Journal of Animal Science, 2021, Vol. 99, No. 7, 1–8

doi:10.1093/jas/skab147
Advance Access publication May 5, 2021
Received: 3 December 2020 and **Accepted:** 30 April 2021
Ruminant Nutrition

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Effects of protein supplementation to steers consuming low-quality forages on greenhouse gas emissions

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Abstract

Providing supplements that enhance the efficiency of feed utilization can reduce methane (CH_4) emissions from ruminants. Protein supplementation is widely used to increase intake and digestion of low-quality forages, yet little is known about its impact on CH_4 emissions. British-cross steers (n = 23; initial body weight [**BW**] = 344 ± 33.9 kg) were used in a three-period crossover design to evaluate the effect of protein supplementation to beef cattle consuming low-quality forage on ruminal CH_4, metabolic carbon dioxide (CO_2) emissions, forage intake, and ruminal fermentation. Steers individually had ad libitum access to low-quality bluestem hay (4.6% crude protein [**CP**]) and were provided supplemental protein based on (dry matter basis): cottonseed meal (**CSM**; 0.29% of BW daily; 391 g/d CP), dried distillers grains with solubles (**DDGS**; 0.41% of BW daily 563 g/d CP), or none (**CON**). Urea was added to DDGS to match rumen degradable protein provided by CSM. Ruminal CH_4 and metabolic CO_2 fluxes were obtained 2.4 ± 0.4 times per steer daily using an automated open-circuit gas quantification system (GreenFeed emission monitoring system; C-Lock Inc., Rapid City, SD). Forage intake increased ($P < 0.01$) with protein supplementation; however, no difference in forage intake ($P = 0.14$) was observed between CSM and DDGS treatments. Flux of CO_2 (g/d) was greater ($P < 0.01$) for steers fed CSM and DDGS than for steers fed CON. Steers supplemented with CSM had greater ($P < 0.01$) CH_4 emissions (211 g/d) than DDGS (197 g/d) both of which were greater ($P < 0.01$) than CON (175 g/d). Methane emissions as a proportion of gross energy intake (**GEI**) were lowest ($P < 0.01$) for DDGS (7.66%), intermediate for CSM (8.46%) steers, and greatest for CON (10.53%). Steers fed DDGS also had the lowest ($P < 0.01$) ruminal acetate:propionate ratio (3.60), whereas CSM (4.89) was intermediate, and CON (5.64) steers were greatest. This study suggests that the common practice of supplementing protein to cattle consuming low-quality forage decreases greenhouse gas emissions per unit of GEI.

Key words: cattle, low-quality forage, methane, protein

Abbreviations

ADF	acid detergent fiber
AIA	acid-insoluble ash
BW	body weight
CP	crude protein
CSM	cottonseed meal
DDGS	dried distillers grains with soluble
DM	dry matter
DMI	DM intake
GEI	gross energy intake
NDF	neutral detergent fiber
OM	organic matter
VFA	volatile fatty acid;
Y_m	methane energy as a percent of GEI

Introduction

Forage-based beef cattle production systems present a significant opportunity to reduce methane (CH_4) emissions as the vast majority of beef industry emissions are from forage-based cow–calf or stocker segments (Pelletier et al., 2010; Beauchemin et al., 2011; Lupo et al., 2013). Forages represent a significant source (73%) of global ruminant feed (Mottet et al., 2017). Structural carbohydrate (cellulose + hemicellulose) proportion in diets was a good predictor of CH_4 output across six experiments (Holter and Young, 1992). Also, modest gains by cattle consuming diets based on low-quality forages result in greater days needed to reach market weight and, consequently, a greater CH_4 producer (NASEM, 2016).

Forages are an important component of beef cattle diets. Rotz et al. (2019) estimated that 82% of feed consumed to produce 1 kg of beef are forages. Forage quality in pasture systems is dynamic, and data on the impacts of forage quality on methane emissions remain scarce. Boadi and Wittenburg (2002) measured methane from cows grazing timothy pastures at four stages of maturity and reported that methane emissions per unit of digestible organic matter (**OM**) consumed were greatest for the low-quality diets.

