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Dehydrated citrus pulp alters feedlot performance of crossbred heifers during the receiving period and modulates serum metabolite concentrations before and after an endotoxin challenge[1]

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ABSTRACT: English × Continental heifers ($n = 180$) were sourced in 2 loads (219.3 ± 16.0 and 221.4 ± 16.4 kg, respectively) from commercial auction barns to study the effects of feeding dehydrated citrus pulp (DCP) on feedlot performance of newly received heifers. A completely randomized block design was used with BW nested within arrival load and blocked by BW into 3 dietary treatments (36 pens, 5 heifers/pen, 12 blocks, 3 pens/block, and 12 pens/treatment). Treatment diets contained 1) 0% DCP (control diet [CON]), 2) 10% DCP, or 3) 20% DCP on a DM basis. Diets containing DCP were exchanged with steam-flaked corn on a 1:1 basis. Cattle were fed a 63, 73, and 83% concentrate diet from d 0 to 28, d 28 to 42, and d 42 to 56, respectively. Over the 56-d trial period, as the amount of dietary DCP increased, DMI decreased ($P = 0.01$), ADG decreased ($P < 0.01$), and G:F decreased ($P = 0.02$). From d 0 to 28, there was no difference in the observed minus the predicted NEg of the diet ($P = 0.73$); from d 28 to 42, there was a linear increase in NEg favoring DCP treatments ($P < 0.01$); and from d 42 to 56, there was a linear decrease in NEg against the DCP treatments ($P < 0.01$). At the conclusion of the trial, a subset of heifers ($n = 22$; 307.89 ± 3.32 kg on d 63) were used to evaluate blood metabolite concentrations before and after a lipopolysaccharide (LPS) challenge. On d 63, heifers were fitted with jugular catheters and moved into individual stalls. On d 64, heifers were intravenously challenged with LPS (0.5 µg/kg BW), and blood samples were collected every 0.5 h from –2 to 8 h and at 24 h relative to the LPS challenge (0 h). Serum glucose, serum urea nitrogen (SUN), and NEFA concentrations were determined. Cattle lost less weight at both 24 and 72 h after the LPS challenge with increasing DCP percentage ($P < 0.01$). Glucose ($P = 0.12$) and NEFA ($P = 0.13$) concentrations did not differ before the LPS challenge; however, there was a treatment effect for SUN, with elevated concentrations of SUN in CON cattle ($P < 0.01$). After the LPS challenge, DCP-fed cattle had reduced glucose, elevated NEFA, and reduced SUN concentrations ($P \leq 0.01$). Results indicate that dietary DCP modulated metabolite concentrations in heifers following an endotoxin challenge and affected feedlot performance when incorporated in receiving diets in replacement of corn. Future studies will need to address strategies to increase DMI or explore levels of DCP less than 10% in the diet of newly received heifer calves.

Key words: acute phase response, dehydrated citrus pulp, feedlot cattle, receiving cattle

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INTRODUCTION

Citrus production is a major agricultural commodity in many tropical regions across the globe. Juicing of citrus fruits creates byproducts including fresh citrus pulp, citrus silage, dehydrated citrus pulp (**DCP**), citrus meal and fines, citrus molasses, citrus peel liquor, and citrus activated sludge (Bampidis and Robinson, 2006). Citrus byproducts are used in livestock diets to offset disposal costs and simultaneously provide an economical feedstuff alternative. The citrus peel and pulp are chopped, dried, and pelleted to create DCP. Dehydration reduces transportation costs and extends shelf life.

Compositionally, DCP has elevated pectin and decreased starch content when compared with corn (Bampidis and Robinson, 2006). Pectin is a structural carbohydrate; however, pectin is rapidly degradable in the rumen (Gradel and Dehority, 1974). Consequently, DCP is typically perceived as an energy concentrate feedstuff that possesses bulky, roughage-like characteristics. The fermentation of DCP yields lesser amounts of lactate with little to no change in ruminal pH (Villarreal et al., 2006). Therefore, DCP replacement of starch-rich concentrates in the diet may yield a positive associative effect on fiber digestion while cattle are being systematically transitioned to finishing diets. Plus, a positive immunomodulatory role may be postulated given the increased concentration of ascorbic acid and proven antimicrobial activities of DCP (Callaway et al., 2008). Equivalent performance has been observed when DCP replaces corn in the diet (Caparra et al., 2007); however, studies with adequate experimental units in a feedlot setting are lacking. The objectives were 1) to determine if DCP inclusion as a concentrate replacement impacted feedlot performance and morbidity during a 56-d receiving period and 2) to determine if DCP affected glucose, serum urea nitrogen (**SUN**), or NEFA concentrations in response to a lipopolysaccharide (**LPS**) challenge.

MATERIALS AND METHODS

All procedures involving live animals were approved (number 10085-11) by the Texas Tech University Animal Care and Use Committee.

