtshausen et al., 2009), but the low saponin contents of the analyzed hydroponic fodders precluded them from being a key driver for ruminal CH_4 mitigation in this study (Supplemental Table S8).

Malate Varies According to Genetic, Chronological, and Environmental Influences

The information derived from the GAM model suggested that for the hydroponic fodder to effectively mitigate CH_4 production, the malate content must be sufficiently high to exceed the positive effect exerted from NDF and starch contents. The concentration of malate could be influenced by factors such as cultivar and growth conditions. The alfalfa seedlings grown in this study belong to the Cannelle cultivar, which exhibited far lower malate contents than the Alfagraze cultivar reported in Callaway et al. (1997). The Alfagraze cultivar was developed by polycrossing 30 parental plants to provide high-yielding, grazing-tolerant alfalfa pastures for livestock (Bouton et al., 1991). The difference in malate contents between the aforementioned Alfagraze cultivar and the Cannelle cultivar in this study could be due to either genetical differences between cultivars or environmental differences, as the high-malate Alfagraze cultivar was not cultivated in hydroponic conditions. The age of seedlings might affect different plant species in various ways. This can be seen from forage pea, which increased in malate as it developed, whereas soybean initially decreased and then increased as it developed. Therefore, additional research is necessary to identify the optimal species, cultivar, growth conditions, and harvest timing to acquire a high-malate fodder.

It may be impractical to reduce CH_4 emissions in animals using hydroponic fodders without a significantly high level of malate, given its relatively minor influence on CH_4 emissions. For instance, the 16% CH_4 mitigation in beef cattle was achieved by supplementing 7.5% of pure malate (Foley et al., 2009). Incidentally, the Alfagraze cultivar of alfalfa seedling also contains 7.5% DM malate (Callaway et al., 1997). Thus, to achieve the same amount of 7.5% DM pure malate diet for beef cattle using Alfagraze cultivar of alfalfa seedlings would require the diet to be composed almost completely of alfalfa, which could bring forth its own set of problems.

Malate supplementation may also have drawbacks in a dairy context. The positive control resulted in 29.4% decrease in CH_4 production, the highest in this study, but it also concomitantly decreased IVOMD, ME, butyrate molar proportions, total gas, and H_2 and CO_2 production. The numerical decrease in blood butyrate (a precursor of milk fat) and milk fat (g/d) observed when malate was added to the diet (Devant et al., 2007) suggested that higher amounts of malate could lead to decreased milk quality.

Potential Consequences of Silage Replacement

The intent of the SR groups was to test whether it is feasible to replace forage such as silage with hydroponic fodder, as some farmers in drought-prone regions are already doing (Ningoji et al., 2021). Two of the high-malate fodders, alfalfa d-10 20R and rye 20R, were selected to assess whether the SR would reduce CH_4 production. The replacement of 80% silage greatly amplified the aforementioned confounding effects from malate, NDF, and starch on ruminal CH_4 production, resulting in an even larger degree of CH_4 reduction from alfalfa d-10 SR and the highly elevated CH_4 production from rye d-9 SR. The larger amount of available energy from starch could have enhanced the fermentation, increasing total gas and CH_4 production, IVOMD, total VFA concentration, butyrate molar proportions, and ME observed from rye d-9 SR. This suggested that hydroponic fodder as a silage substitute may appear tempting, but nutritional and environmental challenges must be overcome. In addition to the diet imbalance problems described, the nutritional parameters of the selected hydroponic fodders have shown that all hydroponic feeds have a low raw fiber content. Because ruminants need structured feed for harmonious ruminal digestion (Oba and Allen, 1999), the low raw fiber and respective physically effective fiber contents could be challenging in a substitution scenario. However, as concentrates are rich in protein and starch, a combination of high-protein and high-starch hydroponic fodder could perhaps advantageously replace expensive concentrates in some places and should be further explored.