Protein supplementation is a well-known feeding strategy for enhancing the utilization of low-quality forages. Multiple experiments (McCollum and Galyean, 1985; Köster et al., 1996, Mathis et al., 2000; Bohnert et al., 2011) report positive effects of supplemental protein on low-quality forage intake, digestibility, and N utilization. Dried distillers grains with solubles (**DDGS**) are a common protein-rich feedstuff that is an alternative protein supplement to traditional oilseed meals such as cottonseed meal (**CSM**) for stocker cattle or beef cows. Additionally, DDGS contain 8% to 12% fat (NASEM, 2016), and providing additional fat in the diet has been considered as a potential CH_4 mitigant (Beck et al., 2018) as fat is toxic to methanogens (Johnson and Johnson, 1995; Beauchemin et al., 2007).

Therefore, this study's objectives were to identify the effects of protein supplementation of either CSM or DDGS on greenhouse gas emissions and energy losses of steers fed low-quality forage. We hypothesize that supplementing beef cattle diets based on low-quality forage with additional protein will increase energy intake and decrease methane produced per unit of gross energy intake (**GEI**).

Materials and Methods

All procedures used for these experiments involving animal care were approved by the West Texas A&M/CREET Institutional Animal Care and Use Committee (IACUC no. 05-01-14).

Cattle and experimental design

A total of 23 British cross-bred steers (initial body weight [**BW**] 344 ± 33.9 kg) were utilized in a three-period crossover design to minimize animal effects on treatment response. Steers were stratified by BW to treatment and randomly assigned to a diet sequence, resulting in 23 observations per treatment. Each period was 28 d, partitioned into 23 d of adaptation, 5 d of fecal collection, and 7 d of CO_2 and CH_4 flux measurement. Steers were weighed before feeding at the start of the experiment and at the end of each experimental period to monitor BW changes to have supplementation offered to be consistent with BW percentage targets. Steers were individually fed using Calan gates (American Calan, Northwood, New Hampshire) and were adapted to the gates for approximately 28 d before the initiation of the study.

Treatments

All steers were fed low-quality hay, predominately big bluestem (*Andropogon* spp.), and were supplemented with (Table 1) either 1) no added protein (**CON**), 2) protein from CSM at 0.29% of BW daily (**CSM**), or 3) a protein source of predominately DDGS and urea supplemented at 0.41% of BW daily (**DDGS**). A small amount (3.24% inclusion in supplement, dry matter [**DM**] basis) of urea was added to the DDGS supplement so that the quantity of ruminally degradable N supplied was similar for CSM and DDGS treatments. Each morning, steers were first offered their assigned supplement at 0800 hours. The hay was delivered 30 min to 1 h thereafter or immediately if steers had eaten all remaining supplements. Hay was fed to target ad libitum intake, and refusals averaged 10.6% of the hay offered each day (as-fed basis). Steers had ad libitum access to freshwater.

Measurements

Enteric CH_4 and metabolic CO_2 were measured up to six times daily using an automated open-circuit gas quantification system (GreenFeed emission monitoring [**GEM**] system; C-Lock Inc., Rapid City, SD). Programming and operation were similar to the study of Ebert et al. (2017). Steers were attracted to the

Table 1. Ingredient and nutrient composition of the supplements, hay, and pellet mixture offered

Ingredient, % of DM	CON	CSM	DDGS	Hay[1]	Pellet[2]
Cottonseed hulls	42.19				
CSM		91.78			
DDGS			90.80		
Molasses	8.44				
Limestone	33.76	5.26	3.81		
Salt	10.55	1.64	1.19		
Urea			3.24		
Titanium dioxide	5.06	1.32	0.95		
Nutrient composition[3] % of DM					
DM, %	91.0	90.8	89.9	92.8	91.2
OM, %	49.5	89.3	90.0	91.4	91.4
CP, %	5.0	39.4	43.2	4.6	11.0
Ether extract, %	0.7	2.0	11.4	0.9	2.9
NDF, %	39.2	30.3	33.1	75.9	63.4
ADF, %	34.2	19.0	19.8	48.9	51.8
Acid detergent lignin, %	8.3	5.0	6.1	5.5	15.0
Starch, %	0.9	3.0	2.1	—	9.2

[1]Bluestem hay.
[2]Pellet offered to steers through GreenFeed (C-Lock INC, Rapid City, SD) system.
[3]Analyzed by Dairy One Inc (Ithaca, NY).

