RUMINANT NUTRITION

Supplementing *Yucca schidigera* extract to mitigate frothy bloat in beef cattle receiving a high-concentrate diet

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Abstract

This experiment compared incidence of frothy bloat, as well as ruminal, physiological, and performance responses of beef heifers receiving a bloat-provoking diet and supplemented with *Yucca schidigera* extract. Sixteen ruminally cannulated Angus-influenced heifers were ranked by body weight (BW) and assigned to 4 groups of 4 heifers each. Groups were enrolled in a replicated 4 × 4 Latin square design containing 4 periods of 28 d, and a 21-d washout interval between periods. Groups were assigned to receive no *Y. schidigera* extract (CON), or *Y. schidigera* extract at (as-fed basis) 1 g/heifer daily (YS1), 2 g/heifer daily (YS2), or 4 g/heifer daily (YS4). During each period, heifers ($n = 16/\text{treatment}$) were housed in individual pens, and fed a sorghum (*Sorghum bicolor* L.)-based bloat-provocative diet at 2% of their BW. Diet and treatments were individually fed to heifers, twice daily in equal proportions (0700 and 1600 hours). Heifers were assessed for bloat score (0 to 5 scale, increasing according to bloat severity) 3 hr after the morning feeding. Blood samples were collected on days 0, 7, 14, 21, and 28 prior to (0 hr) and at 3, 6, and 9 hr relative to the morning feeding. Rumen fluid samples were collected at the same time points on days 0 and 28. Orthogonal contrasts were tested to determine whether inclusion of *Y. schidigera* extract (0, 1, 2, or 4 g/heifer daily) yielded linear or quadratic effects, and explore an overall effect of *Y. schidigera* extract supplementation (CON vs. YS1 + YS2 + YS4). Rumen fluid viscosity was impacted quadratically by *Y. schidigera* extract inclusion ($P = 0.02$), being greatest in YS1, followed by YS2, and equivalent between CON and YS4 heifers. Heifers receiving *Y. schidigera* extract had greater ($P \leq 0.05$) rumen propionate, iso-valerate, and valerate concentrations, as well as less ($P < 0.01$) acetate : propionate ratio compared with CON heifers. Inclusion of *Y. schidigera* extract linearly increased ($P \leq 0.04$) average daily gain and feed efficiency. No other treatment effects were noted ($P \geq 0.19$) including bloat score (1.07 ± 0.03 across treatments), ruminal protozoa count, plasma concentrations of cortisol, haptoglobin, urea N, total protein, and rumen concentration of total volatile fatty acids. Supplementing *Y. schidigera* extract up to 4 g/d favored rumen propionate concentrations and linearly increased growth and feed efficiency but failed to mitigate incidence of frothy bloat in beef heifers consuming a grain-based bloat-provocative diet.

Key words: beef heifers, frothy bloat, physiology, rumen, *Yucca schidigera* extract

Abbreviations

ADG	average daily gain
BW	body weight
PUN	plasma urea nitrogen
VFA	volatile fatty acids

Introduction

Bloat is a common digestive disorder in cattle fed high-concentrate diets, resulting from excessive gas accumulation and increased pressure in the rumen (Cheng et al., 1998). Frothy bloat is the predominant type of bloat in grain-fed cattle, when excessive gas trapped in the ruminal fluid becomes frothy and inhibits eructation (Meyer and Bryant, 2017). This disorder seldom results in cattle mortality but yields severe economic impacts due to treatment expenses and impaired animal performance (Nagaraja et al., 1998). Hence, management to prevent or alleviate the incidence of frothy bloat is critical for optimal welfare and productivity of cattle fed high-concentrate diets (Meyer and Bryant, 2017).

Strict bunk management, quality control of dietary ingredients, and addition of feed-grade antibiotics to high-concentrate diets have proven beneficial in reducing bloat incidence (Meyer and Bryant, 2017). With increased public pressure and restrictions regarding the use of feed-grade antimicrobials in livestock systems (US Food and Drug Administration, 2015), alternative feeding strategies to prevent health and digestive disorders in grain-fed cattle are warranted. One example is the use of plant-derived feed additives such as *Yucca schidigera* extract (Sousa et al., 2019). This additive has been shown to improve rumen fermentation by decreasing population of protozoa and bacteria such as *Streptococcus bovis* (Wallace et al., 1994), which are associated with the etiology of bloat in grain-fed cattle (Cheng et al., 1998). Moreover, *Y. schidigera* extract has natural surfactant properties known to modulate bloat in cattle (Clarke and Reid, 1972), although surfactants have been mostly effective preventing pasture bloat (Nagaraja et al., 1998; Meyer and Bryant, 2017).

Supplementing the *Y. schidigera* extract at 2 g/d (as-fed basis) improved in situ ruminal forage digestibility and microbial N flow in forage-fed steers (McMurphy et al., 2014a), as well as feed efficiency and average daily gain (ADG) in feedlot receiving cattle (Sousa et al., 2019). These latter authors did not report bloat incidence in their study, and the impacts of *Y. schidigera* extract on bloat in cattle fed high-concentrate diets remain unknown. Bloat-provoking diets have been used to examine feed additives with potential to prevent or mitigate bloat in grain-fed cattle (Bartley et al., 1983). Therefore, we hypothesized that supplementing *Y. schidigera* extract will decrease the incidence of frothy bloat in cattle receiving a grain-based bloat-provoking diet. To investigate this hypothesis, this experiment compared incidence of frothy bloat, as well as ruminal, physiological, and performance responses of beef heifers receiving a bloat-provoking diet and supplemented with *Y. schidigera* extract.

