Productive and physiological responses of feeder cattle supplemented with *Yucca schidigera* extract during feedlot receiving[1]

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ABSTRACT: This experiment evaluated the effects of supplementing a saponin-containing feed ingredient, manufactured from purified extract of *Yucca schidigera* [Micro-Aid (**MA**); DPI Global; Porterville, CA], on performance, health, and physiological responses of receiving cattle. A total of 105 recently weaned Angus x Hereford calves (75 steers and 30 heifers), originating from eight cow-calf operations, were obtained from an auction facility on day −2 and road transported (800 km; 12 h) to the experimental facility. Immediately after arrival on day −1, shrunk BW was recorded and calves were grouped with free-choice access to grass hay, mineral supplement, and water. On day 0, calves were ranked by sex, source, and shrunk BW, and allocated to one of 21 pens (5 calves/pen; being one or two heifers within each pen). Pens were assigned to receive a total mixed ration (**TMR**) and one of three treatments (as-fed basis): (1) 1 g/calf daily of MA (**M1**; n = 7), (2) 2 g/calf daily of MA (**M2**; n = 7), or (3) no MA supplementation (**CON**; n = 7). Calves received the TMR to yield 15% (as-fed basis) orts, and treatments were top-dressed from days 0 to 59. Calves were assessed for bovine respiratory disease (**BRD**) signs and TMR intake was recorded for each pen daily. Calves were vaccinated against BRD pathogens on days 0 and 21. Final shrunk BW was recorded on day 60, and blood samples were collected on days 0, 2, 6, 10, 14, 21, 28, 34, 45, and 59. ADG was greater (P = 0.03) in M2 vs. M1 and CON (1.53, 1.42, and 1.42 kg/day, respectively), and similar (P = 0.95) between M1 and CON calves. No treatment effects were detected for TMR intake (P = 0.52), whereas feed efficiency was greater ($P \leq 0.05$) in M2 vs. M1 and CON calves (213, 200, and 204 g/kg, respectively) and similar (P = 0.40) between M1 and CON calves. No treatment effects were detected (P = 0.39) for diagnosis of BRD signs. The number of antimicrobial treatments required upon BRD diagnosis was greater ($P \leq 0.01$) in CON vs. M1 and M2 (1.40, 1.05, and 1.10 treatments, respectively), and similar (P = 0.60) between M1 and M2 calves. No other treatment effects were detected ($P \geq 0.23$), including circulating concentrations of hormones and metabolites, serum antibody titers to BRD pathogens, and mRNA expression of innate immunity genes in whole blood. Collectively, results from this experiment suggest that MA supplementation at 2 g/animal daily enhances performance and response to BRD treatment in high-risk cattle during feedlot receiving.

Key words: innate immunity, nutrition, performance, receiving cattle, respiratory disease, saponins

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INTRODUCTION

Beef cattle are exposed to a multitude of stress challenges during feedlot receiving, including road transport, commingling with different animals, and exposure to novel diets and environments (Duff and Galyean, 2007). The combination of these stressors is known to stimulate neuroendocrine and inflammatory responses that impair cattle immunocompetence and productivity (Cooke, 2017). Accordingly, incidence of bovine respiratory disease (**BRD**) is elevated during feedlot receiving, despite vaccination against BRD pathogens and efforts to minimize stress (Snowder et al., 2006; Wilson et al., 2017).

Prophylactic medication with feed-grade antimicrobials was a conventional strategy to mitigate BRD incidence and enhance growth in receiving cattle (Samuelson et al., 2016). With increased restrictions regarding the use of feed-grade antimicrobials in livestock systems (US Food and Drug Administration, 2015), alternative dietary strategies that enhance cattle performance and immunity are warranted. These include the use of non-antibiotic feed ingredients that enhance immune and rumen function, such as Micro-Aid (**MA**; DPI Global; Porterville, CA). This additive is manufactured from a purified extract of the *Yucca schidigera* plant, and contains ~18% saponins (DM basis; Singer et al., 2008). Saponins provide immunostimulatory properties by enhancing antibody and lymphocyte response to antigens (Shi et al., 2004), while also improving rumen fermentation by decreasing the protozoa population and modulating particulate passage rate (Goetsch and Owens, 1985; Hristov et al., 2004).

Supplementing MA to forage-fed beef steers at 1 or 2 g/d (as-fed basis) improved in situ ruminal forage digestibility (McMurphy et al., 2014a) but not ADG (McMurphy et al., 2014b), whereas MA supplementation at 2 g/d increased microbial N flow (McMurphy et al., 2014a). However, the effects of MA supplementation on performance or immune parameters in receiving cattle still warrant investigation. Based on this rationale, we hypothesized that MA supplementation is a dietary strategy to improve cattle immunocompetence and productivity during feedlot receiving. To investigate this hypothesis, this experiment compared performance, health, and physiological responses of feeder cattle supplemented with MA during a 60-d receiving period.

MATERIALS AND METHODS

This experiment was conducted at the Oregon State University, Eastern Oregon Agricultural Research Center (Burns, OR). All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee (#4973).

Animals and Treatments

One hundred and five Angus x Hereford recently weaned calves (75 steers and 30 heifers) were purchased from a commercial auction facility (Producers Livestock Marketing Association; Vale, OR) and utilized in this experiment (days 0 to 60). Cattle originated from eight cow-calf operations located in Eastern Oregon and Western Idaho, and no previous health or management history was available. On the day of purchase (day −2; 1800 hours), cattle were loaded into a double-deck commercial livestock trailer (Legend 50' cattle liner; Barrett LLC., Purcell, OK) at the auction facility and transported for 800 km to simulate the stress of a long haul (Cooke et al., 2013). During transport, the driver stopped once after 6 h of driving to rest for 60 min, whereas total transport time was 12 h. Cattle remained in the truck throughout the 12-h transportation period. Minimum, maximum, and average environmental temperatures during transport were −1, 13, and 7 °C, respectively, whereas average humidity was 71% and no precipitation was observed.