Whether a similar effect would be observed from rye d-9 SR on CH_4 production in vivo is another story, as Jonker et al. (2016) found that, contrary to the well-buffered in vitro experiment, the inclusion of starch above 20% in vivo actually reduced CH_4 production, likely due to the changes in rumen pH. Therefore, whether such SR with rye d-9 hydroponic fodder would increase CH_4 production in vivo would requires further validation.

Replacing silages with d-10 alfalfa in the basal diet did not affect OM digestibility but increased NH_3 levels. The

elevated NH_3 in this study could be an overestimate due to the limitation of the batch culture system, as it does not account for the removal of metabolic end products via excretion or rumen absorption. Nonetheless, this indicates that replacing silages with less-fibrous hydroponic fodder could cause an imbalance in rumen carbohydrate and protein utilization if the fodder is high in protein. This is because NH_3 typically accumulates in ruminal fluid when intake exceeds microbial protein synthesis capacities (Roffler and Satter, 1975). Elevated NH_3 from N imbalances can contribute to environmental issues, as urine from grazing ruminants serves as a significant pollution source. From the urine patch, approximately 2% of urine nitrogen was converted into nitric oxide, 13% volatilized as ammonia, and 20% leached into the ground as nitrate (Selbie et al., 2015). Therefore, without balancing N and carbohydrate, mitigating CH_4 could increase the emission of other pollutants.

Moreover, relying solely on hydroponic fodder may be difficult due to ruminant animals' high DMI requirements, unless regional climate prevents acquiring fresh forage or silage. Germinating seeds do not increase DM quantity, demanding significant resources to meet nutrition needs through hydroponics. Although high malate content in hydroponic fodder could theoretically reduce CH_4 if used in small amount (Graham, 2008), it remains less effective unless exceeding the malate levels of the Alfagraze cultivar studied by Callaway et al. (1997). However, under certain conditions, using forage pea d-10 and rye d-9 SR might improve milk fat due to an increased molar proportion of butyrate, a milk fat precursor.

CONCLUSIONS

The CH_4-mitigating effect of malate is well known. In this study, we confirmed a negative correlation between ruminal CH_4 and malate supplied through hydroponic fodders, even with relatively low malate levels, which suggests that the CH_4-mitigating effect of malate persisted in hydroponic fodder. Further research should focus on identifying optimal genetic, chronological, and environmental conditions to enhance malate content, thereby paving the way for broader use of hydroponic fodder in dairy cattle diets, with the potential to mitigate enteric CH_4 mitigation. Although complete silage replacement with hydroponic fodder did not adversely affect OM digestibility, caution is advised regarding nitrogen balance, especially if the hydroponic fodder is rich in protein, as it could inadvertently substitute one form of pollution for another. Exploration on malate-containing hydroponic fodder in combination with existing CH_4 mitigating strategies for ruminant animals can be a potential avenue.

NOTES

This study received no external funding. Author contributions are as follows: conceptualization: Yang Li and Mutian Niu; methodology: Yang Li, Melissa Terranova, Rong Peng, and Monika Mocsai; data analysis: Yang Li, Kai Wang, and Mutian Niu; investigation: Carmen Kunz, Yang Li, and Mutian Niu; data curation: Yang Li; writing: Yang Li and Mutian Niu; supervision: Mutian Niu and Emmanuel Frossard; project management: Yang Li. The data that support the findings of this study are available from the corresponding author upon reasonable request. Supplemental material for this article is available at https://doi.org/10.6084/m9.figshare.27203064.v1. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to, and the appropriate ethical review committee approval has been received from the Cantonal Veterinary Office, Zürich, Switzerland. The authors confirm that they have followed European Union standards for the protection of animals used for scientific purposes. The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: %pt = absolute percentage molar proportion; 20R = replacement of 20% of the basal diet; EE = ether extract; GAM = generalized additive model; IVOMD = in vitro OM digestibility; SR = silage replacement.

REFERENCES

Andersen, R. 2009. Nonparametric methods for modeling nonlinearity in regression analysis. Annu. Rev. Sociol. 35:67–85. https://doi.org/10.1146/annurev.soc.34.040507.134631.

