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Abstract

Incorporation of legumes into forage systems has been a widely adopted strategy to increase pasture productivity and forage nutritive value, while reducing N inputs. Considering the population growth, and the diminishing land resources for food production, the need to increase the food supply will have to be balanced with the environmental impact of these systems, particularly their carbon footprint. Enteric methane production represents the largest source of greenhouse gas emissions from livestock. Certain forage legumes have evolved plant secondary compounds, such as tannins and other polyphenols, which have been associated with reductions in enteric methane emissions. Studies were conducted at Utah State University (USU), and at the University of Florida, North Florida Research and Education Center (UF-NFREC) to assess *in vivo* methane emissions in grazing cattle, using the SF_6 tracer technique. At USU, cattle grazing pastures of Birdsfoot trefoil (*Lotus corniculatus;* BFT) emitted less methane per unit of dry matter consumed when compared with cattle fed a totally mixed ration (50% barley grain, 25% alfalfa hay, and 25% corn silage) in *ad libitum* amounts. However, emissions in cattle grazing BFT did not differ from those grazing the legume Cicer milkvetch (*Astragalus cicer*), or a traditional pasture-finishing system based on Meadow brome (*Bromus riparius*). At UF-NFREC, three livestock-forage systems were tested during three consecutive years to determine the effects of including the legume Rhizoma peanut (*Arachis glabrata* Benth.; BHR) in bahiagrass pastures (*Paspalum notatum* Flügge) fertilized (BH) or not (BHF) with N during the warm season. No differences were observed in methane emissions (g d^{-1}), or in methane emission intensity. From the legumes grazed in these experiments, only BFT contains significant concentrations of tannins. Thus, the potential to mitigate livestock enteric methane emissions by grazing legumes appears to be directly related to the presence of tannins.

Introduction

The ability to convert fiber into high-value animal protein has always been the main advantage of ruminants, and one the ecosystem services of greater relevance in the context if a growing global population and food demand. However, the production of enteric methane is a necessary byproduct of these systems. As production increases in an attempt to meet global demand, more pressure is placed on these systems to be more sustainable in terms of their carbon footprint. Most of the successful strategies proposed to decrease enteric methane involve daily feeding of additives or supplementation under confinement situations. In the U.S., the cow/calf sector contributes to 58% of the greenhouse gas emissions, with 61% of that attributed to enteric methane (Lupo et al., 2013). The cow/calf segment relies mostly on grazing, and this is a common feature across diverse geographic regions. Thus, the segment of the beef production industry that relies mostly on grazing, has the greatest contribution in terms of greenhouse gas emissions, and also has the least available options for mitigation.

Feeding a tannin -rich diet has been associated with reductions in enteric methane emissions in ruminants (Hristov et al., 2013, Aboagye et al., 2018). Thus, the incorporation of legumes into beef/forage systems may be one of the few alternatives to decrease enteric methane emissions under grazing conditions. We hypothesize that the inclusion of legumes in grazing pastures may decrease the carbon footprint of beef/forage systems, either by decreasing enteric methane emissions, increasing productivity of the system, or both. The objective of this study was to assess the effects of adding legumes to grazing systems in terms of animal and forage performance, and enteric methane emissions in two contrasting regions of the U.S.: the southeast coastal plains, and the western plains.

Methods and Study Site

Exp. 1 - Grazing Study in the University of Florida

The experiment was conducted at the University of Florida, North Florida Research and Education Center (NFREC), during the cool- and warm-season for three consecutive years (2016-2018). Only data from the warm seasons will be reported in this study.

Treatments consisted of three year-round forage systems, distributed in a randomized complete block design with three replicates, for a total of nine experimental units. The first system (Grass+N) included N-fertilized (112 kg N ha^{-1} yr^{-1}) 'Argentine' bahiagrass (*Paspalum notatum*) pastures during the warm-season, overseeded with a mixture (56 kg ha^{-1} of each) of FL 401 cereal rye (*Secale cereal* L.) and RAM oat (*Avena sativa* L.) during the cool-season with a second application of 112 kg N ha^{-1} yr^{-1}. Total annual fertilization for this treatment was 224 kg N ha^{-1} yr^{-1}. System 2 (Grass + clover) included unfertilized bahiagrass pastures during the warm-season, overseeded with a similar rye-oat mixture, plus a mixture of clovers: 16.8 kg ha^{-1} of 'Dixie' crimson (*Trifolium incarnatum* L.), 6.7 kg ha^{-1} of 'Southern Belle' red clover (*Trifolium pretense* L.), and 3.4 kg ha^{-1} of ball clover (*Trifolium nigrescens* Viv.), fertilized with 34 kg N ha^{-1} during the cool-season. System 3 (Grass+CL+RP) included 'Ecoturf' rhizoma peanut (*Arachis glabrata* B.) and bahiagrass pastures during the warm-season, overseeded with a similar rye-oat mixture and a mixture of clovers (14 kg ha^{-1} of Dixie crimson, 5.5 kg ha^{-1} of Southern Belle red, and 2.8 kg ha^{-1} of ball clover) during the cool-season.