On day −1 of the experiment (0600 hours), cattle were unloaded at the Eastern Oregon Agricultural Research Center, immediately weighed (initial shrunk BW = 220 ± 2 kg), and maintained as a single group (160 × 100 m paddock) for 24 h with free-choice alfalfa-grass hay, water, and a commercial mineral mix (described in Table 1). On day 0, calves were ranked according to sex, source, and shrunk BW, and allocated to one of 21 drylot pens (7 × 15 m; 5 calves/pen, being one or two heifers per pen), in a manner that pens had equivalent initial shrunk BW and calves from at least three different sources to simulate the stress of comingling (Step et al., 2008). Pens were assigned to receive a total-mixed ration (**TMR**) and one of three treatments (as-fed basis): (1) 1 g/calf daily of MA (**M1**; $n = 7$), (2) 2 g/calf daily of MA (**M2**; $n = 7$), or (3) no MA supplementation (**CON**; $n = 7$). Pens were assigned to treatments in a manner such that M1, M2, and CON were balanced for initial shrunk BW, cattle sex and source, and contained calves from each of the eight cow-calf operations. According to the manufacturer (DPI Global), MA is a plant-derived feed additive for use in animal feeds, manufactured from a purified extract *Y. schidigera* that grows in

Table 1. Ingredient composition and nutrient profile of total mixed ration offered during the experiment (days 0 to 59)[1]

Item	A	B	C	D
Ingredient, % DM basis				
Grass hay	74.5	58.2	37.0	33.7
Cracked corn	17.5	35.0	54.6	58.2
Soybean meal	7.2	6.0	7.7	7.4
Mineral mix[2]	0.80	0.80	0.70	0.70
Nutrient profile,[3] DM basis				
NE_m, Mcal/kg	1.38	1.55	1.76	1.80
Net energy for growth, Mcal/kg	0.80	0.95	1.14	1.17
Neutral detergent fiber, %	46.8	39.3	29.5	27.9
Crude protein, %	13.7	13.1	13.6	13.5

[1]A = days 0 to 8; B = days 9 to 19; C = days 20 to 33; and D = days 34 to 59. Calves had free-choice access to the total mixed ration and water throughout the experimental period. The total mixed ration was offered in a manner to yield 15% (as fed basis) orts from the previous feeding, via a vertical feed mixer (NDEco S600; New Direction Equipment; Sioux Falls, SD) into concrete feed bunks (7.5 m of linear bunk space per pen).

[2]Cattleman's Choice (Performix Nutrition Systems, Nampa, ID) containing 14% Ca, 10% P, 16% NaCl, 1.5% Mg, 3,200 mg/kg of Cu, 65 mg/kg of I, 900 mg/kg of Mn, 140 mg/kg of Se, 6,000 mg/kg of Zn, 136,000 IU/kg of vitamin A, 13,000 IU/kg of vitamin D3, and 50 IU/kg of vitamin E.

[3] Based on nutritional profile of each ingredient, which were analyzed via wet chemistry procedures by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Calculations for NE_m and growth used the equations proposed by NRC (1996).

the southwest United States and Mexico, and contains 97% DM and 181.6 g of saponin per kilogram of DM (Singer et al., 2008). The administration rates of MA herein were according to manufacturer's recommendations and previous research with growing beef cattle (McMurphy et al., 2014a,2014b)

From days 0 to 59, cattle had free-choice access to water and TMR (Table 1), which was offered twice daily (0800 and 1300 hours). The TMR was offered in a manner to yield 15% (as-fed basis) orts from the previous feeding, via a vertical feed mixer (NDEco S600; New Direction Equipment; Sioux Falls, SD) into concrete feed bunks (7.5 m of linear bunk space per pen). Four TMR diets were used during the experimental period (Table 1). The MA was mixed with soybean meal (1.25 kg/pen; as-fed basis) and top-dressed daily into the morning TMR feeding of M1 and M2 pens. Soybean meal was also top-dressed into the morning TMR feeding of CON pens (1.25 kg/pen; as-fed basis), without the addition of MA. On day 0, cattle were vaccinated against *Clostridium* and *Mannheimia haemolytica* (One Shot Ultra 7; Zoetis Florham Park, NJ), *bovine herpesvirus-1*, bovine viral diarrhea complex, *parainfluenza3 virus*, and *bovine respiratory syncytial virus* (Bovi-Shield Gold 5; Zoetis), and were administered an anthelmintic (Dectomax; Zoetis). On day 21, cattle were re-vaccinated against *Clostridium* (Ultrabac 8; Zoetis), *bovine herpesvirus-1*, bovine viral diarrhea complex, *parainfluenza3 virus*, and *bovine respiratory syncytial virus* (Bovi-Shield Gold 5; Zoetis). Cattle did not receive growth-promoting implants during the experimental period.

Sampling

Samples of TMR ingredients were collected weekly, pooled across all weeks, and analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). All 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), and neutral detergent fiber using a-amylase and sodium sulfite (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp.). Calculations for NE_m and NE_g used the equations proposed by NRC (1996). Nutritional profile of the TMR is described in Table 1.

Animals were weighed and processed for sampling in a Silencer Chute (Moly Manufacturing, Lorraine, KS) mounted on Avery Weigh-Tronix load cells (Fairmount, MN; readability 0.45 kg). Shrunk BW was recorded on day 60, after 16 h of water and feed withdrawal. The scale was calibrated prior to each weighing procedure by using standards of known weight. Shrunk BW values from days −1 and 60 were used to calculate ADG during the experiment. Intake of TMR (DM basis) was evaluated from days 0 to 59 from each pen by collecting and weighing offered and non-consumed TMR daily (0700 hours), whereas all the non-consumed TMR from each pen was discarded. Samples of offered and non-consumed TMR were dried for 96 h at 50 °C in forced-air ovens for DM calculation. Daily TMR intake of each pen was divided by the number of cattle within each pen, and expressed as kilogram per animal per day. Total BW gain (in grams, based on shrunk BW values) and total TMR intake (in kg, DM basis) of each pen during the experimental period were used for feed efficiency (**G:F**) calculation, and reported as grams of BW gained per kilogram of DM consumed.