GreenFeed unit with a bait-feed reward, which consisted of equal parts calf starter pellets (Nutrena; Cargill, Amarillo, TX) and pelleted cottonseed hulls (Livestock Nutrition Center, Altus, OK; Table 1). Feed was blended together and fed through a single hopper machine. The mixture was used as bait-feed because it was low in protein and starch but still palatable, with the overall goal of not influencing the protein treatments' response. Measurements from the GreenFeed averaged 2.4 ± 0.4 times per steer daily. Pellet nutrient composition is reported in Table 1. The GreenFeed was programmed to provide steers access to bait-feed every 4 h. The GreenFeed unit was calibrated weekly using known standards of CH_4, CO_2, and N. Fecal grab samples (200 to 300 g) were collected from steers once daily, 3 h after feeding, during the last 5 d of each period, and a steer within period composite was made for later lab analysis.

On the last day of each period, ruminal fluid was collected via oral lavage from each steer before feeding (Lodge-Ivey et al., 2009). The sampling device consisted of passing a metal sampling probe (approximately 7.3 cm length × 0.7 cm width; Precision Machine Co. Inc., Hasting, NE) connected to approximately 3 m of poly tubing (0.4 cm i.d.) through a Frick speculum. The other end of the poly tube was connected to one of the two ports affixed to a rubber stopper fitted into the top of a 100-mL polypropylene bottle. The other port was connected to a 0.5-m piece of vinyl tubing that was attached to a continuous vacuum supplied by an electric diaphragm pump (Gast model DOA, Gast Mfg. Inc., Benton Harbor, MI). Approximately, 100 mL of ruminal fluid was collected from each steer. Any particles lodged in the sampling probe were retained in the sample. From this, duplicate 45 mL aliquots were snap-frozen using liquid N and stored at −80 °C until analysis of volatile fatty acid (**VFA**) concentrations in the samples.

Laboratory analyses

Samples of hay, supplements, orts, and feces were dried and ground through a 1-mm screen in a No. 2 Wiley Mill (Thomas Scientific; Swedesboro, NJ) before laboratory analysis. DM of feces, orts, hay, and supplements was determined by drying at 60 °C in a forced-air oven for 48 h. OM content was determined by ashing a subsample in a muffle furnace at 600 °C for 16 h. Gross energy of diets, feces, feed ingredients, and feed orts were determined using a bomb calorimeter (Model 6400, Parr Instrument Company, Moline, IL). Neutral detergent fiber (**NDF**) content of hay, supplements, orts, and feces was determined using an Ankom Semi-Automated Fiber Analyzer (Model A2000; Ankom, Inc.; Macedon, NY: Vogel et al., 1999). Hay, supplements, and the pelleted mixture used in the GreenFeed were also analyzed by wet chemistries on previously oven-dried and ground composite for acid detergent lignin (AOAC, 1990; method 973.18) and crude protein (**CP**; Dairy One, 2015) at a commercial laboratory (Dairy One Inc., Ithaca, NY).

Fecal output was intended to be estimated by dosing 10 g of TiO_2 steer * day^{-1} through the supplements. However, low consumption of the marker supplement by animals in the CON group precluded the use of TiO_2 as an external marker of fecal output. Thus, internal markers of acid-insoluble ash (**AIA**; Van Keulen and Young, 1977; Thonney et al., 1979), indigestible NDF, and indigestible acid detergent fiber (**ADF**; Waller et al., 1980) were subsequently evaluated. Of the three, fecal output derived using AIA as a marker led to the lowest coefficient of variation and was subsequently used in digestibility calculations. Ruminal VFA concentrations were determined on a gas chromatograph (Agilent 7890; Agilent Technologies, Santa Clara, CA) using procedures reported by Erwin et al. (1961).

Calculations

Digestibility was calculated by dividing fecal output, determined using the AIA procedure, by DM intake (**DMI**) subtracted from 100 (Merchen, 1988). A 2-d lag was assumed in corresponding DMI to fecal output. Emission data from GEM were first filtered by removing samples that were not at least 3 min in length or when mass air flow was less than 26.0 L/s. Steers had continuous access to the GEM throughout each period of the experiment. The simple average of CH_4 and CO_2 emission values collected by the GEM unit for the last 7 d of each period (period day 20 through 26) were used in the calculations. Only emission data from this time frame were used to synchronize digestibility and emission estimates.