Materials and Methods

This experiment was conducted at the Texas A&M, Nutrition & Physiology Center (College Station, TX). All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Texas

A&M AgriLife Research, Agriculture Animal Care and Use Committee (#2018-0099).

Animals and treatments

Sixteen ruminally cannulated yearling, nulliparous, nonpregnant Angus-influenced heifers were assigned to this experiment. Heifer unshrunk body weight (BW) was recorded for 3 consecutive days prior to the beginning of the experiment and averaged to represent pretrial BW (224 ± 4 kg). Heifers were ranked by pretrial BW and assigned to 4 groups of 4 heifers each, in a manner that all groups had equivalent pretrial BW. Groups were enrolled in a replicated 4×4 Latin square design containing 4 periods of 28 d, and a 21-d washout interval between periods. During each period (days 0 to 28), heifers were housed in an enclosed barn in individual pens (2×4 m) with ad libitum access to water and fed a bloat-provocative diet (Tables 1 and 2) at 2% of their BW recorded at the beginning of each period (Bartley

Table 1. Nutrient profile (dry matter basis) of feed ingredients offered to heifers¹

Item ²	DDG	Grass hay (chopped)	Pellet ³
Dry matter, %	88.3	94.1	89.4
Crude protein, %	35.7	8.70	19.9
Neutral detergent fiber, %	32.0	72.4	15.4
Acid detergent fiber, %	14.5	44.6	11.9
Starch, %	5.42	9.00	40.4
Net energy for maintenance, ⁴ Mcal/kg	2.18	0.990	2.04
Net energy for growth, ⁴ Mcal/kg	1.50	0.440	1.39
Ca, %	0.030	0.610	0.860
P, %	1.13	0.170	0.640
Mg, %	0.377	0.130	0.210
K, %	1.20	1.87	1.17
Na, %	0.197	0.064	0.418
Fe, mg/kg	95	221	325
Zn, mg/kg	76.3	30	106
Cu, mg/kg	5.67	8	36
Mn, mg/kg	19.7	72	79
Mo, mg/kg	1.13	1.30	1.40
Co, mg/kg	0.116	0.360	1.52
Se, mg/kg	0.833	0.070	0.540

¹During each experimental period (days 0 to 28), heifers received (as-fed) at diet at 2% of their initial BW containing 75% hay + 25% pellet from days 0 to 2, 50% of each ingredient from days 3 to 5, 25% hay and 75% pellet from days 6 to 8, 15% hay and 85% pellet from days 9 to 11, and 100% pellet from days 11 to 28. DDG = dried distiller's grain.

²Analyzed via wet chemistry procedures by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Calculations for net energy for maintenance and growth used the equations proposed by the NRC (2000).

³Containing (as-fed basis) 58.1% of cracked sorghum (*Sorghum bicolor* L.) grain, 21.4% of dehydrated alfalfa hay, 15.5% of soybean meal, 2.5% liquid molasses, 2% mineral mix, and 0.5% pellet binder. The mineral mix contained 14% Ca, 7% P, 13% NaCl, 0.27% K, 0.4% Mg, 0.25% Cu, 0.003% Se, 0.99% Zn, 90.91 IU/kg of vitamin A, 9.09 IU/kg of vitamin D3, and 0.045 IU/kg of vitamin E (Purina Animal Nutrition, Shoreview, MN).

⁴Calculated with the following equations (NRC, 2000): net energy for maintenance = $(1.37 \times \text{ME}) - (0.138 \times \text{ME}^2) + (0.0105 \times \text{ME}^3) - (1.12)$; net energy for gain = $(1.42 \times \text{ME}) - (0.174 \times \text{ME}^2) + (0.0122 \times \text{ME}^3) - (0.165)$; ME = $(0.82 \times \text{DE})$; 1 kg of TDN = 4.4 Mcal of DE. Given that ME = metabolizable energy; DE = digestible energy; TDN = total digestible nutrients calculated based on the study by Weiss et al. (1992).

Table 2. Composition and nutrient profile of bloat-provocative diets offered to heifers during a 28-d experimental period

Item	Day of the experimental period				
	0 to 2	3 to 5	6 to 8	9 to 11	11 to 28
Ingredients, % (as-fed basis)					
Grass hay (chopped)	75	50	25	15	0
Pellet ²	25	50	75	85	100
Nutrient profile ¹ (dry matter basis)					
Dry matter, %	92.9	91.8	90.6	90.1	89.4
Crude protein, %	11.4	14.1	17.0	18.1	19.9
Neutral detergent fiber, %	58.7	44.6	30.2	24.3	15.4
Acid detergent fiber, %	36.7	28.7	20.4	17.0	11.9
Starch, %	16.6	24.3	32.2	35.5	40.4
Net energy for maintenance, Mcal/kg	1.24	1.50	1.77	1.88	2.04
Net energy for growth, Mcal/kg	0.669	0.902	1.143	1.241	1.39
Ca, %	0.670	0.731	0.795	0.821	0.860
P, %	0.283	0.399	0.518	0.566	0.640
Mg, %	0.149	0.169	0.189	0.197	0.210
K, %	1.70	1.53	1.35	1.28	1.17
Na, %	0.149	0.236	0.326	0.363	0.418
Fe, mg/kg	246	272	298	309	325
Zn, mg/kg	48.3	67.0	86.2	94.1	106
Cu, mg/kg	14.7	21.6	28.7	31.6	36
Mn, mg/kg	73.7	75.4	77.2	77.9	79
Mo, mg/kg	1.32	1.35	1.37	1.38	1.40
Co, mg/kg	0.639	0.925	1.218	1.338	1.52
Se, mg/kg	0.183	0.299	0.418	0.466	0.540