Cattle were observed daily for BRD signs by two trained evaluators blinded to treatment assignment, according to the DART system (Zoetis) with the

modifications described by Step et al. (2008) and Wilson et al. (2015). Briefly, cattle were assigned a severity score from 0 to 4, with 0 being an animal with no visual BRD signs. Cattle were diagnosed with BRD signs when assigned a severity score of 1 or 2 and had rectal temperature ≥40.0 °C (GL M-500, GLA Agricultural Electronics, San Luis Obispo, CA), or were assigned severity scores of 3 or 4 regardless of rectal temperature. Within pens, only cattle assigned a severity score of 1 and 2 were processed for rectal temperature, whereas only cattle diagnosed with BRD signs received antimicrobial treatment. The first antimicrobial treatment administered was gamithromycin 150 mg/mL (Zactran; Merial, Duluth, GA) at 1 mL/25 kg of BW subcutaneously. A moratorium was observed (10-d moratorium for severity scores 1 and 2, or 4-d moratorium for severity scores 3 and 4) after gamithromycin administration before a second antimicrobial treatment was administered. Cattle diagnosed with BRD signs after the first moratorium period were administered florfenicol 300 mg/mL (Nuflor; Merck Animal Health, Madison, NJ) at 1 mL/7.6 kg of BW subcutaneously. A 4-d moratorium was observed regardless of severity score, and cattle diagnosed with BRD signs would receive ceftiofur crystalline free acid 200 mg/mL (Excede; Zoetis) at 1 mL/30.3 kg of BW at the base of the ear. However, none of the animals utilized herein required a third antimicrobial treatment to recover from BRD, and no animals were removed from this experiment due to BRD.

Blood samples were collected from all calves on days 0, 2, 6, 10, 14, 21, 28, 34, 45, and 59 of the experiment at 0700 hours, before the first TMR feeding of the day, into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing either no additive or freeze-dried sodium heparin for serum and plasma collection, respectively. Blood samples were also collected from 2 steers per pen, which were randomly selected on day −1, into PAXgene tubes (BD Diagnostics, Sparks, MD) for whole blood RNA extraction. These samples were collected on days 0, 2, 6, 10, and 14 for mRNA expression analysis of innate immunity genes (Table 2) to assess response during the initial 2 wk of feedlot receiving, when cattle are coping with the stressors associated with feedlot entry (Cooke, 2017; Lippolis et al., 2017). Unshrunk BW was recorded concurrently with blood sampling, using the same scale and calibration procedures described for shrunk BW.

Laboratorial Analysis

Plasma and serum samples. After collection, all blood samples were placed immediately on ice, centrifuged (2,500 × *g* for 30 min; 4 °C) for plasma or serum harvest, and stored at −80 °C on the same day of collection. Samples collected from days 0 to 28 were analyzed for plasma cortisol (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA), serum non-esterified fatty acids (**NEFA**; colorimetric kit HR Series NEFA-2; Wako Pure Chemical Industries Ltd. USA, Richmond, VA) and plasma haptoglobin concentrations (Cooke and Arthington, 2013), given that these responses return to baseline levels in receiving cattle within 4 wk after feedlot entry (Cooke, 2017). Plasma samples collected on days 0, 14, 28, 45, and 59 were analyzed for insulin and insulin-like growth factor I (**IGF-I**) concentrations (Immulite 1000; Siemens Medical Solutions Diagnostics) to metabolically assess cattle nutritional status throughout the experimental period (Hess et al., 2005). Plasma samples collected from days 0 to 14 were analyzed for plasma tumor necrosis factor-α (**TNFα**; bovine TNF-α ELISA kit #ELB-TNFα-1; RayBiotech, Inc., Norcross, GA), as cytokines are expected to return to baseline levels within 2 wk after feedlot entry (Cooke, 2017). The intra- and inter-assay CV were, respectively, 1.9% and 8.6% for haptoglobin, 4.3% and 5.2% for NEFA, and 4.7% and 6.3% for TNFα. Plasma cortisol, insulin, and IGF-I concentrations were analyzed within a single assay. The intra-assay CV was, respectively, 4.2% for cortisol, 1.7% for insulin, 0.9% for IGF-I. Serum samples collected from 2 calves per pen not diagnosed with BRD signs during the experiment were selected for analysis of antibody titers against BRD pathogens, to ensure that this response was associated with vaccine efficacy rather than pathogenic infection (Callan, 2001). More specifically, serum samples collected on days 0, 10, 21, and 45 were analyzed for antibody titers against *bovine respiratory syncytial virus* (**BRSV**), *bovine herpesvirus-1* (**BHV-1**), *bovine viral diarrhea virus-1* (**BVD-1**), and *parainfluenza-3 virus* (**PI3**) using virus neutralization tests, and for antibodies against *M. haemolytica* via a quantitative agglutination test (Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo, TX).