Methane emissions were measured on the two tester steers from each experimental unit (pasture) using the sulfur hexafluoride (SF_6) tracer gas technique described by Johnson et al., (2007). Dry matter intake was measured as described by Pinares-Patiño et al. (2016). Total fecal excretion was calculated by the marker dilution technique using Cr_2O_3 and TiO_2 as indigestible external markers, and in vitro digestibility was measured from composited hand-plucked samples from each pasture. Average daily gain (ADG) was determined by differences in animal weights at the beginning and end of each grazing season.

Exp. 2 - Grazing Study in Utah State University

Fifteen 2-year-old Angus heifers [541.09 kg BW ± 30 kg (Mean ± SD)] were randomly assigned to one of three treatment pastures: (1) Birdsfoot Trefoil, *Lotus corniculatus* (BFT), a tannin-containing legume; (2) Cicer Milkvetch, *Astragalus cicer* (CMV), a control non-bloating legume of similar nutritional and agricultural characteristics to BFT but without tannins; and (3) Meadow Brome, *Bromus riparius* (MB), a high-quality grass. Each treatment had 5 spatial replications (experimental plot that represented the experimental unit of the design). Each replication was randomly divided into three paddocks (64 × 57m; 0.3648 ha), seeded with BFT, CMV and MB. One heifer was assigned to graze in each paddock (N=5 animals/pasture). Heifers were allowed to graze in one-twelfth of the paddocks, and they were moved to a new section every 3.5 days. Cows grazed their respective pastures for 77 days, during three sampling periods of 9 days each.

Exp. 3 Confinement Study in Utah State University

Five 2-year-old Angus cows [2017 BW, 526.83 kg ± 18.71 kg; 2018 BW, 563.44 kg ± 83.61 kg (Mean ± SD)] were randomly assigned to individual adjacent pens (measuring 10 x 5 m) inside a covered barn to receive a TMR ration (25% of Alfalfa hay, 25% Corn silage and 50% Chopped barley).

The experiment was performed during two consecutive years, in two (2017) and three (2018) sampling periods of 9 days each. Enteric methane emissions and ADG were measured as described in Exp. 1. Intake was assessed through estimates of fecal output (using Cr_2O_3 as an external marker; Kolver et al., 1998), and digestibility (NIRS, AOAC,1990). Urine samples were analyzed for urinary nitrogen contents and blood serum samples for urea nitrogen (BUN) (Lagrange et al., 2020).

Statistical analyses

Exp. 1 was analyzed as a randomized complete block design using PROC Mixed of SAS (SAS Inst., Cary, NC), with treatment and season as fixed effects, and block and year as random effects. For Exp. 2 and Exp. 3, response variables were analyzed as a split-plot design with repeated measures. In both experiments, cows (random factor) were the whole plot units with treatment (pasture species; ration) as a fixed factor and day, period and year (confinement experiment) as the repeated measures. The variance-covariance structure used was the one that yielded the lowest Bayesian information criterion.

Results

Exp. 1 - Grazing Study in the University of Florida

Methane emissions and emissions intensity are shown in Table 1. No effect of treatment ($P \geq 0.18$) was observed on DMI, CH_4 emissions, or methane emissions intensity.

Table 1. Dry matter intake (DMI) and enteric methane emissions from beef steers during the warm- season; 2016 to 2018.

Item	Treatment[1] Grass+N	Grass+clover	Grass+CL+RP	SE[2]	*P*-value
DMI[3], kg d^{-1}	6.8	6.3	7.6	0.54	0.24
DMI[3], as % of BW	1.79	1.67	2.04	0.150	0.25
CH_4 g $steer^{-1}$ d^{-1}	117	113	101	24.8	0.90
CH_4 $BW^{-(0.75)}$	1.4	1.4	1.2	0.73	0.91
CH_4 g ha^{-1} d^{-1}	548	447	359	96.2	0.40
CH_4 g kg of DMI^{-1}	24.1	24.2	17.4	5.4	0.61
CH_4 g kg of ADG^{-1}	397	448	225	85.1	0.18

[1] Grass+N = N-fertilized (112 kg N ha^{-1}) bahiagrass pastures; Grass+clover = unfertilized bahiagrass pastures; Grass+CL+RP = rhizoma peanut and bahiagrass pastures.
[2] SE = Standard deviation from the observations in 3 consecutive years (2016, 2017 and 2018).
[3] Dry matter intake was measured only during 2016 and 2017, using Cr_2O_3 and TiO_2 as fecal output markers.