¹Containing (as-fed basis) 58.1% of cracked sorghum (*Sorghum bicolor* L.) grain, 21.4% of dehydrated alfalfa hay, 15.5% of soybean meal, 2.5% liquid molasses, 2% mineral mix, and 0.5% pellet binder. The mineral mix contained 14% Ca, 7% P, 13% NaCl, 0.27% K, 0.4% Mg, 0.25% Cu, 0.003% Se, 0.99% Zn, 90.91 IU/kg of vitamin A, 9.09 IU/kg of vitamin D3, and 0.045 IU/kg of vitamin E (Purina Animal Nutrition, Shoreview, MN).

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³Calculated with the following equations (NRC, 2000): net energy for maintenance = $(1.37 \times \text{ME}) - (0.138 \times \text{ME}^2) + (0.0105 \times \text{ME}^3) - (1.12)$; net energy for gain = $(1.42 \times \text{ME}) - (0.174 \times \text{ME}^2) + (0.0122 \times \text{ME}^3) - (0.165)$; ME = $(0.82 \times \text{DE})$; 1 kg of TDN = 4.4 Mcal of DE. Given that ME = metabolizable energy, DE = digestible energy, TDN = total digestible nutrients calculated based on the study by Weiss et al. (1992).

et al., 1983). Diet was fed daily (0700 and 1600 hours) in equal proportions. Throughout the washout interval, heifers received water, chopped grass hay, and mineral mix (Table 1) for ad libitum consumption. Heifers were maintained as a single group in a drylot pen (20 × 20 m) during the initial 14 d of the washout interval and returned to individual pens for the remaining 7 d. It should be noted that diets offered herein did not contain ionophores or other feed additives traditionally used to prevent or alleviate bloat in cattle (Meyer and Bryant, 2017).

At the beginning of each period (day 0), groups were assigned to receive 1 of 4 treatments: (1) no *Y. schidigera* extract supplementation (CON, $n = 16$), (2) *Y. schidigera* extract at 1 g/heifer daily (as-fed basis; YS1, $n = 16$), (3) *Y. schidigera* extract at 2 g/heifer daily (as-fed basis; YS2, $n = 16$), or (4) *Y. schidigera* extract at 4 g/heifer daily (as-fed basis; YS4, $n = 16$). The proper dose of *Y. schidigera* extract (-Aid; DPI Global, Porterville, CA) was mixed with dried distiller's grain and manually blended into the diet to create the CON, YS1, YS2, YS4 treatments. Heifers received half of their daily dose of *Y. schidigera* extract during each feeding of the day (50 g/heifer of the mix per feeding; as-fed basis). Dried distiller's grain without *Y. schidigera* was manually blended during each feeding of CON heifers (50 g/heifer per feeding; as-fed basis). Heifers completely consumed their diets within an 8-hr period.

Sampling

Samples of feed ingredients were collected prior to the beginning of each period, pooled across all period, and analyzed for

nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Heifer unshrunk BW was recorded on days -3, -2, and -1 relative to the beginning of each period and averaged for initial BW. Pretrial BW was used as initial BW for period 1. Final body was calculated by averaging heifer BW on days 28, 29, and 30 relative to the beginning of each period. Heifers were assessed daily (days 0 to 28) for frothy bloat score 3 hr after the morning feeding (Bartley et al., 1983; Neibarger and Nagaraja, 1988; Coe et al., 1996) according to Bartley (1965) where 0 = no froth nor abdominal distention; 1 = slight froth but no pressure nor abdominal distention; 2 = definite froth with sufficient pressure to expel froth but no abdominal distention; 3 = definite froth with sufficient pressure to cause abdominal distention on the left side; 4 = definite froth with sufficient pressure to cause abdominal distention on the left and right side; 5 = definite froth, severe abdominal distention, animal in severe distress, terminal unless pressure is relieved by opening the cannula cap. Heifers did not receive any additional treatments if diagnosed with bloat, regardless of the score given.

Blood samples were collected from all heifers on days 0, 7, 14, 21, and 28 of each period, immediately prior to (0 hr) and at 3, 6, and 9 hr relative to the morning feeding (0700 hours). Blood was collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing freeze-dried sodium heparin. Hair samples were collected from the tail-switch of all heifers (Schubach et al., 2017), concurrently with the blood sampling prior to feeding (0700 hours). Hair was collected from an area that had not been

previously sampled. Hair was clipped using scissors as close to the skin as possible, and the hair material closest to the skin (1 cm of length, 100 mg of weight) was collected.

Rumen samples were collected via rumen cannula from all heifers on days 0 and 28 of each period, immediately prior to (0 hr) and at 3, 6, and 9 hr relative to the morning feeding (0700 hours). Whole rumen contents were extracted as in [Cagle et al. \(2020\)](#) using a suction strainer, and ruminal pH measured immediately after collection (Orion STAR A221 pH meter, Thermo Fisher Scientific, Waltham, MA). Rumen samples (~200 mL) were strained through 8 layers of cheesecloth for fluid extraction, which was stored into individual stainless-steel thermoses to maintain both temperature and an anaerobic environment and transported to the laboratory for further processing.

