In Vitro Methane Production from Heifers Offered Four Bermudagrass Cultivars

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Abstract

Though bermudagrass (*Cynodon dactylon* [L.] Pers.) is one of the predominant warm-season perennial forage supporting the southeastern United States livestock production systems, little is known about its influence on parameters of ruminal metabolism, including carbon loss as methane. With the multitude of cultivars of this grass that have been developed and released, one may question whether the physiological cultivar differences will manifest varying results in digestive efficiency and subsequent methane emissions. Thus, the objective of this study was to evaluate *in vitro* methane (CH_4) production as influenced by four bermudagrass cultivars. Ruminally-fistulated heifers (n = 4) were assigned randomly to one of four bermudagrass cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]) for four 30-d *in vivo* periods in a Latin square design. On d 28 of each period, rumen fluid was collected from each heifer for use in CH_4 production evaluation. Samples of each bermudagrass, corresponding to the cultivar fed, were weighed into duplicate 10-mL serum bottles and incubated at 39°C for 0, 2, 4, and 24 h. Following incubation, headspace samples were assayed for CH_4 concentrations by gas chromatography. There was an interaction of cultivar and time (P < 0.01). There was no difference among cultivars (P < 0.05) at 0, 2, or 4 h of incubation. After 24 h of incubation, however, CH_4 concentrations were greater (P < 0.05) from T44 and T85 (7.7 and 6.2 mmol/L, respectively) than from RUS and COS (3.4 and 3.0 mmol/L, respectively). Results are interpreted to mean that cultivar type has an influence on the potential CH_4 production of bermudagrass.

Introduction

Greenhouse gas (GHG) emissions have become a major concern of livestock production systems. Methane and N_2O have global warming potentials of 23 and 296 CO_2-eq, respectively (IPCC 2006). Enteric fermentation from grazing livestock is implicated in the production of 27% of all CH_4 emissions in the United States (EPA 2022). The southeastern United States is home to approximately 42% (38.5 million head) of all cattle nationally and approximately 37% (14.5 million head) of cows and heifers that calved from January 2021 to January 2022 managed on forage-based systems (NASS 2022). In southeastern United States beef production systems, cattle are managed on pasture for the majority of their life cycle (Troxel et al. 2014). The predominant source of enteric CH_4 production in beef cattle is the consumption by cattle of feedstocks dense in cell wall material (e.g., when cattle are grazing) (Pinares-Patiño et al. 2003).

Not only does CH_4 represent an environmental concern, but it is also an energetic loss to the production system (Johnson and Johnson 1995). This loss in production efficiency has stimulated research on CH_4 mitigation strategies, especially nutritional manipulation. Factors such as forage quality and type can influence enteric CH_4 production (Eugène et al. 2021). Bermudagrass, the predominant warm-season perennial grass in the Southeast, accounts for approximately 14 million ha in the United States (Vendramini et al. 2019). Much effort has been devoted to improving these forages for better livestock efficiency (Taliaferro et al. 2004). However, to date, there have been few investigations into the effects of bermudagrass cultivars on ruminal fermentation, especially enteric methane production beef production systems. Thus, the objective of this study was to evaluate *in vitro* methane production from beef cattle as influenced by four bermudagrass cultivars.

Methods

The *in vivo* metabolism experiment was conducted as a 4 × 4 Latin square. Ruminally-fistulated *Bos taurus* heifers (n = 4) were assigned randomly to one of four bermudagrass cultivars (COS, RUS, T44, or T85) for four 30-d *in vivo* periods (21 d adaptation and 9 d collection). The accompanying *in vitro* CH_4 production experiment was conducted using the design of the *in vivo* experiment with the addition of incubation time (0, 2, 4, or 24 h). Laboratory duplicates served as observational units within the *in vivo* experimental unit.