PAXgene samples. Total RNA was extracted using the PAXgene Blood RNA Kit (Qiagen, Valencia, CA). Quantity and quality of isolated RNA were assessed via UV absorbance (NanoDrop Lite; Thermo Fisher Scientific, Wilmington, DE) at 260 nm and 260/280 nm ratio, respectively (Fleige and Pfaffl, 2006). All samples had a 260/280 nm ratio between 1.8 and 2.0; hence, appropriate for cDNA synthesis (Fleige and Pfaffl, 2006). Extracted

Table 2. Primer sequences, accession number, and reference for all gene transcripts analyzed by reverse transcription-PCR

Target gene	Primer sequence	Accession no.	Source
Cyclooxygenase-2			
Forward	AATCATTCACCAGGCAAAGG	AF031699	Silva et al. (2008)
Reverse	TAGGGCTTCAGCAGAAAACG		
Tumor necrosis factor α			
Forward	AACAGCCCTCTGGTTCAAAC	NM_173966	Riollet et al. (2000)
Reverse	TCTTGATGGCAGACAGGATG		
L-selectin			
Forward	GACACTTCCCTTCAGCCGTAC	NM_174182.1	Playford et al. (2014)
Reverse	AGTTCTTTGCTTCTTCAGTGAGAG		
Interleukin-8			
Forward	ACACATTCCACACCTTTCCAC	NM_173925.2	Kliem et al. (2013)
Reverse	ACCTTCTGCACCCACTTTTC		
Interleukin-8 receptor			
Forward	CGGGTCATCTTTGCTGTCG	NM_174360.3	Playford et al. (2014)
Reverse	ATGAGGGTGTCCGCGATC		
CCL5			
Forward	GCCCTGCTGCTTTGCCTATAT	NM_175827.2	Buza et al. (2003)
Reverse	TCCACCCTAGCTCAACTCCAA		
β-Actin			
Forward	CTGGACTTCGAGCAGGAGAT	AY141970	Gifford et al. (2007)
Reverse	GGATGTCGACGTCACACTTC		
β2-Microglobulin			
Forward	GGGCTGCTGTCGCTGTCT	NM_173893	Silva et al. (2008)
Reverse	TCTTCTGGTGGGTGTCTTGAGT		

RNA (120 ng) was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit with random hexamers (Applied Biosystems, Foster City, CA). Real-time reverse transcription-PCR was completed using the Fast SYBR Green Master Mix (Applied Biosystems) and gene-specific primers (20 pM each; Table 2) with the StepOne Real-time PCR system (Applied Biosystems). Following incubation at 95 °C for 10 min, 40 cycles of denaturation (95 °C for 15 s) and annealing/synthesis (60 °C for 2 min) were completed. Each RNA sample was analyzed in triplicate, and the absence of genomic contamination was verified by including a fourth reaction lacking exposure to the reverse transcriptase. At the end of each reverse transcription-PCR, amplified products were subjected to a dissociation gradient (95 °C for 15 s, 60 °C for 30 s, and 95 °C for 15 s) to verify the amplification of a single product by denaturation at the anticipated temperature. A portion of the amplified products were purified with the QIAquick PCR purification kit (Qiagen Inc., Valencia, CA) and sequenced at the Oregon State University – Center for Genome Research and Biocomputing to verify the specificity of amplification. All amplified products represented only the genes of interest. Responses from genes of interest were quantified based on the threshold cycle (C_T), the number of PCR cycles required for target amplification to reach a predetermined threshold. The C_T responses from genes of interest were quantified based on C_T and normalized to the geometrical mean of C_T values from *β2-microglobulin* and *β-actin* (Vandesompele et al., 2002). The CV for the geometrical mean of *β2-microglobulin and β-actin* C_T values across all samples was 2.7%. Results are expressed as relative fold change ($2^{-\Delta\Delta CT}$) as described by Ocón-Grove et al. (2008).

Statistical Analysis

Pen was considered the experimental unit for all analyses. Quantitative data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), whereas binary data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc.) with a binomial distribution and logit link function. All data were analyzed using Satterthwaite approximation to determine the denominator df for tests of fixed effects, with pen(treatment) and calf(pen) as random variables, whereas TMR intake and G:F used pen(treatment) as the random variable. Model statements for initial and final BW, ADG, G:F, and morbidity-related results contained the effects of treatment and calf sex as an independent covariate.

Model statements for TMR intake, cumulative BRD signs, unshrunk BW, and blood variables contained the effects of treatment, day, the resultant interaction, and calf sex as an independent covariate. Blood variables were analyzed using results from day 0 as an independent covariate. Calf source was also included as an independent covariate for mRNA expression analysis of innate immunity genes and antibody titers against BRD pathogens, given that sampling for these analyses did not account for cattle source. The specified term for all repeated statements was day, with pen(treatment) as subject for TMR intake and calf(pen) as subject for all other analyses. The covariance structure used was first-order autoregressive, which provided the smallest Akaike information criterion and hence the best fit for all variables analyzed. All results are reported as covariately adjusted least square means, and separated using PDIFF. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Repeated measures are reported according to main treatment effect if the treatment × day interaction was $P > 0.10$. Moreover, *P*-values relative to individual treatment comparisons via PDFF are provided when the main treatment effect was $P \leq 0.10$.

RESULTS

Performance Responses

As designed, initial BW (day −1) was similar ($P = 0.97$) among treatments (Table 3). ADG was greater ($P = 0.03$) in M2 vs. M1 and CON calves, and similar ($P = 0.95$) between M1 and CON calves (Table 3; main treatment effect, $P = 0.05$). However, no treatment effects were detected ($P \geq 0.49$) for final shrunk BW (day 60; Table 3) or unshrunk BW measurements (Figure 1). No treatment effects were detected for TMR intake ($P = 0.52$; Table 3), whereas G:F was greater ($P \leq 0.05$) in M2 vs. M1 and CON calves, and similar ($P = 0.40$) between M1 and CON calves (Table 3; main treatment effect, $P = 0.04$).

Health Responses

No treatment effects were detected ($P = 0.39$) for incidence of cattle diagnosed with BRD signs and treated at least once during the experiment (Table 4), which were mainly observed during the initial 21 d of feedlot receiving (Figure 2, day effect, $P < 0.01$). However, the incidence of cattle that required two antimicrobial treatments was greater ($P \leq 0.05$) in CON vs. M1 and M2, and similar ($P = 0.66$) between M1 and M2 calves (Table 4; main treatment effect, $P = 0.04$). Accordingly, the number of antimicrobial treatments required upon diagnosis of BRD signs was greater ($P \leq 0.01$) in CON vs. M1 and M2, and similar ($P = 0.60$) between M1 and M2 calves (Table 4; main treatment effect, $P = 0.01$). No incidence of mortality was observed during the experiment.