Laboratorial analysis

Feed samples

Samples were analyzed by wet chemistry procedures for concentrations of crude protein (method 984.13; [AOAC, 2006](#)), acid detergent fiber (method 973.18 modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp., Fairport, NY; [AOAC, 2006](#)), neutral detergent fiber using α -amylase and sodium sulfite ([Van Soest et al., 1991](#); modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp.), starch (YSI 2700 SELECT Biochemistry Analyzer; YSI Inc., Yellow Springs, OH), macro and trace minerals using inductively coupled plasma emission spectroscopy ([Sirois et al., 1991](#)), and Se (method 996.16; [AOAC, 2006](#)). The equations used to calculate net energy for maintenance and gain, and the nutritional profile of feed ingredients and diets are described in [Table 1](#).

Plasma and hair samples

Blood samples were placed immediately on ice after collection, centrifuged (2500 $\times$ g for 30 min; 4 °C) for plasma harvest, and stored at -80 °C on the same day of collection. All samples were analyzed for cortisol (radioimmunoassay kit #07221106, MP Biomedicals, Santa Ana, CA; [Colombo et al., 2019](#)) and haptoglobin ([Cooke and Arthington, 2013](#)). Plasma samples collected on days 0, 14, and 28 were analyzed for plasma urea N (PUN) and total protein (Carysta High Volume Chemistry Analyzer; Zoetis). The intra- and inter-assay coefficient of variation were, respectively, 3.9% and 4.4% for cortisol, 5.3% and 7.2% for haptoglobin, 2.1% and 2.3% for total protein, and 1.7% and 2.3% for PUN. Cortisol was extracted from hair samples and analyzed by [Schubach et al. \(2020\)](#). The intra- and interassay coefficient of variation for hair cortisol were 7.7% and 8.9%, respectively.

Rumen samples

A 5-mL subsample of each rumen fluid sample was transferred into individual falcon tubes containing 1 mL of metaphosphoric acid, and stored at -20 °C on the same day of collection. These samples were processed and analyzed for volatile fatty acid (VFA) profile as described by [Cappelozza et al. \(2013\)](#). A 10-mL subsample of each rumen fluid sample was analyzed for viscosity using SV-10/SV-100 Vibro Viscometer (A&D Company Ltd., Tokyo, Japan) by [Pitta et al. \(2016\)](#). A third subsample of rumen fluid was analyzed for protozoa counts as described by [Cagle et al. \(2020\)](#) using a Sedgewick Rafter Counting Chamber (Hausser Scientific, Horsham, PA).

Statistical analysis

Heifer was considered the experimental unit for all analyses. All data were analyzed with the MIXED procedure of SAS

(SAS Inst. Inc., Cary, NC), using Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects, and heifer(group) and group as random variables. The model statement used for heifer BW, ADG, and feed efficiency contained the effects of treatment and period. The model statement used for feed intake, bloat score, and hair cortisol concentrations contained the effects of treatment, day, the treatment $\times$ day interaction, and period as independent variable. The specified term for these repeated statements was day, with heifer(group $\times$ treatment $\times$ period) as the subject, and autoregressive as covariance structure based on the Akaike information criterion. The model statement used for all plasma and rumen fluid variables contained the effects of treatment, day, hour, all resultant interactions, and period as an independent variable. The specified term for these repeated statements was hour, with heifer(group $\times$ treatment $\times$ period $\times$ day) as the subject, and autoregressive as covariance structure based on the Akaike information criterion. Results from plasma variables, ruminal fluid variables, and hair cortisol from day 0 were averaged and used as independent covariate in each, respectively, analysis. All results are reported as least square means, or covariately adjusted least square means when model contained independent variables, and separated using least square differences. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10 . Orthogonal contrasts were tested to determine whether inclusion of *Y. schidigera* extract (0, 1, 2, or 4 g/heifer daily) yielded linear or quadratic effects, and to explore overall effect of *Y. schidigera* extract supplementation (CON vs. YS1 + YS2 + YS4). Contrast coefficients were generated using the IML procedure of SAS (SAS Inst. Inc.). The contrasts described above were chosen given their relevance to our hypothesis and limited to 3 contrasts as 4 experimental treatments were investigated herein ([Kaps and Lamberson, 2017](#)). If multiple contrasts are significant ($P \leq 0.05$), the contrast with the greatest F-value and smallest P-value was discussed.

Results

Bloat score and incidence of each individual score during the experiment were not impacted by treatments ($P \geq 0.15$; [Table 3](#)). Bloat score increased across treatments with the advance of each experimental period (day effect, $P < 0.01$; [Figure 1](#)). Rumen pH and ruminal protozoa count ([Table 4](#)) were also not affected by treatments ($P \geq 0.39$; [Table 4](#)). Rumen fluid viscosity was quadratically impacted by *Y. schidigera* extract inclusion ($P = 0.02$), being greatest in YS1 heifers, followed by YS2 heifers, and equivalent between CON and YS4 heifers ([Table 4](#)). Day and hour effects, as well as day $\times$ hour interactions were detected ($P \leq 0.05$) for these parameters and are described in [Supplementary Figure 1](#).

Rumen fluid concentrations of acetate, butyrate, isobutyrate, and total VFA were not affected ($P \geq 0.12$) by treatments ([Table 4](#)). Heifers receiving *Y. schidigera* extract had greater ($P \leq 0.05$) ruminal concentrations of propionate, isovalerate, and valerate, as well as less ($P < 0.01$) acetate : propionate ratio compared with CON heifers ([Table 4](#)). Day and hour effects, as well as day $\times$ hour interactions were detected ($P \leq 0.05$) for VFA responses and are described in [Supplementary Figure 2](#).