Cattle

Two hundred thirteen English × Continental heifers were sourced from auction barns in the central Texas area. Heifers arrived in 2 separate loads on July 21 and 23, 2011, at the Texas Tech University Beef Center in New Deal, Texas. Upon arrival, heifers were housed in large, dirt-surfaced lots and provided ad libitum access to sudangrass hay and were processed the following morning. Initial processing of both groups included 1) measurement of individual BW (Pearson squeeze chute [Pearson Livestock Equipment, Thedford, NE] set on 4 electronic load cells [Gallagher Smart Scale Systems, North Kansas City, MO; accuracy of ±0.9072 kg]; scales were calibrated with 454 kg of certified weights [Texas Department of Agriculture, Austin, TX] before use), 2) individual identification by ear tag, 3) vaccination with a bovine rhinotracheitis–bovine virus diarrhea–parainfluenza3 virus–bovine respiratory syncytial virus modified live virus vaccine (Vista 5; Merck Animal Health, DeSoto, KS), 4) vaccination with a clostridial bacterin toxoid (Vision 7 with SPUR; Merck Animal Health), 5) treatment for internal and external parasites (Ivomec injectable; Merial, Duluth, GA), and 6) administration of an antibiotic (Micotil; Elanco Animal Health, Greenfield, IN). After processing, the heifers were returned to the large, dirt-surfaced lots and continued to be provided ad libitum access to sudangrass hay and water until the initiation of the trial on July 25, 2011 (d 1). On d 14 of the study, heifers were implanted with Component TE-IH with Tylan (80 mg trenbolone acetate and 8 mg estradiol with 29 mg tylosin tartrate as a local antibacterial; Elanco Animal Health) and also revaccinated with Vista 5 (Merck Animal Health).

Experimental Design

The initial processing BW for load 1 and load 2 were 188.7 ± 18.0 (126 heifers) and 225.2 ± 22.2 kg (87 heifers), respectively, before sorting. Heifers beyond 2 SD from their load average for BW were scrutinized against for selection for the study. Heifers that appeared temperamental, lame, or unthrifty or appeared to have excessive *Bos indicus* influence were also eliminated. Ultimately, a total of 180 heifers were enrolled in the study (219.3 ± 16.0 kg for load 1 and 221.4 ± 16.4 kg for load 2). A completely randomized block design was used. Heifers were blocked by BW nested within their respective load. There were 5 blocks in load 1 and 7 blocks in load 2. Within a block, 3 treatments were randomly assigned to pens (36 pens, 5 heifers/pen, 12 blocks, 3 pens/block, and 12 pens/treatment). Treatments were as follows: 1) a control diet (**CON**) containing 0% DCP pellets, 2) a diet containing 10% DCP pellets on a DM basis (10% DCP), and 3) a diet containing 20% DCP pellets on a DM basis (20% DCP). The DCP pellets were guaranteed to contain no more than 1.5% lime (Texas Citrus Exchange, Mission, TX). The diets containing DCP were formulated to be exchanged with steam-flaked corn on a 1:1 basis. Diets were formulated to meet or exceed NRC (2000) recommendations for nutrients (Table 1). Heifers were fed a 63% concentrate diet from d 0 to 28, a 73% concentrate diet from d 28 to 42, and an 83% concentrate diet from d 42 to trial completion (d 56).

Table 1. Formulated composition of treatment diets[1]

Category	Starter diet[2]			Transition diet[3]			Finishing diet[4]		
	CON	10% DCP	20% DCP	CON	10% DCP	20% DCP	CON	10% DCP	20% DCP
Ingredient, %[5]									
Steam-flaked corn	46.7	36.7	26.7	56.4	46.4	36.4	65.9	55.9	45.9
Dehydrated citrus pulp[6]	0.0	10.0	20.0	0.0	10.0	20.0	0.0	10.0	20.0
Alfalfa hay, ground	24.0	24.0	24.0	17.5	17.5	17.5	11.0	11.0	11.0
Cottonseed hulls	13.0	13.0	13.0	9.5	9.5	9.5	6.0	6.0	6.0
Cottonseed meal	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7
Molasses	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Tallow	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Supplement premix[7]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Urea	0.4	0.4	0.4	0.5	0.5	0.5	0.6	0.6	0.6
Limestone	0.2	0.2	0.2	0.5	0.5	0.5	0.8	0.8	0.8
Item[8]									
CP, %	14.7	14.3	13.9	14.5	14.1	13.7	14.4	14.0	13.6
NDF, % of DM	26.4	26.7	27.0	20.5	20.8	21.2	14.6	14.9	15.2
DM, %	86.1	86.4	86.9	88.7	87.5	88.7	83.6	84.4	85.8
NEm, Mcal/kg	1.82	1.78	1.75	1.93	1.90	1.87	2.05	2.01	1.98
NEg, Mcal/kg	1.17	1.14	1.12	1.27	1.25	1.22	1.38	1.35	1.32

[1]CON = control diet (containing 0% dehydrated citrus pulp [DCP] pellets). The 10% DCP diet contains 10% DCP pellets and the 20% DCP diet contains 20% DCP pellets.