Statistical analysis

Data pertaining to digestibility, energy loss, and daily CO_2 and CH_4 flux were analyzed in a mixed linear model using the Mixed procedure of SAS (SAS Institute; Cary, NC). Steer was a random effect and both period and treatment were fixed effects. Mean separation was determined via the pdiff option. Response variables with $P \leq 0.05$ were considered a significant difference among treatment means, whereas $0.10 \geq P \geq 0.05$ was considered a numerical trend.

Results and Discussion

Intake and digestibility

Supplementing CP from CSM or DDGS increased ($P < 0.01$; Table 2) forage DM intake by 33% compared with steers receiving CON. McCollum and Galyean (1985) noted that forage intake increased by 27% when 800 g of CSM was provided daily to steers offered ad libitum access to prairie hay (CP = 4.6%). By design, steers consumed more DDGS supplement than CSM supplement ($P < 0.05$). The total DMI of CON steers was 36% less ($P < 0.01$) than the average DMI of CSM- and DDGS-supplemented steers. Similarly, Bohnert et al. (2011) reported a 58% increase in total DMI when supplementing soybean meal to steers consuming low-quality warm-season grass hay. Total DMI of DDGS and CSM treatments were not different ($P > 0.05$). Cole et al. (2020) reported no difference in DMI of low-quality forage diets supplemented with CSM (0.67 kg/d). The lack of intake difference could be explained by alfalfa's addition to the low-quality forage diet, which had a CP concentration of 7.35% (DM basis). In general, forage intake response to supplemental protein is greater when forage CP is below 7% (Köster et al.,1996).

On average, intake of apparently digested DM (g/d) was 53% greater ($P < 0.05$) when steers were supplemented with protein from either the CSM or DDGS groups than when not supplemented. Cole et al. (2020) also reported increased DM digestion when CSM was supplemented with low-quality forage diets. Apparently digested DM intake was 309 g ($P < 0.05$) greater when DDGS was supplemented than when CSM was supplemented. Apparent digestibility, as a percent of DMI, was greater ($P < 0.05$) when DDGS was supplemented instead of CSM or CON. The 410 g/d difference in supplement DM intake could explain differences in g/d of DM apparently digested and digestibility as a percentage of intake.

Bohnert et al. (2011) noted greater DM digestibility when steers consuming low-quality forage were supplemented with soybean meal. The lack of differences in DM digestibility of CON and CSM treatments may be due to changes in digesta passage caused by differences in DMI (DelCurto et al., 1990).

Table 2. Intake, nutrient excretion, and digestibility by steers offered low-quality forage and provided supplemental protein

Item	CON[1]	CSM[1]	DDGS[1]	SEM[2]
DM				
Supplement intake, g/d	186[a]	992[b]	1,302[c]	24.6
Hay intake, g/d	3,723[a]	5,168[b]	4,983[b]	258.1
GreenFeed pellet intake, g/d	849[a]	750[b]	803[ab]	25.3
Total intake, g/d	4,759[a]	6,910[b]	7,088[b]	270.2
Fecal excretion, g/d	2,482[a]	3,584[b]	3,453[b]	160.2
Apparent digested, g/d	2,277[a]	3,326[b]	3,635[c]	140.5
Apparent digestibility, % of intake	48.5[a]	48.6[a]	51.9[b]	1.14
OM				
Total intake, g/d	4,291[a]	6,301[b]	6,471[b]	248.0
Fecal excretion, g/d	2,064[a]	3,023[b]	2,901[b]	135.1
Apparent digested, g/d	2,227[a]	3,278[b]	3,569[b]	143.2
Apparent digestibility, % of intake	52.4	52.5	55.7	1.17
CP				
Calculated dietary %	6.45[a]	10.87[b]	13.14[c]	0.281
Supplement intake, g/d	9[a]	391[b]	563[c]	9.5
Degradable CP intake, g/d[3]	0[a]	205[b]	181[c]	4.2
Hay intake, g/d	195[a]	265[b]	254[b]	10.8
GreenFeed pellet intake, g/d	93[a]	82[b]	88[b]	2.8
Total intake, g/d	297[a]	738[b]	905[c]	17.8
NDF				
Total intake, g/d	3,774[a]	4,890[b]	4,926[b]	199.2
Fecal excretion, g/d	1,321[a]	2,007[b]	1,880[b]	90.0
Apparent digested, g/d	2,452[a]	2,884[b]	3,046[b]	131.8
Apparent digestibility, % of intake	65.6[a]	59.6[b]	62.2[b]	1.04