Plasma concentrations of cortisol, haptoglobin, PUN, total protein, and hair cortisol concentrations were not impacted ($P \geq 0.20$) by treatments ([Table 5](#)). Day and hour effects, as well as day $\times$ hour interactions were also detected ($P \leq 0.05$) for these variables and are described in [Supplementary Figure 3](#). Heifer

Table 3. Overall bloat score (0 to 5; Bartley, 1965), and incidence of each score, of rumen-cannulated heifers receiving a bloat-inducing diet, and supplemented or not (CON; $n = 16$) with *Yucca schidigera* extract (Micro-Aid; DPI Global, Porterville, CA) at (as-fed basis) 1 g/heifer daily (YS1; $n = 16$), 2 g/heifer daily (YS2; $n = 16$), or 4 g/heifer daily (YS4; $n = 16$)¹

Item	CON	YS1	YS2	YS4	SEM	Contrasts (P-value) ²		
						M inclusion	Linear	Quadratic
Mean bloat score	1.21	1.25	1.32	1.27	0.08	0.20	0.38	0.19
Score 0, %	38.1	39.3	33.1	36.0	2.81	0.52	0.38	0.44
Score 1, %	11.0	8.83	12.8	9.91	1.89	0.79	0.93	0.60
Score 2, %	42.0	42.5	42.3	45.8	3.77	0.63	0.31	0.70
Score 3, %	8.51	7.95	11.1	8.36	3.38	0.71	0.87	0.29
Score 4, %	0.392	2.07	0.076	0.00	0.621	0.60	0.15	0.15

¹Heifers were assigned to a 4 × 4 Latin square design, containing 4 period of 28 d each and a washout interval of 14 d between periods.

Heifers received diets at 2% of their initial BW (as-fed basis), and completely consumed their diets within an 8-hr period. Feed efficiency was calculated using total BW gain and total feed intake of each heifer within each period. Bloat was scored daily 3 hr after the morning feeding.

²Orthogonal contrasts were tested to determine an overall effect of *Y. schidigera* extract supplementation (M inclusion; CON vs. YS1 + YS2 + YS4), and if inclusion of *Y. schidigera* extract (0, 1, 2, or 4 g/heifer daily) yielded linear or quadratic effects.

Figure 1. Bloat score (0 to 5 scale; Bartley, 1965) in rumen-cannulated heifers receiving a bloat-inducing diet, and supplemented or not (CON; $n = 7$) with *Y. schidigera* extract (Micro-Aid; DPI Global, Porterville, CA) at (as-fed basis) 1 g/heifer daily (YS1; $n = 16$), 2 g/heifer daily (YS2; $n = 16$), or 4 g/heifer daily (YS4; $n = 16$). Heifers received the bloat-inducing diet at 2% of their BW per day, which was fed twice daily (0700 and 1600 hours) in equal proportions. Bloat was scored daily 3 hr after the morning feeding. Orthogonal contrasts were tested to determine an overall effect of *Y. schidigera* extract supplementation (CON vs. YS1 + YS2 + YS4), and if inclusion of *Y. schidigera* extract (0, 1, 2, or 4 g/heifer daily) yielded linear or quadratic effects. No treatment differences were noted ($P \geq 0.44$). A day effect was noted ($P < 0.01$) as bloat score increased with the advance of the experimental period.

BW during the experiment was not influenced ($P \geq 0.24$) by treatments (Table 6). However, inclusion of *Y. schidigera* extract linearly increased ($P \leq 0.04$) heifer ADG and feed efficiency (Table 6).

Discussion

The experimental model and bloat-provocative diet used herein were successful in inducing frothy bloat, given the increasing bloat score noted in heifers across treatments as the experimental period advanced. No incidence of extreme bloat (e.g., score 5) and only few cases of bloat score 4 were noted (Table 3), in accordance with previous research using a similar diet and reporting bloat induction without extreme cases (Bartley et al., 1983; Neibarger and Nagaraja, 1988; Coe et al., 1996). Contrary to our hypothesis, *Y. schidigera* extract supplementation failed to alleviate frothy bloat in cattle receiving a grain-based bloat-provocative diet. Despite its

surfactant properties and potential impacts in reducing bloat (Cheng et al., 1998; Nagaraja et al., 1998), *Y. schidigera* extract also contains ~18% saponins (Singer et al., 2008) that yield a foaming effect (Cheeke, 2000). Foaming and subsequent frothy bloat are associated positively with rumen fluid viscosity (Cheng et al., 1998; Pitta et al., 2016). Rumen fluid viscosity was greatest in YS1 heifers, followed by YS2 heifers, and equivalent between CON and YS4 heifers. The reason to why rumen fluid viscosity decreased according to dietary inclusion of *Y. schidigera* extract is unknown and deserves investigation. These differences were not sufficient to increase bloat incidence in YS1 and YS2 heifers. Nonetheless, rumen fluid viscosity increased with the advance of the experiment (day 0 vs. day 28) and upon feeding of the bloat-provocative diet, corroborating that bloat incidence and rumen fluid viscosity are positively associated (Pitta et al., 2016).