[2]The starter diet was fed from d 0 to 28.

[3]The transition diet was fed from d 28 to 42.

[4]The finishing diet was fed from d 42 to 56.

[5]Percentage inclusion in the total diet on a DM basis.

[6]Chemical analysis of DCP (DM basis): 91.5% DM, 5.2% CP, 18.0% NDF, 18.6% ADF, 1.76 Mcal/kg NEm, 1.14 Mcal/kg NEg, and 1.83% Ca; $P = 0.10$%.

[7]Supplement for the diet contained (DM basis): 66.383% cottonseed meal, 0.500% Endox (Kemin Industries, Inc., Des Moines, IA), 0.648% dicalcium phosphate, 10% potassium chloride, 4.167% ammonium sulfate, 15.000% salt, 0.002% cobalt carbonate, 0.196% copper sulfate, 0.083% iron sulfate, 0.003% ethylenediamine dihydroiodide, 0.333% manganese oxide, 0.125% selenium premix (0.2% Se), 0.986% zinc sulfate, 0.010% vitamin A (1,000,000 IU/g), 0.157% vitamin E (500 IU/g), 0.844% Rumensin (176.4 mg/kg; Elanco Animal Health, Indianapolis, IN), and 0.563% Tylan (88.2 mg/kg; Elanco Animal Health). Concentrations in parenthesis are expressed on a 90% DM basis.

[8]The values given are the formulated chemical composition of the diet based on the chemical analysis (Servi-Tech Laboratories, Amarillo, TX) of each individual ingredient used in the diet.

Feeding, Weighing, and Health Monitoring Practices

During the study, heifers were housed in 3 m wide by 9.1 m pipe feedlot pens with a dirt floor and concrete aprons around water troughs and feed bunks. Heifers were provided 60 cm of linear bunk space each. Heifers were fed once daily between 0700 and 0800 h. Before feeding, estimates of the quantity of unconsumed feed remaining in the feed bunk were made and adjustments in feed delivery for each pen were made to guarantee ad libitum access to feed with the target being to leave from 0 to 0.454 kg of feed in the bunk before the following day's delivery. Feed was offered at 95% of the previous day's delivery on each transition day. The feeding order throughout the trial was in numerical pen order. Feed was mixed in a drag type Roto-Mix 84-8 mixer/delivery unit feed wagon (Roto-Mix, Dodge City, KS) pulled by a small tractor. The dry bulk ingredients were unloaded into the mixer first followed by the dry microingredients. These ingredients were mixed for a minimum of 7 rotations. Next, the wet ingredients were added and again the mixer was turned for a minimum of 7 rotations. Subsequently, diets were delivered to the treatment pens by the use of load cells and scale indicator on the Roto-Mix 84-8 unit (±0.454 kg).

Daily feed samples were taken from each diet during the experimental period. Samples were composited weekly for each treatment diet and a subsample was placed in a forced-air oven at 100°C for 24 h for DM determination. Weekly DM concentrations were used to calculate the average DM value for each diet during the experimental period. In addition, each ingredient (steam-flaked corn, DCP, alfalfa hay, cottonseed hulls, and cottonseed meal) was sampled weekly, frozen, and stored. The ingredient samples were submitted to a commercial laboratory (Servi-Tech Laboratories, Amarillo, TX) for chemical analysis. Table 1 displays the formulated chemical composition of the treatment diets.

Individual BW were collected before daily feeding on d 0, 14, 28, 42, and 56 of the trial period. At approximately 0600 h on the morning of each weigh day, feed refusals were collected and weighed. A sample of the remaining feed was dehydrated as described above to determine the DM content. The DMI by each pen

was calculated by subtracting the quantity of dry feed unconsumed at the end of every 14 d from the total dietary DM delivered to each pen during that period.

After feeding, heifers were evaluated for signs of illness or lameness. Heifers demonstrating preliminary signs of illness or severe signs of discomfort and illness were pulled from the home pen and rectal temperature was measured. If body temperature was $\geq 39.7°C$, heifers were treated by subcutaneous injection with Resflor GOLD (florfenicol and flunixin meglumine; Merck Animal Health) at a rate of 6 mL/45.4 kg BW. Heifers that demonstrated severe signs of illness and discomfort were treated regardless of rectal temperature. After proper treatment, all heifers were immediately returned to designated home pen and daily treatment records were kept.