[1]Treatments were: CON, no protein supplement; CSM, CSM at 0.29% of BW daily; DDGS, DDGS + urea supplemented at 0.41% of BW daily.
[2]Pooled SE of the treatment means.
[3]Calculated using tabular ruminal degradable protein values for CSM, DDGS, and urea (NASEM, 2016).
[a–c]Within a row, means with unlike superscripts differ $P < 0.05$.

McCollum and Galyean (1985) reported that passage rates were greater for steers consuming low-quality prairie hay when supplemented with CSM than when fed no protein supplement. Cole et al. (2020) reported an increase in digestibility of low-quality forage diets supplemented with CSM. Diets fed were total mixed rations, and CSM was a substitute for alfalfa and low-quality forage in the control treatment. The substitution of alfalfa for CSM could explain digestibility differences in Cole et al. (2020) and the lack of one in the current experiment.

Total OM intake and fecal OM excretion were greater ($P < 0.05$) for CSM and DDGS than for CON steers. Apparently digested OM (g/d) was the least ($P < 0.05$) for CON steers and this likely resulted from less OM intake for CON steers. Increases in OM intake were reported by Bandyk et al. (2001), who infused casein directly into the rumen or abomasum of steers fed prairie hay (3.4% CP). Minson (1990) reported that voluntary OM intake increased by 40% when additional protein was provided to steers fed low-quality forages. In the current study, OM intake was 49% greater in supplemented steers than in CON. Cattle fed CSM and DDGS consumed more ($P < 0.01$; Table 2) CP than CON, as per experimental design. Protein supplementation is known to stimulate the intake of low-quality forages (Köster et al., 1996; Mathis et al. 2000; Wickersham et al., 2008). Greater CP intake in the DDGS treatment compared with CSM is an artifact of the experimental design. CP intake from hay increased with supplementation ($P < 0.01$), which coincides with an increase in DMI in response to supplementation.

Total intake and excretion of NDF were also greater ($P < 0.05$) when steers were supplemented than in CON steers. In comparison, apparently digested NDF (g/d) was greater ($P < 0.05$) for supplemented steers. However, digestibility as a percent of NDF intake was 7.2% lower ($P < 0.05$) for steers receiving the CSM treatment than the CON or DDGS treatment. Greater NDF intake and excretion would be expected, given the greater forage DMI when protein supplements were fed. Greater NDF digestibility is expected when additional protein is provided to cattle consuming low-quality forage (Köster et al., 1996; Mathis et al., 2000; Bohnert et al., 2011). However, other researchers have reported no differences in NDF digestibility due to protein supplementation (Bandyk et al., 2001; Salisbury et al., 2004). Colucci et al. (1982) stated that the quantity of DM consumed, digesta passage rate, and fermentation rate all play a critical role in fiber digestibility. When ruminal passage rates are increased as a result of greater intakes, fiber digestibility can be decreased. Although the rate of passage and fermentation were not evaluated in the current experiment, decreased NDF digestibility of supplemented steers would agree with the results of Colucci et al. (1982).

Energetics

GEI (Mcal/d; Table 3) from supplement was least ($P < 0.05$) for CON, intermediate for CSM, and greatest ($P < 0.05$) for DDGS. Similarly, GEI from hay was also least for CON; however, no difference ($P > 0.05$) was detected between CSM and DDGS. Subsequently, total GEI was greater ($P < 0.05$) with CSM or DDGS than CON. Ferrell et al. (1999) also reported greater GEI of wethers consuming bromegrass hay (4.3% CP) when supplemented with either urea, soybean meal, or a 50:50 mix of blood and feather meal and is consistent with other research on the impact of protein supplementation of low-quality forages (McCollum and Galyean, 1985; Köster et al., 1996).