Samples of the four bermudagrass cultivars were collected from bales in the *in vivo* period. Hay samples were dried at 55°C for 72 h and ground to pass through a 1-mm screen. Subsamples (0.1 g) of each bermudagrass,

representing the cultivar corresponding to the rumen inoculum were weighed into duplicate 10-mL serum bottles for each incubation timepoint. Following sample addition, 2 mL of buffer solution (Callaway et al. 1997) were added to each bottle under CO_2, then bottles will be sealed with butyl rubber stoppers and aluminium crimp caps and brought to temperature in a gravity convection incubator at 39°C.

On d 28 of the *in vivo* experiment, whole rumen contents (approximately 1,000 mL) were sampled at 4 h relative to feeding (Goering and Van Soest 1970) and collected into pre-warmed (39°C) insulated containers for transport to the Auburn University Ruminant Nutrition Laboratory. Rumen fluid was strained with 4 layers of cheesecloth and added to 125 mL serum bottles under CO_2. These bottles were sealed with butyl rubber stoppers and aluminium crimp caps and kept at 39°C in a gravity convection incubator for separation of rumen liquor and feed particles. A volume (1 mL) of rumen liquor from each heifer was drawn using a syringe and injected into the prepared 10-mL serum bottles (n = 8 per heifer per period) resulting in a final incubation volume of 3 mL. The 0 h timepoint samples were immediately transferred to a refrigerator at 4°C until further analysis. Other samples were allowed to incubate for their prescribed time then transferred to a refrigerator at 4°C to stop fermentation until further analysis. Incubated samples were transported on ice to the Department of Animal and Dairy Sciences at the University of Georgia. Using a gas-sealed syringe, a headspace sample was removed from each bottle and analysed for CH_4 on a Gow Mac thermal conductivity series 550 gas chromatograph (Gow Mac Instrument, Bridgewater, NJ) equipped with a Carbosieve S 8100 column (Supelco, Inc., Bellefonte, PA). Machine parameters for gas flow (N_2; 20 mL/min), column temperature (125°C), and detector temperature (150°C) were set prior to sampling.

Methane data were analysed using generalized linear mixed models procedure (PROC GLIMMIX) of SAS v. 9.4 (SAS Institute Inc., Cary, NC). The fixed effects included treatment, incubation time, and their interaction. Denominator degrees of freedom were adjusted using the 2nd order Kenward-Roger approximation (Kenward and Roger 2009). The random statement included the effects of period, heifer, and replicate within period × heifer × incubation time. Incubation time was identified as a repeated measurement with a first-order autoregressive covariance structure (based on minimum BIC). Means separations were performed based on *F*-protected *t*-tests using the LINES option in the LSMEANS statement of PROC GLIMMIX. Differences among responses were declared when $P < 0.05$, and tendencies were declared when $0.05 \leq P < 0.10$.