AOAC International. 1997. Official Methods of Analysis. Association of Official Analytical Chemists, Arlington, VA.

Bates, D., M. Machler, B. M. Bolker, and S. C. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67:1–48. https://doi.org/10.18637/jss.v067.i01.

Bouton, J. H., S. J. Smith Jr., D. T. Wood, C. S. Hoveland, and E. C. Brummer. 1991. Registration of 'Alfagraze' alfalfa. Crop Sci. 31:479. https://doi.org/10.2135/cropsci1991.0011183X003100020052x.

Callaway, T. R., and S. A. Martin. 1997. Effects of cellobiose and monensin on in vitro fermentation of organic acids by mixed ruminal bacteria. J. Dairy Sci. 80:1126–1135. https://doi.org/10.3168/jds.S0022-0302(97)76039-9.

Callaway, T. R., S. A. Martin, J. L. Wampler, N. S. Hill, and G. M. Hill. 1997. Malate content of forage varieties commonly fed to cattle. J. Dairy Sci. 80:1651–1655. https://doi.org/10.3168/jds.S0022-0302(97)76096-X.

Devant, M., A. Bach, and J. A. García. 2007. Effect of malate supplementation to dairy cows on rumen fermentation and milk production in early lactation. J. Appl. Anim. Res. 31:169–172. https://doi.org/10.1080/09712119.2007.9706655.

Ehrlich, G. G., D. F. Goerlitz, J. H. Bourell, G. V. Eisen, and E. M. Godsy. 1981. Liquid chromatographic procedure for fermentation product analysis in the identification of anaerobic bacteria. Appl. Environ. Microbiol. 42:878–885. https://doi.org/10.1128/aem.42.5.878-885.1981.

Fasiolo, M., R. Nedellec, Y. Goude, C. Capezza, S. N. Wood, and M. M. Fasiolo. 2020. Package 'mgcViz'. Visualisations for generalised