Inclusion of *Y. schidigera* extract also failed to impact rumen pH and protozoa count in heifers receiving the bloat-provocative diet. As expected, rumen pH decreased with the

Table 4. Ruminal responses of rumen-cannulated heifers receiving a bloat-inducing diet, and supplemented or not (CON; $n = 7$) with *Y. schidigera* extract (Micro-Aid; DPI Global, Porterville, CA) at (as-fed basis) 1 g/heifer daily (YS1; $n = 16$), 2 g/heifer daily (YS2; $n = 16$), or 4 g/heifer daily (YS4; $n = 16$)^{1,2}

Item	CON	YS1	YS2	YS4	SEM	Contrasts (P-value) ³		
						M inclusion	Linear	Quadratic
Rumen pH	5.58	5.62	5.51	5.53	0.074	0.72	0.39	0.88
Ruminal protozoa count, 1000 per mL	257	255	268	280	29	0.84	0.50	0.92
Rumen fluid viscosity, mPa s	13.0	17.3	15.6	12.3	1.8	0.23	0.32	0.02
Rumen volatile fatty acids								
Acetate	66.6	67.3	64.2	66.8	2.3	0.83	0.90	0.54
Propionate	25.1	31.9	31.8	31.3	2.4	0.01	0.10	0.09
Butyrate	13.0	13.8	13.3	13.6	0.7	0.53	0.74	0.80
Isovalerate	2.12	2.48	2.48	2.60	0.16	0.03	0.05	0.32
Isobutyrate	1.06	1.19	1.10	1.19	0.05	0.12	0.16	0.85
Valerate	1.61	1.97	1.86	1.76	0.13	0.05	0.72	0.06
Acetate : propionate ratio	3.18	2.73	2.57	2.63	0.18	<0.01	0.04	0.04
Total	110	119	115	117	4	0.13	0.37	0.44

¹Heifers were assigned to a 4 × 4 Latin square design, containing 4 period of 28 d each and a washout interval of 14 d between period. Heifers received a bloat-inducing diet at 2% of their BW per day, which was fed twice daily (0700 and 1600 hours) in equal proportions.

²Rumen fluid samples (50 mL) were collected on days 0 and 28 of each experimental period, immediately prior to (0 hr) and at 3, 6, and 9 hr relative to the morning feeding (0700 hours). Rumen pH was measured using a pH meter concurrently with rumen fluid sampling. Rumen fluid samples collected at 0 and 6 hr were analyzed for viscosity assessment (Pitta et al., 2016) and protozoa count (Caigle et al., 2020), whereas all samples were analyzed for volatile fatty acid profile (Cappelozza et al., 2013). Results obtained on day 0 were averaged and used as independent covariate in each respective analysis.

³Orthogonal contrasts were tested to determine an overall effect of *Y. schidigera* extract supplementation (M inclusion; CON vs. YS1 + YS2 + YS4), and if inclusion of *Y. schidigera* extract (0, 1, 2, or 4 g/heifer daily) yielded linear or quadratic effects.

Table 5. Physiological responses of rumen-cannulated heifers receiving a bloat-inducing diet, and supplemented or not (CON; $n = 7$) with *Y. schidigera* extract (Micro-Aid; DPI Global, Porterville, CA) at (as-fed basis) 1 g/heifer daily (YS1; $n = 16$), 2 g/heifer daily (YS2; $n = 16$), or 4 g/heifer daily (YS4; $n = 16$)^{1,2}

Item	CON	YS1	YS2	YS4	SEM	Contrasts (P-value) ³		
						M inclusion	Linear	Quadratic
Plasma cortisol, ng/mL	4.75	5.01	4.33	4.44	0.39	0.61	0.20	0.81
Plasma haptoglobin, mg/mL	0.261	0.239	0.273	0.199	0.037	0.49	0.18	0.40
PUN, mg/dL	11.8	12.1	11.5	12.3	0.4	0.59	0.34	0.38
Plasma total protein, g/dL	7.53	7.62	7.50	7.59	0.06	0.59	0.76	0.74
Hair cortisol, pg/mg of hair	4.81	4.36	4.68	4.59	0.32	0.46	0.83	0.66

¹Heifers were assigned to a 4 × 4 Latin square design, containing 4 period of 28 d each and a washout interval of 14 d between period. Heifers received a bloat-inducing diet at 2% of their BW per day, which was fed twice daily (0700 and 1600 hours) in equal proportions.

²Blood samples were collected from heifers on days 0, 7, 14, 21, and 28 of each experimental period, immediately prior to (0 hr) and at 3, 6, and 9 hr relative to the morning feeding (0700 hours). Hair samples were collected from the tail-switch by Schubach et al. (2017) at 0700 hours on days 0, 14, and 28 of each experimental period. Results obtained on day 0 were used as independent covariate in each respective analysis.

³Orthogonal contrasts were tested to determine an overall effect of *Y. schidigera* extract supplementation (M inclusion; CON vs. YS1 + YS2 + YS4), and if inclusion of *Y. schidigera* extract (0, 1, 2, or 4 g/heifer daily) yielded linear or quadratic effects.