Physiological Variables

No treatment effects were detected ($P \geq 0.42$) for concentrations of serum NEFA, plasma cortisol, haptoglobin, TNFα, insulin, and IGF-I (Table 4), whereas day effects were detected ($P \leq 0.05$) for all these variables (Table 5). No treatment differences were detected ($P \geq 0.56$) for serum titers against respiratory pathogens (Table 6), which all increased upon vaccination during the experimental period (day effects; $P < 0.01$; Table 7). No treatment effects were detected ($P \geq 0.23$) for blood mRNA expression of *chemokine ligand 5*, *cyclooxygenase 2*, *interleukin 8*, *interleukin 8 receptor*, *L-selectin, and tumor necrosis factor-a* (Table 5), whereas day effects were detected ($P \leq 0.03$) for all blood mRNA expression responses (Table 7).

Table 3. Performance parameters of feeder cattle supplemented or not (**CON**; $n = 7$) with *Yucca schidigera* extract (Micro-Aid; DPI Global, Porterville, CA) at (as-fed basis) 1 g/calf daily (**M1**; $n = 7$) or 2 g/calf daily (**M2**; $n = 7$) during feedlot receiving (days 0 to 59)[1,2]

Item	CON	M1	M2	SEM	*P*-value
Initial BW (day −1; kg)	220	220	221	4	0.97
Final BW (day 60; kg)	307	307	315	4	0.49
ADG, kg/day	1.42[b]	1.42[b]	1.53[a]	0.03	0.05
Feed intake, kg/day; DM basis	7.16	7.33	7.35	0.13	0.52
Feed efficiency, g/kg	204[b]	200[b]	213[a]	3	0.04

[1]Shrunk BW was recorded after road transport (800 km for 12 h) on day −1, and after 16 h of water and feed withdrawal on day 60. ADG was calculated using initial and final shrunk weights. Feed intake was recorded daily from days 0 to 59 by measuring offer and refusals from each pen, divided by the number of calves within each pen, and expressed as kilogram per calf per day. Feed efficiency was calculated using total BW gain (in grams, based on shrunk values) and total feed intake (in kilogram, DM basis) of each pen during the experimental period, and reported as grams of BW gained per kilogram of DM consumed.

[2]The treatment × day interaction was not significant for feed intake ($P = 0.83$); therefore, values are presented as least-square means according to main treatment effect. Within rows, treatment means with different superscripts differ ($P \leq 0.05$).

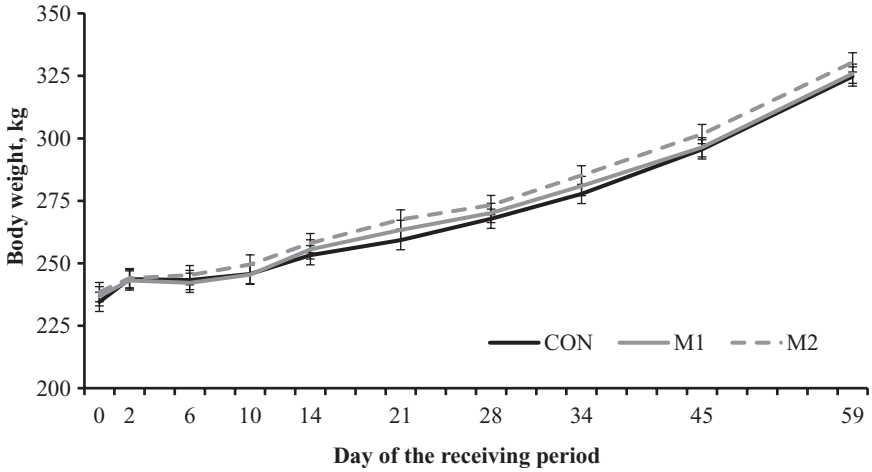

Figure 1. Unshrunk BW in feeder cattle supplemented or not (**CON**; $n = 7$) with *Yucca schidigera* extract (Micro-Aid; DPI Global, Porterville, CA) at (as-fed basis) 1 g/calf daily (**M1**; $n = 7$) or 2 g/calf daily (**M2**; $n = 7$) during feedlot receiving (days 0 to 59). No treatment or treatment × day interaction were detected ($P \geq 0.33$), whereas a day effect was significant ($P < 0.01$).

Table 4. Morbidity and physiological responses in feeder cattle supplemented or not (**CON**; $n = 7$) with *Yucca schidigera* extract (Micro-Aid; DPI Global, Porterville, CA) at (as-fed basis) 1 g/calf daily (**M1**; $n = 7$) or 2 g/calf daily (**M2**; $n = 7$) during feedlot receiving (days 0 to 59)[1]

Item	CON	M1	M2	SEM	*P-value*
Cattle treated for respiratory disease[2], %					
Once	42.9	60.0	54.3	8.8	0.39
Twice	17.1[a]	2.85[b]	5.71[b]	4.68	0.04
Number of antimicrobial treatments required	1.40[a]	1.05[b]	1.10[b]	0.08	0.01
Physiological variables[3]					
Plasma cortisol, ng/mL	23.6	21.6	21.7	1.7	0.63
Plasma insulin, pmol/L	29.8	29.1	33.3	3.0	0.55
Plasma insulin-growth factor I, ng/mL	159	155	149	7	0.42
Plasma haptoglobin, mg/mL	0.37	0.40	0.37	0.05	0.82
Plasma tumor necrosis-α, ng/mL	0.14	0.24	0.35	0.13	0.46
Serum non-esterified fatty acids, μEq/L	0.25	0.25	0.23	0.02	0.67

[1]Values reported are least-square means according to main treatment effect, given that no treatment × day interactions were detected ($P \geq 0.37$). Within rows, treatment means with different superscripts differ ($P \leq 0.05$).