Results and Discussion

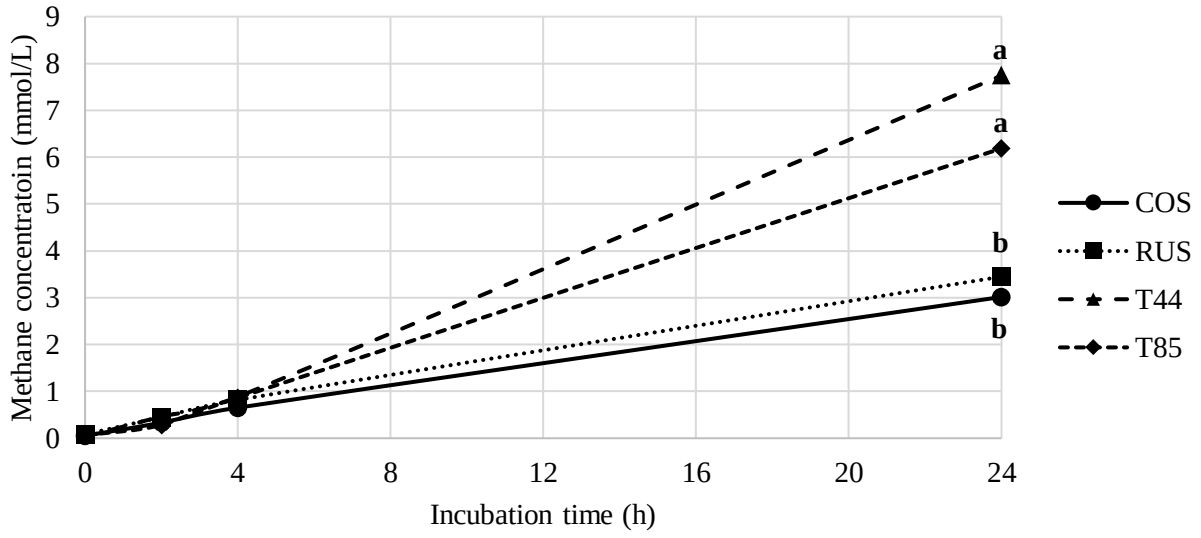

Figure 1 In vitro methane production from beef heifers offered one of four bermudagrass cultivars (Coastal [COS], Russell [RUS], Tifton 44 [T44], or Tifton 85 [T85]).

There was a bermudagrass cultivar and incubation time interaction ($P < 0.01$; Figure 1). At 0, 2, and 4 h, there were no differences ($P > 0.05$) among cultivars for CH_4 concentration. These results are unsurprising and by design, verifying that there was no background interference of CH_4. Muck et al. (2007) found that 56-70% of CH_4 production occurred by 9 h of incubation, while Wang et al. (2020) estimated that 54-59% of CH_4 production occurred by 14 h of incubation in vitro.

After 24 h of incubation, however, CH_4 concentrations were greater ($P < 0.05$) from T44 and T85 (7.7 and 6.2 mmol/L, respectively) than from RUS and COS (3.4 and 3.0 mmol/L, respectively; Figure 1). Cultivar differences are likely due to differences in chemical composition (Benchaar et al. 2001). The breeding programs that produced these cultivars were directed toward improved animal performance, often through a reduction in or alteration of cell wall constituents. Burton and Monson (1988) identified T44 as having lower concentrations of cell wall constituents and greater digestibility. Similarly, T85 was identified to be more digestible than COS due to decreased lignin concentrations and increased concentrations of neutral sugars (Burton et al. 1993). Along these lines, Mandebvu et al. (1998) found that in vitro digestibility of COS was only 53%, comparable to physiologically mature TIF85.

The absolute values of CH_4 produced were less than expected compared to Young et al. (2013) wherein they found CH_4 produced at rates of 10 to 30 mmol/h or 15 to 25 mmol/d when bermudagrass was tested in continuous culture. It is possible that the decreased volume of the incubation vessel (and, thus, the headspace volume) had an effect on the overall concentration of CH_4 measured.

While there is sufficient evidence to suggest cultivar differences, caution should be used in the interpretation of these data. There are many ways in which to describe CH_4 production. In a bermudagrass supplementation experiment, Smith et al. (2020) found no cultivar differences in CH_4 expressed as total production (in this case, mg/L), but differences arose when CH_4 was expressed relative to chemical constituents of the substrate or relative to digestibility. It could be that, over a longer incubation time, COS and RUS may produce similar total CH_4 as T44 and T85 if their respective rates of fermentation are slower. Thus, the differences observed in the current experiment warrant further investigation into the dynamics of fermentation and relative CH_4 production.

Conclusions and/or Implications

In conclusion, we found that bermudagrass cultivar did have an influence on potential methane production in vitro. These findings have an impact on both the environmental and economic sustainability of southeastern United States beef production systems. From an environmental perspective, not only forage species selection, but also cultivar selection, will be critical in the management of methane emissions from livestock operations. From an economic perspective, the energetic inefficiencies of carbon loss through methane will require intensive nutritional management decisions within forage systems. These data will serve as the foundation for further investigation into ruminal fermentation dynamics of southeastern United States forage systems.

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