advance of the experiment and upon feeding across treatments (Cheng et al., 1998; Nagaraja et al., 1998). Protozoa count increased throughout the experiment and decreased 6 hr after feeding across treatments. Given its high grain content and fermentative capacity, the bloat-provocative diet favored the proliferation of protozoa in the rumen (Williams and Coleman, 1997). Although their involvement in feedlot bloat is not fully known (Cheng et al., 1998), protozoa are known to ingest plant chloroplasts that contain antifoaming lipids (Oxford, 1958; Mangan, 1959), whereas cell content of protozoa bursting from excessive starch storage also contribute to ruminal foaming (Clarke, 1965; Jones and Lyttleton, 1972). Supporting our findings, McMurphy et al. (2014a) reported reduced rumen pH and increased ruminal protozoa concentrations in forage-fed steers supplemented with cottonseed and wheat middlings compared

with unsupplemented steers. These authors also reported that inclusion of *Y. schidigera* extract to the supplement (1 or 2 g/steers daily) failed to impact ruminal pH, but reduced protozoa concentration compared with supplemented steers receiving no *Y. schidigera* extract. Hristov et al. (1999) supplemented *Y. schidigera* powder to beef heifers receiving a grain-based diet and reported that rumen pH was not altered, whereas protozoa count was decreased in supplemented heifers. The saponins contained in the *Y. schidigera* extract react with cholesterol in the protozoal cell membrane to stimulate lysis (Cheeke, 2000). Perhaps the bloat-provocative diet used herein favored ruminal protozoa proliferation and altered ruminal environment to an extent that nullified the antiprotozoal effects of *Y. schidigera* extract. In turn, Sliwinski et al. (2002) reported that ruminal protozoa count was not altered by inclusion of *Y. schidigera*

Table 6. Performance parameters of rumen-cannulated heifers receiving a bloat-inducing diet, and supplemented or not (CON; $n = 16$) with *Y. schidigera* extract (Micro-Aid; DPI Global, Porterville, CA) at (as-fed basis) 1 g/heifer daily (YS1; $n = 16$), 2 g/heifer daily (YS2; $n = 16$), or 4 g/heifer daily (YS4; $n = 16$)^{1,2}

Item	CON	YS1	YS2	YS4	SEM	Contrasts (P-value) ³		
						M inclusion	Linear	Quadratic
Initial BW, kg	257.4	257.4	258.2	255.6	4.7	0.86	0.40	0.42
Final BW, kg	266.5	267.6	269.5	268.2	5.2	0.24	0.38	0.27
ADG, kg/day	0.324	0.363	0.420	0.452	0.052	0.08	0.04	0.66
Feed intake, kg/day	5.15	5.15	5.17	5.11	0.094	0.84	0.39	0.41
Feed efficiency, kg/kg	0.064	0.070	0.084	0.091	0.010	0.10	0.04	0.71

¹Heifers were assigned to a 4 × 4 Latin square design, containing 4 period of 28 d each and a washout interval of 14 d between periods.

Heifers received diets at 2% of their initial BW (as-fed basis), and completely consumed their diets within an 8-hr period. Feed efficiency was calculated using total BW gain and total feed intake of each heifer within each period.

²Heifer BW was recorded on days −3, −2, and −1 relative to the beginning of each experimental period, and averaged for initial BW. Final body was calculated by averaging heifer BW on days 28, 29, and 30 relative to the beginning of each experimental period.

³Orthogonal contrasts were tested to determine an overall effect of *Y. schidigera* extract supplementation (M inclusion; CON vs. YS1 + YS2 + YS4), and if inclusion of *Y. schidigera* extract (0, 1, 2, or 4 g/heifer daily) yielded linear or quadratic effects.

extract to rumen fermenters containing a grass silage-based diet. Research is still warranted to determine the impacts of *Y. schidigera* extract on ruminal protozoa population in beef cattle receiving forage-based, grain-based, and bloat-provoking diets.

Rumen VFA profile was also altered by inclusion of *Y. schidigera* extract independently of level, favoring propionate, isovalerate, valerate, and decreasing the acetate : propionate ratio. Similarly, Holtshausen et al. (2009) reported that inclusion of *Y. schidigera* powder to in vitro 24-hr batch ruminal cultures increased propionate and valerate concentrations, decreased acetate : propionate ratio, and did not alter total VFA concentration. Hristov et al. (1999) also reported greater propionate concentrations, similar acetate and total VFA concentrations and reduced acetate : propionate ratio in beef heifers receiving a grain-based diet and supplemented with *Y. schidigera* powder. These outcomes were mostly attributed to the antiprotozoal effects of *Y. schidigera*, as rumen protozoa compete for nutrients and hydrogen with propionate-producing bacteria (Williams and Coleman, 1997). Nonetheless, *Y. schidigera* has also been shown to inhibit growth of rumen bacterial species not involved in propionate synthesis such as *S. bovis* and *Butyrivibrio fibrisolvens* (Wallace et al., 1994), facilitating the proliferation of propionate-producing bacteria (Hristov et al., 1999). This latter rationale corroborates the results from this experiment, as *Y. schidigera* extract inclusion increased rumen propionate concentrations without reducing rumen protozoa counts. The impacts of *Y. schidigera* on valerate synthesis have been variable (Hristov et al., 1999; Santoso et al., 2004; Holtshausen et al., 2009), whereas the increased isovalerate concentration noted herein may be associated with enhanced ruminal protein fermentation from *Y. schidigera* supplementation (Dijkstra, 1994; Santoso et al., 2004). Across treatments, concentrations of all VFA increased and acetate : propionate ratio decreased with the advance of the experiment (day 0 vs. day 28) and after feeding, which was expected given the grain-based composition of the bloat-provocative diet (NASEM, 2016). The hour effects reported herein depict the expected ruminal VFA profile upon feeding of a forage-based diet (day 0) or grain-based diet (day 28; NASEM, 2016).