[2]Cattle was observed daily for signs of bovine respiratory disease according to the DART system (Zoetis, Florham Park, NJ) with the modifications described by Step et al. (2008) and Wilson et al. (2015). If diagnosed with respiratory disease signs, cattle received antimicrobial treatment as described by Wilson et al. (2015).

[3]Blood samples were collected on days 0, 2, 6, 10, 14, 21, 28, 34, 45, and 59 of the experiment, and analyzed for plasma tumor necrosis-α (days 0 to 14), cortisol, haptoglobin, and non-esterified fatty acids (days 0 to 28), as well as insulin and insulin-like growth factor I (**IGF-I**; days 0, 14, 28, 45, and 59). Data were analyzed using results from day 0 as independent covariate.

DISCUSSION

Saponins are naturally occurring compounds in a variety of plants and edible legumes, consisting of one or more oligosaccharides attached to a triterpenoid or steroidal hydrophobic aglycone (Shi et al., 2004). The structures of saponins from different plants are variable and depend on the plant type, sugar content, and composition of the steroid ring (Rao et al., 1995). Nevertheless, the biological functions of saponins remain similar across different vegetable sources (Francis et al., 2002). Given the limited body of research investigating the role of saponins from *Y. schidigera* extract on receiving cattle, results from this experiment are also being contrasted with studies using saponins from other plant sources and administered to different species.

Cattle utilized in this experiment were considered high-risk given that their prior management and health history were not fully known (Lippolis et al. 2017; Wilson et al., 2017; Souza et al., 2018). Moreover, cattle experienced the stress of weaning, auction, transportation, commingling, vaccination, and feedlot entry within a short interval, and the combination of these stressors impacts cattle immunocompetence and performance (Cooke, 2017). Day effects observed across treatments for

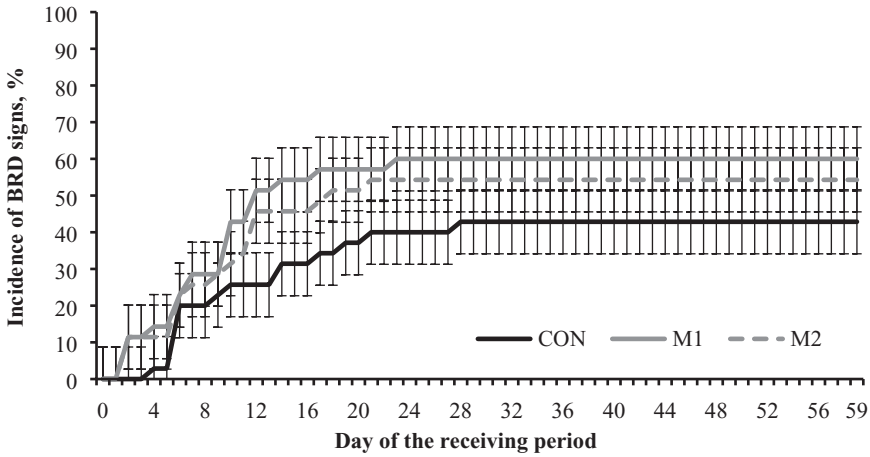

Figure 2. Cumulative incidence of bovine respiratory disease (**BRD**) signs in feeder cattle supplemented or not (**CON**; $n = 7$) with *Yucca schidigera* extract (Micro-Aid; DPI Global, Porterville, CA) at (as-fed basis) 1 g/calf daily (**M1**; $n = 7$) or 2 g/calf daily (**M2**; $n = 7$) during feedlot receiving (days 0 to 59). Cattle were observed daily for BRD signs according to the DART system (Zoetis, Florham Park, NJ) with the modifications described by Step et al. (2008) and Wilson et al. (2015). If diagnosed with respiratory disease signs, cattle received antimicrobial treatment as described by Wilson et al. (2015). Graph represents incidence of cattle diagnosed with BRD signs and treated with antimicrobial at least once during the experiment. No treatment or treatment × day interaction were detected ($P \geq 0.34$), whereas a day effect was significant ($P < 0.01$).

Table 5. Concentrations of plasma cortisol (ng/mL), insulin (pmol/L), insulin-like growth factor I (**IGF-I**; ng/mL), haptoglobin (mg/mL), tumor necrosis-α (**TNFα**; ng/mL), and non-esterified fatty acids (**NEFA**; μEq/L) in feeder cattle during feedlot receiving (days 0 to 59)[1,2]

Day	Cortisol	Insulin	IGF-I	Haptoglobin	TNFα	NEFA
0	29.2^{a}	28.8^{c}	82.6^{d}	0.26^{e}	0.12^{b}	0.42^{a}
2	20.3^{c}	—	—	0.37^{cd}	0.18^{b}	0.36^{b}
6	20.9^{c}	—	—	0.48^{ab}	0.33^{a}	0.31^{c}
10	20.2^{c}	—	—	0.52^{a}	0.22^{ab}	0.22^{d}
14	20.3^{c}	26.9^{c}	131^{c}	0.41^{bc}	0.14^{b}	0.19^{e}
21	23.8^{b}	—	—	0.31^{de}	—	0.19^{e}
28	23.7^{b}	37.6^{a}	162^{b}	0.31^{de}	—	0.18^{e}
45	—	30.3^{bc}	165^{b}	—	—	—
59	—	34.5^{ab}	175^{a}	—	—	—
SEM	1.6	2.7	4.8	0.05	0.07	0.01
P-value	0.01	<0.01	<0.01	0.03	0.05	<0.01

[1]Within columns, values with different superscripts differ ($P \leq 0.05$).