Table 3. Energetic losses by steers offered low-quality forage and provided supplemental protein

Item	CON[1]	CSM[1]	DDGS[1]	SEM[2]
GEI, Mcal/d				
Forage	19.11[a]	26.13[b]	25.04[b]	1.07
Supplement	0.37[a]	4.23[b]	6.25[c]	0.105
Greenfeed pellets	3.74[a]	3.31[b]	3.53[ab]	0.108
Total GEI, Mcal/d	23.22[a]	33.67[b]	34.82[b]	1.24
Fecal energy, Mcal/d	9.99[a]	14.84[b]	14.39[b]	0.65
Digestible energy intake, Mcal/d	13.22[a]	18.83[b]	20.43[c]	0.721
Digestible energy, % of GE	57.52	56.05	59.00	1.100
DE, Mcal/kg of DM	2.87	2.77	2.95	0.086
Metabolic CO_2 production, g/d	4,963[a]	5,632[b]	5,573[b]	229.6
Ruminal CH_4 production, g/d	175.4[a]	211.7[c]	196.9[b]	10.1
Methane energy, Mcal/d	2.33[a]	2.80[c]	2.61[b]	0.074
Methane energy, % of GE (Y_m)	10.53[a]	8.46[b]	7.66[c]	0.365

[1]Treatments were: CON, no protein supplement; CSM, CSM at 0.29% of BW daily; DDGS, DDGS + urea supplemented at 0.41% of BW daily.
[2]Pooled SE of the treatment means.
[a–c]Within a row, means with unlike superscripts differ $P < 0.05$.

Supplementing steers with CSM or DDGS increased ($P < 0.05$) fecal energy (Mcal/d) by 46.3%. Greater energy results from increased throughput of undigested OM in feces, as fecal DM output increased by 41.7% (Table 2) with supplement in the current experiment. Greater fecal OM output was also observed in previous studies that supplemented low-quality forage with various protein sources (Hannah et al., 1991; Köster et al., 1996; Salisbury et al., 2004; Bohnert et al., 2011). Fecal energy is the largest and most variable loss of energy and should be reported in future research focusing on energetics and low-quality forage.

Digestible energy intake (Mcal/d) was least for CON and was greater ($P < 0.05$; Table 3) when steers were supplemented with protein. Increased digestible energy (**DE**) intake is expected given the greater intake of forage and the greater DE concentration in the supplements than the forage. These results agree with the study of Kartchner (1980), who reported increased intake of DE when cows grazing native forage (<7.0% CP) were supplemented with CSM. Between CSM and DDGS, DE intake was 8.49% greater ($P < 0.05$) when DDGS was used as a supplement. This difference is largely explained by the greater GEI from the DDGS supplement. As a percent of GEI, digestible energy was similar ($P > 0.05$) among treatments. The lack of difference in DE as a percentage of GE and DE concentration (Mcal/kg of DM) is supported by the lack of difference in DM and OM digestibility reported in Table 2.

Metabolic CO_2 production (g/d; Table 3) was approximately 12.8% greater ($P < 0.05$) with either CSM or DDGS supplements than in CON steers. Cole et al. (2020) also noted an increase in CO_2 production when steers consuming low-quality forage were provided supplemental protein, explained by increased energy retention. In the current experiment, DE intake increased with supplemental protein, indicating improved energy status as well. Ruminal CH_4 production (g/d) was least ($P < 0.05$) for CON steers, greatest ($P < 0.05$) for CSM supplemented steers, and intermediate ($P < 0.05$) for steers receiving DDGS. Increased CO_2 and CH_4 production with CSM and DDGS treatments are expected, as DMI increased with supplementation. In general, methane production is increased as fermentative activity in forage-fed cattle increases. Charmley et al. (2016) reported a strong linear relationship among DMI, GEI, and methane production across a wide group of forage-fed cattle in Australia.