Lipopolysaccharide Challenge

At the conclusion of the feedlot performance trial (d 56), cattle were left in treatment pens and continually fed their prescribed treatment diets. On d 63, a subset of heifers ($n = 24$; 307.89 ± 3.32 kg on d 63) were randomly selected from the 2 heaviest blocks (4 heifers from each pen for a total of 8 heifers per treatment) to evaluate blood metabolite concentrations before and after a LPS challenge in a completely randomized design. The selected heifers were fitted with jugular vein cannulas. For the jugular cannulation procedure, a small 2- to 3-cm incision was made in the skin to more easily access the jugular vein. Temporary indwelling jugular catheters, consisting of approximately 30.48 cm of sterile Tygon tubing (AAQ04133; U.S. Plastics Corp., Lima, OH; 1.27 mm i.d. and 2.286 mm o.d.) was inserted into the jugular vein using a 14-gauge by 5.08-cm thin-walled stainless steel biomedical needle (3 mm o.d.). The catheter was held in place using tag cement and a 2.08-cm-wide porous surgical tape around the incision site, and then the entire neck region of the heifers were wrapped with vet wrap (Vetrap; 3M Animal Care Products, St. Paul, MN) to ensure stability of the catheterization site. The remaining tubing not inserted into the heifer served as the extension portion of the cannula for collection of blood samples. During these procedures, heifers were restrained in a working chute for approximately 10 to 15 min.

Following these procedures, individual heifer BW was recorded and heifers were moved to a facility that contained individual stalls (2.13 m long by 0.76 m wide) that housed the heifers for the duration of the LPS challenge. Heifers were placed so that treatments were alternated by stall. During the challenge, heifers had ad libitum access to their treatment diet and water. Individual feed intake was recorded daily and 1 feed sample was taken per treatment diet and placed in a forced-air oven at 100°C for 24 h for DM determination. The extension tubing of the cannula was extended above the stall to allow researchers to collect blood throughout the study without disturbing the heifers, despite whether the heifers were standing or lying down.

On d 64, whole blood samples were collected into Sarstedt blood tubes (Sarstedt Inc., Newton, NC) containing no additive every 0.5 h beginning 2 h before and continuing 8 h after intravenous administration of LPS (0.5 μg/kg BW; *Escherichia coli* O111:B4; Sigma-Aldrich, St. Louis, MO) and again at 24 h after the LPS challenge (Bernhard et al., 2012). Whole blood was allowed to clot for 30 min and serum was collected after centrifugation at $1,250 \times g$ for 20 min at 4°C. Serum was stored at −80°C until analyzed for glucose, SUN, and NEFA concentrations. Before administration of LPS, the jugular cannula became dislodged from one 10% DCP heifer and one 20% DCP heifer; therefore, data presented represents 22 heifers (CON, $n = 8$; 10% DCP, $n = 7$; and 20% DCP, $n = 7$). Twenty-four hours after the LPS challenge, jugular cannulas were removed, cattle were removed from the stalls, and a BW was recorded. Subsequently, cattle were commingled back in their original home pens and continuously fed their treatment diets. At 72 h after the LPS challenge, an additional final BW was recorded to evaluate the change in BW during the post-LPS period.

Serum Analysis

Glucose concentrations were determined by modification of the enzymatic Autokit Glucose (Wako Diagnostics, Richmond, VA) for a 96-well format. Briefly, 300 μL of prepared working solution was added to 2 μL of serum or prepared standards in a 96-well plate. Plates were incubated at 37°C for 5 min and then read using a plate reader at 505 nm. Concentration of glucose was determined by comparing unknown samples with a standard curve of known glucose concentrations. The minimum detectible concentration was 3.8 mg/dL and the intra- and interassay CV were 10.2 and 11.1%, respectively.

Concentrations of NEFA were determined by modification of the enzymatic HR Series NEFA-HR (2) assay (Wako Diagnostics) for a 96-well format. Briefly, 200 μL of the prepared Color Reagent A were added to 5 μL of serum or prepared standards in a 96-well plate. Plates were incubated at 37°C for 5 min and then read using a plate reader at 550 nm. Next, 100 μL of prepared Color Reagent B was added to the 96-well plate. Plates were incubated for an additional 5 min and read for a second time using a plate reader at 550 nm. Concentrations of NEFA were determined by comparing unknown samples with a standard curve of known NEFA concentrations. The minimum detectable concentration was 0.0014 mmol/L and the intra- and interassay CV were 13.6 and 17.4%, respectively.

Concentrations of SUN were determined by a colorimetric assay according to the manufacturer's directions (K024-H1; Arbor Assays, Ann Arbor, MI) by comparison of unknowns with standard curves generated with known concentrations of urea nitrogen. The minimum detectable SUN concentration was 0.065 mg/dL and the intra- and interassay CV were 6.9 and 6.5%, respectively.