[2]Blood samples were collected on days 0, 2, 6, 10, 14, 21, 28, 34, 45, and 59 of the experiment, and analyzed for plasma tumor necrosis-α (days 0 to 14), cortisol, haptoglobin, and non-esterified fatty acids (days 0 to 28), as well as insulin and IGF-I (days 0, 14, 28, 45, and 59).

plasma cortisol, haptoglobin, and TNFα validate that cattle experienced an adrenocortical and subsequent acute-phase response during feedlot receiving (Cooke et al., 2011; Rodrigues et al., 2015). Similar outcomes were noted for mRNA expression of whole blood genes, indicating immune activation upon feedlot entry as these encode key inflammatory components of the innate immune system (Abbas and Lichtman, 2014). All these stress-induced inflammatory processes are linked with the BRD complex in receiving cattle (Berry et al., 2004; Cooke, 2017) supporting the incidence of BRD signs observed in the present experiment, which is comparable to research conducted at commercial receiving yards (Snowder et al., 2006; Marques et al., 2016). Collectively, the experimental model adopted herein represented the stress and health challenges that commercial feeder cattle typically experience during feedlot receiving in the United States (Duff and Galyean, 2007).

Supplemental MA increased cattle ADG when included at 2 g/calf daily (M2), but not when included at the lower dose (1 g/calf daily; M1). This outcome should be primarily attributed to increased G:F feed efficiency in M2 cattle, given that TMR intake, incidence of BRD signs, and all physiological responses did not differ among treatment groups. McMurphy et al. (2014a) reported that MA supplementation at 1 and 2 g/day (as-fed basis) improved in situ rumen DM and neutral detergent fiber digestibility in beef steers, without impacting total feed intake. Authors associated these outcomes with decreased rumen particulate passage rate in MA-supplemented cattle, as saponins from *Y. schidigera* have foam-forming characteristics that may increase rumen fluid viscosity (Cheeke, 2000). Supplementing MA at 1 and 2 g/day (as-fed basis) also reduced ruminal protozoa concentrations in McMurphy et al. (2014a), given that saponins react with cholesterol in the protozoal cell membrane to stimulate lysis (Hristov et al., 1999; Cheeke, 2000). In turn, decreased rumen protozoa may enhance G:F by reducing methane emissions, shifting ruminal fermentation from acetate toward propionate production, and increasing microbial N flow to the lower gastrointestinal

Table 6. Serum titers against respiratory pathogens and mRNA expression in whole blood from feeder cattle supplemented or not (**CON**; $n = 7$) with *Yucca schidigera* extract (Micro-Aid; DPI Global, Porterville, CA) at (as-fed basis) 1 g/calf daily (**M1**; $n = 7$) or 2 g/calf daily (**M2**; $n = 7$) during feedlot receiving (days 0 to 59)[1]

Item	CON	M1	M2	SEM	*P*-value
Serum antibodies, titer log 2					
Mannheimia haemolytica	9.21	9.19	9.05	0.21	0.78
Parainfluenza-3 virus	6.10	5.87	5.61	0.35	0.56
Bovine respiratory syncytial virus	4.84	4.46	4.70	0.50	0.83
Bovine viral diarrhea virus-1	3.29	3.10	3.17	0.36	0.92
Bovine herpesvirus-1	1.95	1.94	1.92	0.38	0.99
Blood mRNA expression, fold effect					
Chemokine ligand 5	4.83	3.93	3.62	0.54	0.23
Cyclooxygenase 2	6.95	7.51	6.66	0.75	0.64
Interleukin 8	11.6	9.68	13.8	2.08	0.27
Interleukin 8 receptor	20.7	23.6	19.7	2.7	0.50
L-selectin	2.61	2.84	2.90	0.25	0.62
Tumor necrosis-α	6.26	6.06	6.07	0.82	0.98

[1]Blood mRNA expression reported as in Ocón-Grove et al. (2008). Values reported are least-square means according to main treatment effect, given that no treatment × day interactions were detected ($P \geq 0.55$).

[2]Blood samples were collected on days 0, 2, 6, 10, 14, 21, 28, 34, 45, and 59 of the experiment and analyzed for serum titers (days 0, 10, 21, and 45) and whole blood mRNA expression (days 0 to 14). Data were analyzed using results from day 0 as independent covariate. On day 0, cattle were vaccinated against *Clostridium* and *Mannheimia haemolytica* (One Shot Ultra 7; Zoetis Florham Park, NJ), *bovine herpesvirus-1*, bovine viral diarrhea complex, *parainfluenza3 virus*, and *bovine respiratory syncytial virus* (Bovi-Shield Gold 5; Zoetis), and were administered an anthelmintic (Dectomax; Zoetis). On day 21, cattle were re-vaccinated against *Clostridium* (Ultrabac 8; Zoetis), *bovine herpesvirus-1*, bovine viral diarrhea complex, *parainfluenza3 virus*, and *bovine respiratory syncytial virus* (Bovi-Shield Gold 5; Zoetis).

Table 7. Serum titers against *Mannheimia haemolytica* (**MH**), parainfluenza-3 virus (**PI3**), bovine respiratory syncytial virus (**BRSV**), bovine viral diarrhea virus-1 (**BVD-1**), and bovine herpesvirus-1 (**BHV**), and whole blood mRNA expression of *chemokine ligand 5* (**CCL5**), *cyclooxygenase 2* (**COX2**), *interleukin 8* (**IL8**), *interleukin 8 receptor* (**IL8R**), *L-selectin* (**SELL**), and *tumor necrosis-α* (**TNFα**) in feeder cattle during feedlot receiving (days 0 to 59)[1,2]