Bloat can yield acute and chronic stress in cattle according to its severity (Lippke et al., 1972), which in turn elicit inflammatory and acute-phase responses (Cooke, 2017). For these reasons, concentrations of cortisol (hair and plasma) and haptoglobin (plasma) were evaluated herein to determine whether

Y. schidigera extract would impact such responses by alleviating bloat incidence. Cortisol and haptoglobin concentrations were not impacted by inclusion of *Y. schidigera* extract to the bloat-provocative diet, corroborating the bloat score results. Across treatments, plasma concentrations of haptoglobin and cortisol increased until day 14 of the experimental period but returned to baseline levels by day 28, suggesting that heifers experienced transient adrenocortical and acute-phase responses (Cooke, 2017). Hour effects were variable for plasma cortisol but show that plasma haptoglobin concentrations decreased after feeding, which to our knowledge has not been reported before and provide novel insight in the bovine haptoglobin response. Hair cortisol concentrations has been used as biomarker of chronic stress in cattle (Schubach et al., 2020) and increased with the advance of the experiment across treatments. This outcome can be associated with bloat incidence and heightened chronic stress but also with the confinement of heifers into individual pens (Schubach et al., 2017). Nonetheless, the impacts of bloat on stress-induced physiological and inflammatory responses deserve consideration, particularly due to the role of these responses on cattle immunocompetence (Cooke, 2017).

Supplementing *Y. schidigera* to cattle has also been shown to increase microbial N flow to the lower gastrointestinal tract, by decreasing rumen protozoa population and predation of bacteria (Wallace et al., 1994; Hristov et al., 1999). McMurphy et al. (2014a) reported that microbial N flow to the small intestine was increased by supplementing *Y. schidigera* extract at 2 g/d to forage-fed steers, suggesting greater supply of metabolize protein available for duodenal absorption. Accordingly, PUN and plasma total protein were evaluated herein but were not impacted by inclusion of *Y. schidigera* extract to the bloat-provocative diets, which agrees with the lack of differences in rumen protozoa count across treatments. Nonetheless, McMurphy et al. (2014a) also failed to report differences in blood urea N in their experiment, despite differences noted in microbial N flow. Hristov et al. (1999) reported similar PUN concentrations in grain-fed beef heifers supplemented or not with *Y. schidigera* powder, despite differences in protozoa count. Perhaps PUN and total protein concentrations did not capture the potential benefits of *Y. schidigera* extract on ruminal microbial synthesis herein and in previous research (McMurphy et al., 2014a; Hristov et al., 1999). Concentrations of PUN increased with the advance of the experiment and upon feeding across treatments, as expected given the crude protein content of the bloat-provocative diet (Tables 1 and 2; Broderick and Clayton, 1997).

Plasma total protein concentrations were decreased when heifers received the bloat-provocative diet, and hour effects varied with the advance of the experiment. These outcomes may suggest hindered protein absorption and altered hepatic and kidney function due to bloating (Meyer and Bryant, 2017).

The main objective of this experiment was to investigate the role of *Y. schidigera* extract on frothy bloat in grain-fed cattle, as the productive benefits of supplementing this additive to growing beef cattle were recently investigated others (McMurphy et al., 2014b; Sousa et al., 2019). Nevertheless, heifer BW, ADG, and feed efficiency were evaluated because these productive responses are impacted by bloat (Nagaraja et al., 1998). Heifer ADG was linearly increased with the inclusion of *Y. schidigera* extract to the diet and attributed to a linear improvement in feed efficiency as feed intake was fixed at 2% of heifer initial BW. Supporting these findings, Sousa et al. (2019) reported that supplementing 2 g/d of *Y. schidigera* extract to feedlot receiving cattle improved their ADG and feed efficiency with no impacts on feed intake. These outcomes were credited to improved rumen fermentation conditions due to reduced rumen protozoa population (Sousa et al., 2019), leading to decreased methane emissions, increased ruminal propionate production, and greater microbial N flow to the lower gastrointestinal tract (Wallace et al., 1994; Hristov et al., 1999). However, Sousa et al. (2019) did not evaluate any of these ruminal parameters, nor the inclusion of *Y. schidigera* extract at 4 g/animal daily. The *Y. schidigera* extract was included herein at 1 g, 2 g, or 4 g/heifer daily as experimental dosages for bloat prevention, which increased rumen propionate concentrations but not in a linear fashion. Rumen protozoa count was not impacted by *Y. schidigera* extract, and any potential benefits to microbial N flow to the small intestine were not captured by plasma total protein and PUN concentrations. Hence, research is warranted to determine the biological mechanisms by which supplementing *Y. schidigera* extract up to 4 g/d linearly increased ADG and feed efficiency of grain-fed beef heifers.

In conclusion, supplementing *Y. schidigera* extract at 1 g, 2 g, or 4 g/d failed to mitigate incidence of frothy bloat in beef heifers consuming a grain-based bloat-provocative diet. Accordingly, *Y. schidigera* extract did not reduce ruminal responses associated with bloat etiology, including rumen fluid viscosity, protozoa count, and total VFA concentration (Cheng et al., 1998; Nagaraja et al., 1998). In turn, *Y. schidigera* extract inclusion linearly increased heifer ADG because of improved feed efficiency, corroborating previous research from our group (Sousa et al., 2019). These latter outcomes were independent of bloat incidence and cannot be fully explained by ruminal and physiological responses evaluated herein. Therefore, research is warranted to further investigate the biological and productive benefits of *Y. schidigera* extract to beef cattle receiving high-concentrate diets, including supplementation of this feed additive at 4 g/animal daily to feedlot cattle.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

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Conflict of Interest Statement

M.J.R. is employed by the funder of this project (Distributors Processing, Inc., Porterville, CA) and contributed to research design and data interpretation. However, the principal investigator (R.F.C.) and all other authors of this manuscript declare no real or perceived conflicts of interest.

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