Statistical Analyses

All performance data during the 56-d period were analyzed as a completely randomized block design using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). Treatment was included as a fixed effect and block was included as a random effect. Morbidity data were analyzed on a pen basis as binomial proportions using the GLIMMIX procedure of SAS. The initial trial BW was unshrunk whereas all other BW measurements were shrunk for statistical analysis. Three orthogonal contrasts were analyzed within the individual SAS programs for performance and morbidity: 1) CON versus the average of the 2 DCP treatment groups, 2) linear effect when increasing the DCP percentage from 0% DCP to 20% DCP, and 3) quadratic effect when increasing the DCP percentage concentration from 0% DCP to 20% DCP. A P-value of ≤ 0.05 was considered significant. A generalized quadratic solution was used to calculate the observed NEg of the diet using actual DMI and actual initial and final shrunk BW for the given period following equations outlined by the NRC (2000). Heifers were estimated to have a final shrunk BW at their targeted endpoint of 544 kg and it was assumed that they would grade Choice. The predicted NEg of the diet was based on the formulated dietary values displayed in Table 1. The predicted NEg value of the diet was subtracted from the observed NEg, meaning that greater values would indicate that heifers performed better than anticipated on the given diet.

Glucose, SUN, and NEFA data were analyzed using the MIXED procedure of SAS specific for repeated measures with treatment, time, and time × treatment interaction included as fixed effects. Individual heifer served as the experimental unit. An autoregressive covariance structure was applied. Specific treatment comparisons were made using the PDIFF option in SAS. A P-value of ≤ 0.05 was considered significant and $P \leq 0.10$ was considered a tendency.

RESULTS

Feedlot Performance

Performance data (BW, ADG, DMI, and G:F) are reported in Table 2. There were no source × treatment

interactions for any of the performance variables presented ($P \geq 0.15$). There was no difference in the initial BW between treatments at the beginning of the trial ($P = 0.65$). Body weight linearly decreased ($P \leq 0.01$) at every recorded interval as the dietary concentration of DCP increased. On d 56, the final BW were 304.8, 292.1, and 284.5 kg for the CON, 10% DCP, and 20% DCP treatments, respectively. During the time that the starter diet was fed, d 0 to 28, a linear decrease ($P < 0.01$) in ADG was observed as the dietary concentration of DCP increased. Alternatively, during the time the transition diet was fed (d 28 to 42), there was a tendency for a linear decrease ($P = 0.07$) in ADG favoring the inclusion of DCP. Nevertheless, when the heifers were transitioned to the finishing diet (d 42 to 56), there was a linear decrease ($P = 0.02$) in ADG for cattle consuming diets with increasing concentrations of DCP. Overall, although the response in ADG was inconsistent dependent on the concentrate level of the diet, a linear decrease in ADG was observed from d 0 to 56 as the inclusion concentration of DCP increased ($P < 0.001$; 1.50, 1.28, and 1.13 kg, respectively).

The response in ADG was largely a result of the response observed in DMI. During the period in which the starter diet was fed (d 0 to 28), there was a linear decrease ($P < 0.01$) in DMI as the dietary concentration of DCP increased. There was an 18.3% decrease in DMI during this period when comparing the CON group with the 20% DCP group (5.75 vs. 4.69 kg, respectively). Once again, during the period in which the intermediate diet was fed (d 28 to 42), there was a linear decrease in DMI as the dietary level of DCP increased ($P = 0.02$). Nevertheless, the decrease in DMI between the CON group and the 20% DCP group narrowed to a margin of 7.1% (7.06 vs. 6.56 kg, respectively). During the period in which the finishing diet was fed (d 42 to 56), there was a tendency for a linear decrease in DMI as the dietary concentration of DCP increased ($P = 0.08$) and, once again, the percentage decrease in DMI narrowed to 4.4% between the CON and 20% DCP group (8.25 vs. 7.89 kg, respectively). Collectively, over the entire duration of the 56-d trial period, as the amount of dietary DCP increased, DMI decreased ($P < 0.01$; 6.70, 6.13, and 5.96 kg, respectively).

When the starter diet was fed, from d 0 to 28, a linear decrease ($P < 0.01$) in G:F was documented as the inclusion level of DCP increased in the treatment diets. In contrast, when the transition diet was fed, from d 28 to 42, there was a linear increase ($P = 0.01$) in G:F in favor of the treatments with an increasing concentration of DCP. However, when the finishing diet was fed, from d 42 to 56, there was a linear decrease in G:F as the dietary concentration of DCP increased ($P = 0.04$) with a numerically wide margin of difference between the

Table 2. Effects of dehydrated citrus pulp (DCP) concentration on feedlot performance and morbidity during a 56-d receiving period[1]