additive models. CRAN. Accessed May 10, 2024. https://CRAN.R-project.org/package=mgcViz.
Foley, P. A., D. A. Kenny, J. J. Callan, T. M. Boland, and F. P. O'Mara. 2009. Effect of DL-malic acid supplementation on feed intake, methane emission, and rumen fermentation in beef cattle. J. Anim. Sci. 87:1048–1057. https://doi.org/10.2527/jas.2008-1026.
FAO (Food and Agriculture Organization of the United Nations). 2021. The State of the World's Land and Water Resources for Food and Agriculture—Systems at Breaking Point. Synthesis report 2021. Accessed May 10, 2024. https://www.fao.org/3/cb7654en/cb7654en.pdf.
Gebremedhin, W. K. 2015. Nutritional benefit and economic value of feeding hydroponically grown maize and barley fodder for Konkan Kanyal goats. IOSR J. Agric. Vet. Sci. 8:24–30.
Graham, I. A. 2008. Seed storage oil mobilization. Annu. Rev. Plant Biol. 59:115–142. https://doi.org/10.1146/annurev.arplant.59.032607.092938.
Holtshausen, L., A. V. Chaves, K. A. Beauchemin, S. M. McGinn, T. A. McAllister, N. E. Odongo, P. R. Cheeke, and C. Benchaar. 2009. Feeding saponin-containing *Yucca schidigera* and *Quillaja saponaria* to decrease enteric methane production in dairy cows. J. Dairy Sci. 92:2809–2821. https://doi.org/10.3168/jds.2008-1843.
Hook, S. E., A. D. G. Wright, and B. W. McBride. 2010. Methanogens: Methane producers of the rumen and mitigation strategies. Archaea 2010:1–11. https://doi.org/10.1155/2010/945785.
IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Page 151. Core Writing Team, R. K. Pachauri, and L. A. Meyer, ed. IPCC, Geneva, Switzerland.
Jafari, S., G. Y. Meng, M. A. Rajion, M. F. Jahromi, and M. Ebrahimi. 2016. Manipulation of rumen microbial fermentation by polyphenol rich solvent fractions from papaya leaf to reduce green-house gas methane and biohydrogenation of C18 PUFA. J. Agric. Food Chem. 64:4522–4530. https://doi.org/10.1021/acs.jafc.6b00846.
Janssen, P. H. 2010. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Anim. Feed Sci. Technol. 160:1–22. https://doi.org/10.1016/j.anifeedsci.2010.07.002.
Jonker, A., K. Lowe, S. Kittelmann, P. H. Janssen, S. Ledgard, and D. Pacheco. 2016. Methane emissions changed nonlinearly with graded substitution of alfalfa silage with corn silage and corn grain in the diet of sheep and relation with rumen fermentation characteristics in vivo and in vitro. J. Anim. Sci. 94:3464–3475. https://doi.org/10.2527/jas.2015-9912.
Kidane, T., and A. Dagnachew. 2022. Retrospective impact assessment for pilot hydroponic green fodder production in Sekota Woreda, Amhara Regional State, Ethiopia. Biophys. Econ. Sustain. 7:9. https://doi.org/10.1007/s41247-022-00103-3.
Knapp, J. R., G. L. Laur, P. A. Vadas, W. P. Weiss, and J. M. Tricarico. 2014. Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. J. Dairy Sci. 97:3231–3261. https://doi.org/10.3168/jds.2013-7234.
Le, A. V., S. E. Parks, M. H. Nguyen, and P. D. Roach. 2018. Improving the vanillin-sulphuric acid method for quantifying total saponins. Technologies (Basel) 6. https://doi.org/10.3390/technologies6030084.
Martin, S. A., and M. N. Streeter. 1995. Effect of malate on in vitro mixed ruminal microorganism fermentation. J. Anim. Sci. 73:2141–2145. https://doi.org/10.2527/1995.7372141x.
Menke, K. H., and H. Steingass. 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Dev. 28:7–55.
Muller, R. A., and E. A. Muller. 2017. Fugitive methane and the role of atmospheric half-life. Geoinformatics and Geostatistics: An Overview 5:1–7. https://doi.org/10.4172/2327-4581.1000162.
NASEM (National Academies of Sciences, Engineering, and Medicine). 2016. Attribution of Extreme Weather Events in the Context of Climate Change. National Academies Press, Washington, DC. https://doi.org/10.17226/21852.
Ningoji, S. N., M. N. Thimmegowda, S. Tulja, and B. G. Vasanthi. 2021. Hydroponics fodder production—An innovative approach for sustainable livestock production under varied climatic distress. Mysore J. Agric. Sci. 55:1–11.