	Serum antibody titers (titer log 2)					Blood mRNA expression (fold effect)					
Day	MH	PI3	BRSV	BVD-1	BHV	CCL5	COX2	IL8	IL8R	SELL	TNFα
0	8.17^{d}	2.82^{c}	1.37^{d}	0.84^{d}	0.21^{c}	4.66^{ab}	7.16^{b}	12.5^{a}	26.6^{a}	2.76^{a}	6.68^{ab}
2	—	—	—	—	—	4.08^{b}	8.61^{a}	6.88^{b}	16.7^{c}	2.37^{b}	7.18^{a}
6	—	—	—	—	—	5.07^{a}	5.34^{c}	14.8^{a}	22.3^{ab}	2.63^{ab}	5.17^{c}
10	8.48^{c}	3.99^{b}	3.08^{c}	1.01^{c}	0.50^{c}	4.32^{b}	6.83^{b}	14.2^{a}	18.3^{bc}	3.00^{a}	6.14^{b}
14	—	—	—	—	—	4.14^{b}	7.21^{b}	13.6^{a}	22.2^{ab}	2.93^{a}	5.69^{c}
21	9.31^{b}	6.25^{a}	4.73^{b}	2.32^{b}	2.81^{a}	—	—	—	—	—	—
45	9.75^{a}	6.75^{a}	5.56^{a}	5.63^{a}	2.36^{b}	—	—	—	—	—	—
SEM	0.19	0.36	0.37	0.35	0.25	0.37	0.65	2.28	2.57	0.19	0.61
P-value	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	<0.01	0.03	<0.01

[1]Within columns, values with different superscripts differ ($P \leq 0.05$). Blood mRNA expression reported as in Ocón-Grove et al. (2008).

[2] Blood samples were collected on days 0, 2, 6, 10, 14, 21, 28, 34, 45, and 59 of the experiment and analyzed for serum titers (days 0, 10, 21, and 45) and whole blood mRNA expression (days 0 to 14). On day 0, cattle were vaccinated against *Clostridium* and MH (One Shot Ultra 7; Zoetis Florham Park, NJ), BHV, BVD complex, PI3, and BRSV (Bovi-Shield Gold 5; Zoetis), and were administered an anthelmintic (Dectomax; Zoetis). On day 21, cattle were re-vaccinated against *Clostridium* (Ultrabac 8; Zoetis), BHV, BVD complex, PI3, and BRSV (Bovi-Shield Gold 5; Zoetis).

tract (Wallace et al., 1994; Hristov et al., 1999). Accordingly, McMurphy et al. (2014a) reported that microbial N flow was increased by MA supplementation, but mainly when it was provided at 2 g/day (as-fed basis). Together, results from the present experiment and by McMurphy et al. (2014a) suggest that MA should be supplemented at 2 g/day to yield rumen fermentation benefits that translate into improved G:F and ADG in receiving cattle.

Saponins are recognized as potent immune stimulators and used as adjuvants for human and animal vaccines (Ellis et al., 2005; Skene and Sutton, 2006; Sun et al. 2009). Chavali and Campbell (1987) reported that dietary saponin

supplementation increased the resistance of mice to rabies virus. Contrary to this rationale, however, MA supplementation failed to mitigate incidence of BRD signs in this experiment. Nonetheless, M1 and M2 cattle diagnosed with BRD signs required less antimicrobial treatments to recover from sickness compared with CON. These outcomes imply that MA supplementation partially improved cattle immunocompetence, but do not explain increased performance of M2 cattle as such health benefits were observed for both MA-including treatments. One of the key immunostimulatory properties of saponins is to enhance antibody and lymphocyte response to antigens (Kensil, 1996; Shi et al., 2004), including stimulation of Th1 immune response and production of cytotoxic T-lymphocytes against exogenous pathogens (Sun et al., 2009). Saponins may also enhance immunity by increasing the uptake of antigens by the intestine and other membranes (Das et al., 2012). Therefore, MA supplementation likely enhanced the ability of cattle to recover from BRD upon antimicrobial administration, although research is warranted to properly elucidate the immunological benefits of MA supplementation.

Another mechanism by which saponins stimulate the immune system is by inducing the production of cytokines and inflammatory components (Francis et al., 2002). Supplementing saponins has also been shown to enhance vaccine efficacy in poultry, such as greater antibody titer response to vaccination against infectious bursal disease (Zhai et al., 2014). However, MA supplementation did not modulated inflammatory and acute-phase responses associated with feedlot receiving herein. These include mRNA expression of innate immunity genes in whole blood, and plasma concentrations of haptoglobin, cortisol, and TNFα. Serum antibody titers against BRD pathogens were also not impacted by MA supplementation, but increased across treatments during the experiment, which denotes acquired humoral immunity to these antigens upon vaccination (Richeson et al., 2008). Likewise, MA supplementation did not modulate circulating concentrations of insulin, IGF-I, and NEFA, which are metabolic markers of nutritional status in ruminants (Hess et al., 2005). Although the specific impacts of MA and saponins on these variables are mostly undetermined, one could expect increased circulating concentrations of insulin and IGF-I in M2 cattle due to their greater ADG and G:F (Ellenberger et al., 1989). Yet, plasma concentrations of insulin and IGF-I increased while serum NEFA concentrations decreased across treatments, given greater nutrient intake and growth as the experiment advanced (Lippolis et al., 2017). Collectively, the physiological variables evaluated herein failed to elucidate the biological mechanisms by which MA supplementation improved performance (M2) and response to BRD treatment (M1 and M2); perhaps these occurred without substantial impacts on systemic inflammatory, humoral, and metabolic responses.

In summary, this experimental model represented the stress and health challenges that commercial feeder cattle experience during feedlot receiving, resulting in substantial incidence of BRD signs. Supplementing MA at 2 g/d increased feedlot receiving ADG due to enhanced G:F, whereas the same outcome was not observed when MA was supplemented at 1 g/d. Moreover, supplementing MA at 1 or 2 g/d did not prevent the incidence of BRD signs, but reduced the need for antimicrobial treatments in cattle diagnosed with BRD signs. None of the inflammatory, humoral, and metabolic responses evaluated herein were impacted by MA; hence, research is still warranted to investigate the physiological impacts of supplementing this ingredient to receiving cattle. Nevertheless, results from this experiment suggest that MA supplementation at 2 g/animal daily may enhance performance and immunocompetence of high-risk cattle during feedlot receiving.

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