Item	DCP 0%	DCP 10%	DCP 20%	SEM[2]	Contrast, P-value 0 vs. DCP	Linear	Quadratic
BW, kg							
d 0	220.5	220.5	221.1	4.5	0.65	0.44	0.66
d 14	235.5	233.4	230.9	4.2	0.03	0.01	0.87
d 28	260.3	252.8	248.7	5.0	<0.01	<0.01	0.38
d 42	278.6	274.3	270.6	5.0	0.01	0.01	0.91
d 56	304.8	292.1	284.5	5.9	<0.01	<0.01	0.49
ADG, kg							
d 0 to 28	1.42	1.15	0.99	0.065	<0.01	<0.01	0.46
d 28 to 42	1.31	1.54	1.57	0.099	0.05	0.07	0.40
d 42 to 56	1.87	1.27	1.00	0.267	0.02	0.02	0.59
d 0 to 56	1.50	1.28	1.13	0.696	<0.01	<0.01	0.54
DMI, kg							
d 0 to 28	5.74	5.02	4.69	0.144	<0.01	<0.01	0.20
d 28 to 42	7.06	6.64	6.56	0.171	0.01	0.02	0.31
d 42 to 56	8.25	7.84	7.89	0.167	0.04	0.08	0.19
d 0 to 56	6.70	6.13	5.96	0.130	<0.01	<0.01	0.12
G:F							
d 0 to 28	0.247	0.230	0.208	0.099	0.01	<0.01	0.85
d 28 to 42	0.184	0.235	0.243	0.015	0.01	0.01	0.24
d 42 to 56	0.227	0.163	0.125	0.347	0.05	0.04	0.74
d 0 to 56	0.225	0.210	0.191	0.011	0.04	0.02	0.83
Morbidity[3]							
Treated once, %	3.33	5.00	13.33	0.05	0.39	0.18	0.53
Treated twice, %	0	0	0	–	–	–	–

[1]A 63% concentrate diet was fed from d 0 through 28, a 73% concentrate diet was fed from d 28 through 42, and an 83% concentrate diet was fed from d 42 through 56.

[2]Standard error of the treatment means.

[3]Heifers demonstrating preliminary signs of illness or severe signs of discomfort and illness were pulled from the home pen and rectal temperature was measured. If body temperature was ≥39.7°C, heifers were treated by subcutaneous injection with Resflor GOLD (florfenicol and flunixin meglumine; Merck Animal Health, DeSoto, KS) at a rate of 6 mL/45.4 kg BW. Heifers that demonstrated severe signs of illness and discomfort were treated regardless of rectal temperature.

CON group and the 20% DCP group (0.227 vs. 0.125, respectively). Collectively, although feed efficiency varied throughout the trial, there was a linear decrease in G:F as the dietary concentration of DCP increased ($P = 0.02$; 0.225, 0.210, and 0.191, respectively).

Although fiber digestibility was not directly measured in the study, the difference between the observed NEg of the treatment diets compared with the predicted NEg was calculated to provide insight into potential positive associative effects that might be seen with fiber digestion as the level of roughage was systematically decreased during the trial. These data are presented in Table 3. When the starter diet (37% roughage on a DM basis) was fed, from d 0 to 28, there was no significant response detected between treatments ($P = 0.65$ for the quadratic response; 0.41, 0.44, and 0.43 Mcal/kg, respectively). However, from d 28 to 42, when the transition diet (27% roughage on a DM basis) was fed, there was a linear increase in the observed NEg minus the predicted NEg in favor of increasing levels of DCP in the diet ($P \leq 0.01$; –0.08, 0.13, and 0.19, respectively).

The opposite response was seen from d 42 to 56, when the cattle were on the finishing diet (17% roughage on a DM basis), where there was a linear decrease in observed NEg minus the predicted NEg as the concentration of DCP in the diet increased ($P < 0.01$; –0.018, –0.178, and –0.247, respectively).

Morbidity

Morbidity data are presented in Table 2 for the 56-d trial period. The total morbidity rate for all treatments combined was 7.2% with all treatments being based on clinical signs of bovine respiratory disease. Cattle receiving treatment occurred between d 2 and 13 during the time period when the starter diet was fed. No incidences of lameness occurred for the duration of the trial. There was no significant differences in morbidity (treated at least once) between treatments ($P = 0.18$ for the linear response; 3.33, 5.00, and 13.33%, respectively). No cattle were treated more than once and no mortalities occurred during the trial.

Table 3. Observed NEg minus predicted NEg of the treatment diets[1]

Time period[2]	Dehydrated citrus pulp concentration			SEM[3]	Contrast, P-value	
	0%	10%	20%		Linear	Quadratic
d 0 to 28	0.412	0.439	0.429	0.038	0.73	0.65
d 28 to 42	−0.081	0.128	0.186	0.554	<0.01	0.28
d 42 to 56	−0.018	−0.178	−0.247	0.054	<0.01	0.49

[1]A generalized quadratic solution was used to calculate the observed NEg of the diet using actual DMI and actual initial and final shrunk BW for the given period following equations outlined by the NRC (2000). Heifers were estimated to have a final shrunk BW at their targeted endpoint of 544 kg and it was assumed that they would grade Choice. The predicted NEg of the diet was based on the formulated dietary values displayed in Table 1. The predicted NEg value of the diet was subtracted from the observed NEg meaning that greater values would indicate that heifers performed better than anticipated on the given diet. Values are expressed as megacalories per kilogram.

[2]A 63% concentrate diet was fed from d 0 through 28, a 73% concentrate diet was fed from d 28 through 42, and an 83% concentrate diet was fed from d 42 through 56.

[3]Standard error of the treatment means.