Niu, M., E. Kebreab, A. N. Hristov, J. Oh, C. Arndt, A. Bannink, A. R. Bayat, A. F. Brito, T. Boland, D. Casper, L. A. Crompton, J. Dijkstra, M. A. Eugène, P. C. Garnsworthy, M. N. Haque, A. L. F. Hellwing, P. Huhtanen, M. Kreuzer, B. Kuhla, P. Lund, J. Madsen, C. Martin, S. C. McClelland, M. Mcgee, P. J. Moate, S. Muetzel, C. Muñoz, P. O'Kiely, N. Peiren, C. K. Reynolds, A. Schwarm, K. J. Shingfield, T. M. Storlien, M. R. Weisbjerg, D. R. Yáñez-Ruiz, and Z. T. Yu. 2018. Prediction of enteric methane production, yield, and intensity in dairy cattle using an intercontinental database. Glob. Chang. Biol. 24:3368–3389. https://doi.org/10.1111/gcb.14094.
Oba, M., and M. S. Allen. 1999. Evaluation of the importance of the digestibility of neutral detergent fiber from forage: Effects on dry matter intake and milk yield of dairy cows. J. Dairy Sci. 82:589–596. https://doi.org/10.3168/jds.S0022-0302(99)75271-9.
R Core Team. 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Rezaei Ahvanooei, M. R., M. A. Norouzian, A. H. Piray, P. Vahmani, and M. H. Ghaffari. 2024. Effects of monensin supplementation on rumen fermentation, methane emissions, nitrogen balance, and metabolic responses of dairy cows: A systematic review and dose-response meta-analysis. J. Dairy Sci. 107:607–624. https://doi.org/10.3168/jds.2023-23441.
Roffler, R. E., and L. D. Satter. 1975. Relationship between ruminal ammonia and nonprotein nitrogen utilization by ruminants. I. Development of a model for predicting nonprotein nitrogen utilization by cattle. J. Dairy Sci. 58:1880–1888. https://doi.org/10.3168/jds.S0022-0302(75)84803-X.
Russell, J. B., and A. J. Houlihan. 2003. Ionophore resistance of ruminal bacteria and its potential impact on human health. FEMS Microbiol. Rev. 27:65–74. https://doi.org/10.1016/S0168-6445(03)00019-6.
Selbie, D. R., L. E. Buckthought, and M. A. Shepherd. 2015. Chapter 4: The challenge of the urine patch for managing nitrogen in grazed pasture systems. Pages 229–292 in Advances in Agronomy. Vol. 129. D. L. Sparks, ed. Academic Press. https://doi.org/10.1016/bs.agron.2014.09.004.
Shit, N. 2019. Hydroponic fodder production: An alternative technology for sustainable livestock production in India. Explor. Anim. Med. Res. 9:108–119.
Smith, A. M., and S. C. Zeeman. 2006. Quantification of starch in plant tissues. Nat. Protoc. 1:1342–1345. https://doi.org/10.1038/nprot.2006.232.
Soliva, C. R., and H. D. Hess. 2007. Measuring methane emission of ruminants by in vitro and in vivo techniques. Measuring Methane Production from Ruminants. H. P. S. Makkar and P. E. Vercoe, ed. https://doi.org/10.1007/978-1-4020-6133-2_2.
South, J. B. 1996. Changes in organic acid levels during malting. J. Inst. Brew. 102:161–166. https://doi.org/10.1002/j.2050-0416.1996.tb00904.x.
Sun, X. G., Y. Li, K. Giller, C. Kunz, M. Terranova, and M. T. Niu. 2024. Comparative assessment of emulsifiers for in vitro ruminal gas production and fermentation measurements: Tween 80 is a suitable emulsifier. J. Anim. Physiol. Anim. Nutr. (Berl.) 108:680–690. https://doi.org/10.1111/jpn.13924.
Thiex, N., L. Novotny, and A. Crawford. 2012. Determination of ash in animal feed: AOAC Official Method 942.05 revisited. J. AOAC Int. 95:1392–1397. https://doi.org/10.5740/jaoacint.12-129.
Ungerfeld, E. M., and R. J. Forster. 2011. A meta-analysis of malate effects on methanogenesis in ruminal batch cultures. Anim. Feed Sci. Technol. 166:282–290. https://doi.org/10.1016/j.anifeedsci.2011.04.018.
Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Wehner, M. F., J. R. Arnold, T. Knutson, K. E. Kunkel, and A. N. LeGrande. 2017. Droughts, floods, and wildfires. Pages 231–256 in Climate Science Special Report: Fourth National Climate Assessment, Volume I. D. J. Wuebbles, D. W. Fahey, K. A. Hibbard, D. J. Dokken, B. C. Stewart, and T. K. Maycock, ed. U.S. Global Change Research Program, Washington, DC. https://doi.org/10.7930/J0CJ8BNN.

Wood, S., and M. S. Wood. 2015. Package 'mgcv'. R package version 1.29-0. CRAN. Accessed May 10, 2024. https://cran.r-project.org/package=mgcv.

ORCIDS

Yang Li, https://orcid.org/0000-0001-9371-9978
Rong Peng, https://orcid.org/0000-0003-4167-897X
Carmen Kunz, https://orcid.org/0000-0002-1907-9527
Kai Wang, https://orcid.org/0000-0002-6672-1121
Melissa Terranova, https://orcid.org/0000-0003-4152-8429
Mutian Niu, https://orcid.org/0000-0003-4484-4611