Methane energy (mcal/d) was greatest ($P < 0.05$) for CSM, intermediate for DDGS, and least for CON. Moss et al. (2000) stated that increasing energy intake via increasing forage quality or via supplementation is associated with increased digestion resulting in greater energy loss as CH_4. Wolin and Miller (1983) reported that increased CH_4 production by ruminal methanogens is necessary to reduce the greater CO_2 and H_2 produced by cellulolytic bacteria when increased fermentation occurs. In the current experiment, apparently digested DM, OM, and NDF were increased when supplement was provided, representing greater ruminal fermentation. One would expect energy loss from CH_4 to be greater when steers are supplemented. The difference between CSM and DDGS could be attributed to the differences in fat concentration between the two supplements, as fat decreased methane emissions across various diets (Patra, 2013). The greater oil content of DDGS could explain the differences between CSM and DDGS. Fat supplementation has various methane inhibiting actions, including a toxic effect on methanogens, a hydrogen sink through biohydrogenation, greater propionate production, and replacing more fermentable substrates that would increase methane production (Johnson and Johnson, 1995).

Methane energy as a percent of GEI (**Y_m**) was greatest ($P < 0.05$) for CON and was, on average, 23.9% less when supplement was provided. Decreased Y_m in supplemented steers could be explained by greater total energy intake (Johnson and Johnson, 1995; Yan et al., 2000; Beauchemin and McGinn, 2005) by steers in the CSM and DDGS groups than the CON group. Between CSM and DDGS, Y_m was further decreased ($P < 0.05$) when DDGS was supplemented. The lower CH_4 production observed with DDGS than CSM explains the lower Y_m value of DDGS than CSM because GEI was similar between treatments. We hypothesize that increased oil content decreased CH_4 and subsequently Y_m associated with DDGS supplementation. By supplementing cattle with either CSM or DDGS, a smaller proportion of energy intake was lost as CH_4. Therefore, supplementing protein can serve to improve the carbon footprint of cattle grazing low-quality forage as well as improve animal production. Beck et al. (2018) reported reductions in CH_4 emission intensity in beef cattle grazing warm-season forages and consuming high-fat, high-protein supplements. Beck et al. (2018) offered greater amounts of fat (0, 162, 328, 490, 652, or 814 g per day ether extract offered) per day across treatments than the current experiment (148 g/hd/d ether extract in the DDGS treatment).

Absolute values for Y_m (7.66% to 10.65%) observed in this study are generally higher than previously reported for cattle grazing high-forage diets. Johnson and Johnson (1995) noted a possible range in Y_m of 2% to 12%. The International Panel on Climate Change (IPCC, 2006) Tier II methodology recommends a fixed value for Y_m of 6.5% when forage diets are fed and when limited information exists on diet composition (NASEM, 2016). Comparable studies that report Y_m calculated from CH_4 production and intake of cattle consuming low-quality forage are limited. Beck et al. (2019) provided four supplemental fat sources to steers grazing tallgrass prairie, which would contain similar species but slightly greater forage quality (9.6% CP, 72.1% NDF, and 46.1% ADF) to the hay fed in the current study. Beck et al. (2019) observed Y_m of 9.7% in unsupplemented steers, and Y_m by steers provided supplemental fat from whole cottonseed, soybean oil, or ruminal protected fats ranged from 7.1% to 8.0%.

Lassey (2007) summarized the available literature regarding methane production of grazing cattle. Across six studies, CH_4 production was determined in dairy cattle using SF_6, with intake estimated using either energy requirements (IPCC Tier II methodology) or inert markers coupled with in vitro OM digestibility measured on the forage. Estimated voluntary intake ranged from 6.8 to 22 kg per animal daily, with an estimated Y_m ranging from 4.5% to 9.5%. Direct comparisons to the current study and the IPCC review are difficult due to the differences in forage quality, in estimated DMI as a percent of BW, and in the methods used to quantify DMI and CH_4 production. Models to predict CH_4 production based on the dietary ingredients fed to the cattle need to be evaluated. For comparison, we estimated CH_4 production using the equations recommended in the NASEM (2016) (equation 16–8). NASEM (2016) equation 16–8 underpredicted methane production by 36.3 to 21.5 g/d in the present study (predicted = 123.4 g/d CH_4 for CON, 145.5 g/d CH_4 for CSM, and 145.8 g/d CH_4 for DDGS) and also predicted similar values for CSM compared with DDGS. However, the difference in methane production predicted using the NASEM empirical model and measured in the current study was similar between DDGS and CON (21.5 g/d measured vs. 22.4 g/d predicted). In contrast, the difference in methane production between CSM and CON appeared to be greater in the current study than model predictions (22.1 g/d in the NASEM model vs. 36.3 g/d in the current study).