Lipopolysaccharide Challenge

At the time of jugular cannulation (d −1), heifer BW did not differ between treatment groups ($P = 0.37$) before being placed into the barn. At both 24 and 72 h after the LPS challenge, heifers demonstrated a linear reduction in BW loss in favor of treatments with an increasing level of DCP in the diet ($P < 0.01$). At 24 h after the LPS challenge, the CON, 10% DCP, and 20% DCP cattle had a BW change of −13.55, −12.55, and −4.66 kg, respectively. At 72 h after the LPS challenge, the BW change was −4.99, −2.49, and 4.81 kg, respectively. During the 24 h before the LPS challenge, DMI linearly decreased as the level of DCP increased ($P = 0.02$). During the 24-h period after the LPS challenge was administered, DMI had a tendency to decrease as the level of DCP in the diet increased ($P = 0.07$).

Concentrations of the blood metabolites (glucose, NEFA, and SUN) before and after LPS administration are presented in Fig. 1. Before the LPS challenge, no time × treatment interactions were noted for any of the 3 metabolites ($P \geq 0.97$). There were no differences between treatments for glucose ($P = 0.12$) or NEFA ($P = 0.13$) levels. However, SUN ($P < 0.01$) concentrations were different between treatments. Following the LPS challenge, all 3 metabolites demonstrated a time ($P < 0.01$) and treatment ($P \leq 0.01$) effect, but no time × treatment interactions were detected ($P \geq 0.98$). More specifically, glucose was the most elevated for the CON treatment (79.19 ± 1.24 mg/dL) followed by the 20% DCP cattle (78.04 ± 1.33 mg/dL) and the least for the 10% DCP group (73.98 ± 1.33 mg/dL). The concentration of NEFA was the greatest in the 10% DCP group (0.3649 ± 0.0135 mmol/L), intermediate for the 20% DCP cattle (0.3302 ± 0.0132 mmol/L),
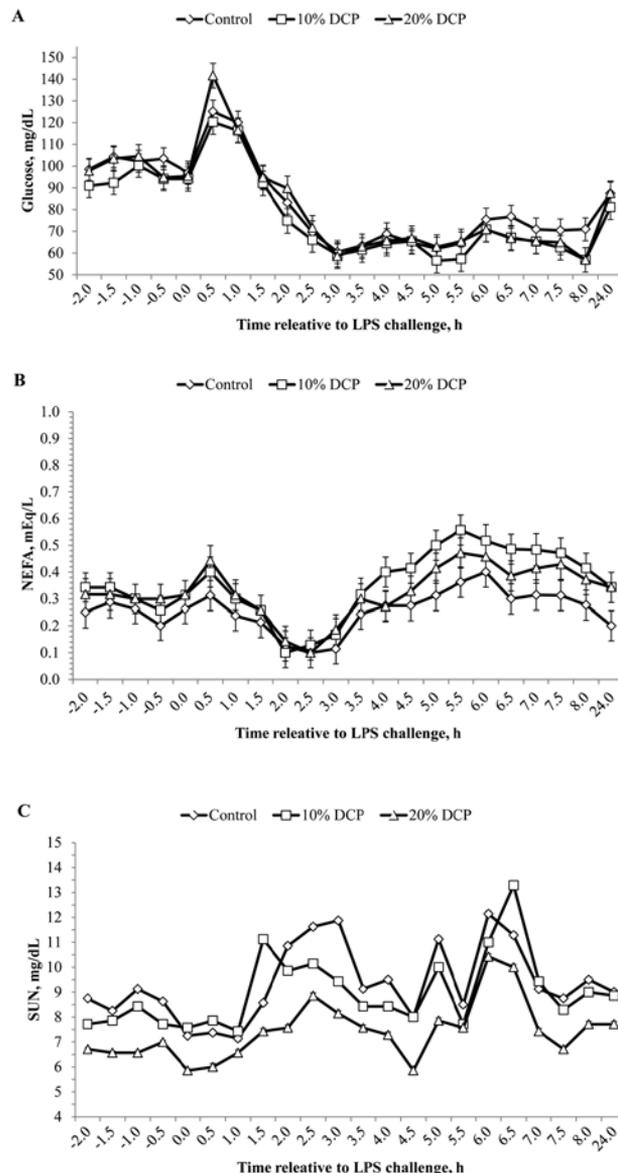