Exp. 2 and Exp 3. - Grazing and Confinement Experiments in Utah State University

Cows grazing BFT (1.9% condensed tannins) showed greater weight gains than cows grazing CMV or MB (P=0.0006), but similar to cows fed the TMR (P=0.5790; Table 2). Methane emissions per unit of intake from cows grazing BFT were lower than emissions from animals consuming the TMR (P=0.074). Methane emissions were comparable among animals grazing CMV (P=0.1180), MB (P=0.6763) or fed the TMR (Table 1). Blood urea nitrogen concentrations were similar in cows grazing BFT or CMV (P=0.1202), but greater than in animals grazing MB or consuming the TMR (P=<.0001). Urinary nitrogen concentrations were similar among the diet treatments (P=0.5266; Table 2).

Table 2. Response variable by animals during Exp. 2 and 3. Means in a row with different letters (a-c) are significantly different at the α = 0.10. SEM: Standard error of the mean.

Grazing Study	**BFT**		**CMV**		**MB**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
DMI, kg/d	14.84 [a]	1.063	13.14[ab]	1.063	10.12 [b]	1.063
DMI/kg LBW	0.13 [a]	0.010	0.12[ab]	0.010	0.09 [b]	0.010
DMI%BW	2.77 [a]	0.238	2.57[ab]	0.238	1.94 [b]	0.238
Methane per day, g/d	283.56	13.254	261.37	13.128	254.28	12.955
Methane/kg DMI	20.55	2.119	21.04	2.119	25.42	2.119
ADG, kg/d	0.70 [a]	0.079	0.18[c]	0.079	0.46 [b]	0.079
BUN, mg/dL	17.80 [a]	0.748	20.06[a]	0.748	8.40 [b]	0.748
Urinary Nitrogen, g/L	4.55	0.536	4.14	0.536	2.87	0.536

Confinement Study	**Overall**		**2017**		**2018**	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
DMI, kg/d	10.87	0.362	7.97[b]	0.463	11.61[a]	0.387
DMI/LBW	0.08	0.003	0.07[b]	0.004	0.09[a]	0.003
DMI%BW	1.78	0.087	1.46[b]	0.098	1.99[a]	0.092
Methane per day, g/d	224.69	12.464	253.09[a]	10.345	212.65[b]	9.124
Methane/kg DMI	28.87	1.966	32.52	3.624	26.43	3.451
ADG, kg/d	0.81	0.069	0.73	0.097	0.88	0.097
BUN, mg/dL	7.06	0.748	-	-	7.06	0.748
Urinary Nitrogen, g/L	3.92	0.536	-	-	3.92	0.536

Discussion

In Exp. 1, despite the numeric reduction of nearly 44% in emissions intensity when including rhizoma peanut in the warm-season forages (397 vs. 225 g of CH4 kg of ADG-[1] for Grass+N and Grass+CL+RP, respectively; P = 0.18), no effects were observed in daily methane emissions. Most likely the lack of effect on CH4 methane emissions observed in this experiment may be related with the fact that unlike other tropical legumes, rhizoma peanut does not contain significant concentrations of tannins (Naumann et al., 2013).

Cows grazing a tannin-containing legume (BFT) showed greater weight gains than cows grazing a non-tannin containing legume (CMV) or a grass (MB), but similar to cows fed a confinement ration with high contents of roughage. This outcome may be explained by the high nutritional quality of BFT comparable to the high-roughage confinement ration. Moreover, tannins from BFT are responsible for an increase in the efficiency of use of dietary protein in the intestines (Mueller-Harvey et al., 2019). The greater ADG observed for animals grazing BFT than for animals grazing MB could also be explained by the greater intakes by cows grazing BFT.

The lower methane emissions per unit of intake In Exp. 2 in cows grazing BFT vs. cows consuming the high-forage TMR suggests a positive effect of condensed tannins or nutrients in BFT on methane abatement (Min et al., 2020). Blood urea nitrogen and urinary nitrogen concentrations were similar in cows grazing tannin-(BFT) or non-tannin (CMV) containing legumes, suggesting that tannins in BFT did not reduce ruminal proteolysis or shifted the site of nitrogen excretion from urine to feces, as reported in previous studies (Lagrange et al., 2020), likely due to the low concentration of tannins (1.9%) in this legume. These results suggest grazing BFT is a viable alternative to high-roughage confinement rations for maintaining beef production with similar or potentially lower levels (i.e., methane emissions) of environmental impact.

In conclusion, incorporation of tannin-containing legumes into grazing systems has the potential to decrease greenhouse gas emissions, reducing the overall carbon footprint of beef production.

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