VFA concentration

Acetate molar concentration (mol/100 mols; Table 4) was greatest ($P < 0.05$) for CON and was lower when CSM or DDGS was fed. The DDGS group had lower ($P < 0.05$) acetate molar concentrations than the CSM group. Conversely, propionate and butyrate molar concentrations were greatest ($P < 0.05$) for DDGS and least ($P < 0.05$) for CON. Subsequently, the acetate-to-propionate ratio was least ($P < 0.05$) for DDGS, intermediate for CSM, and greatest ($P < 0.05$) for CON. It is well established that when concentrates are included in forage-based diets that acetate molar concentrations decrease and propionate molar concentrations increase (Putnam et al., 1966; Rumsey et al., 1970). Köster et al. (1996) reported a linear decrease in acetate molar concentration when greater protein quantities were fed to steers consuming low-quality forage. In a similar experiment, Mathis et al. (2000) observed greater propionate molar concentrations when cattle consuming low-quality forage were provided supplemental protein. The observed greater propionate molar concentrations for DDGS over CSM could be attributed to feeding an additional 310 g of DM in DDGS treatment than CSM. Alternatively, differences in the composition of the supplements could have also contributed to differences in molar concentrations of propionate between CSM and DDGS treatments.

Propionate production via the succinate pathway serves as a hydrogen sink in the rumen, whereas hydrogen is liberated during acetate and butyrate production (Ungerfeld, 2020). The released hydrogens can be utilized by methanogens to reduce CO_2 to CH_4 (Janssen, 2010). Thus, dietary interventions that support increased propionate production will also likely reduce methane production in forage-fed cattle.

Table 4. Effect of supplemental protein on ruminal VFA molar concentrations in steers fed low-quality forage and provided supplemental protein

Item	CON[1]	CSM[1]	DDGS[1]	SEM[2]
Acetate, mol/100 mol	79.1[a]	76.1[b]	69.4[c]	0.56
Propionate, mol/100 mol	14.1[a]	15.6[b]	19.4[c]	0.32
Butyrate, mol/100 mol	5.6[a]	6.7[b]	10.3[c]	0.23
Isobutyrate, mol/100 mol	0.15	0.27	0.05	0.06
Isovalerate, mol/100 mol	0.87	0.73	0.22	0.29
Valerate, mol/100 mol	0.21[a]	0.48[b]	0.67[b]	0.08
A:P[3]	5.64[a]	4.89[b]	3.60[c]	0.11

[1]Treatments were: CON, no protein supplement; CSM, CSM at 0.29% of BW daily; DDGS, DDGS + urea supplemented at 0.41% of BW daily.
[2]Pooled SE of the treatment means.
[3]Acetate to propionate ratio.
[a–c]Within a row, means with unlike superscripts differ ($P < 0.05$).

Summary

Providing a protein supplement to steers fed a low-quality forage resulted in increased DM, OM, and NDF intake as expected and agrees with previous literature. The apparent digestibility of OM as a percent of OM intake was not impacted by supplementation. Gross energy intake increased when the supplement was provided to steers. Although greater amounts of energy were recovered in the feces, DE intake was still greater for supplemented steers. As expected, ruminal methane production (g/d) was increased due to supplementation; however, methane loss was greatest for the control steers when expressed as a percent of GEI. These results demonstrate that as GEI increased, the portion of energy lost as methane was decreased due to protein supplementation. Thus, the common practice of supplementing protein to cattle grazing low-quality forage would also be expected to affect ruminants' carbon footprint positively.

Acknowledgments

We acknowledge the contribution from the USDA-ARS Conservation and Production Res. Lab, Bushland, TX 79012, in cooperation with Texas A&M AgriLife Research. The mention of trade names or commercial products in this article is solely for the purpose of providing scientific information and does not imply recommendation or endorsement by the USDA. The funding was provided by United States Department of Agriculture to Project No. 2012-02355 through the National Institute for Food and Agriculture's Agriculture and Food Research Initiative, Regional Approaches for Adaptation to and Mitigation of Climate Variability and Change.

Conflict of interest statement

The authors have no conflicts of interest to report.

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