Figure 1. The effect of dehydrated citrus pulp (DCP) supplementation on A) serum glucose, B) NEFA, and C) serum urea nitrogen (SUN) concentrations in response to a lipopolysaccharide (LPS) challenge. English × Continental heifers ($n = 22$; 307.89 ± 3.32 kg) were separated into 3 treatments receiving a diet that added 0% DCP (control diet [CON]; $n = 8$), 10% DCP ($n = 7$), or 20% DCP ($n = 7$) on a DM basis continuously for 64 d before the LPS challenge. Blood samples were collected at 0.5-h intervals from −2 to 8 h and at 24 h relative to an LPS challenge (0.5 μg/kg BW) at time 0 h. Before or after the LPS challenge, there were no time × treatment interactions ($P \geq 0.97$) for any of the parameters. After the LPS challenge, there was a time ($P < 0.01$) and treatment effect ($P \leq 0.01$) for all parameters. After the LPS challenge, glucose (panel A) was the most elevated for the CON treatment (79.19 ± 1.24 mg/dL) followed by the 20% DCP cattle (78.04 ± 1.33 mg/dL) and the least for the 10% DCP group (73.98 ± 1.33 mg/dL). After the LPS challenge, the concentration of NEFA (panel B) was the greatest in the 10% DCP group (0.3649 ± 0.0135 mmol/L), intermediate for the 20% DCP cattle (0.3302 ± 0.0132 mmol/L), and the least in the CON treatment (0.2564 ± 0.0126 mmol/L). After the LPS challenge, the concentration of SUN (panel C) was the greatest in the CON treatment (9.496 ± 0.025 mg/dL) followed by the 10% DCP group (9.310 ± 0.027 mg/dL) and the least for the 20% DCP group (7.587 ± 0.027 mg/dL). Data presented as least squares means ± SEM.

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DISCUSSION

The available literature is mixed as to the level of performance observed when substituting corn with DCP in feedlot cattle, especially in regards to intake. Henrique et al. (1998) examined effects of replacing corn with DCP in a corn silage–based diet but also examined doing so at 2 different concentrate levels (20 and 80% concentrate) in 28 young growing bulls. In the low concentrate diets, DCP or corn was included at a rate of 7%, and in the high concentrate diet, it was included at a rate of 65% as a proportion of the concentrate. Between the high concentrate diets, the cattle fed corn were recorded with increased DMI, ADG, and G:F compared with bulls fed DCP. Between bulls fed the DCP, the performance of bulls was greater in the low-concentrate versus the high-concentrate diet. Vijchulata et al. (1980) investigated exchanging corn for DCP at relatively high levels as the primary energy source in 2 separate experiments (treatments diets contained ≤15% roughage on an as-fed basis) with 32 steers during an 83- and a 64-d trial. In the first study, DCP was fed at an exchange rate of 40% and a 9.3% decrease in DMI was observed; however, no differences were noted for ADG and G:F. In the second experiment, DCP was exchanged at a 60% rate and no differences were witnessed for DMI, ADG, or G:F. Chen et al. (1981) conducted a finishing trial (diets contained 15% roughage) with 40 yearling steers, feeding citrus condensed molasses solubles (**CCMS**) while replacing either corn or citrus pulp at levels of 0, 7, 14, or 21% on an as-fed basis. The level of CCMS had no effect on ADG, DMI, or G:F. Independent of the level of CCMS, when comparing the treatment diets containing exclusively corn versus the treatment diets containing DCP, there was no difference reported for ADG, DMI, or G:F. Interestingly, the DCP-based diets replaced corn at a rate of 40% on as-fed basis. These results are in contrast to the study herein, where ADG and G:F were suppressed during the last 14 d when the finishing diet was fed with increasing levels of DCP. However, in agreement, both studies did not observe a decrease in DMI during the finishing phase.

Perhaps DMI is heavily influenced by the age and weight of cattle and roughage level of the diet when fed DCP as the steers reported in Chen et al. (1981) were heavier, yearling steers that were already stepped up to the finishing diet. In our study, DMI linearly decreased between treatments as DCP inclusion rate was increased; however, the magnitude of difference decreased be-

tween treatments the longer the cattle were on feed. Therefore, lightweight calves may initially struggle to accept DCP, potentially due to palatability-related factors, but this appears to diminish over time as the cattle become more acclimated to DCP. Palatability-related factors might include the physical size of the pellet (the diameter of the pellet used herein was 1.59 cm), texture and density of the pellet, or taste differences between citrus sources. Karadeniz (2004) reported that dramatic differences exist in titratable acidity between species and varieties of citrus (10.42 to 58.94%). In the current study, the DCP was guaranteed to contain no more than 1.5% lime, but no other guarantees were made relative to the contribution of different citrus sources.

Observations from several studies have implied that a positive associative effect on fiber digestibility may be accomplished when partially replacing corn with DCP. Dehydrated citrus pulp contains a high concentration of pectins, which are primarily utilized by ruminococci and *Bacteroides ruminicola* and do not yield lactic acid during fermentation (Strobel and Russell, 1986). Pascual and Carmona (1980) reported in 2 separate finishing trials with wether lambs that as the proportion of DCP in the concentrate increased from 0 to 60%, there was an apparently linear increase in the digestibility coefficient of ADF. A similar trend was documented by Ben-Ghedalia et al. (1989) in a feeding study with rams when increasing levels of DCP from 20.4 to 84.4% in a barley-based diet. Bueno et al. (2002) observed an increase in both NDF and ADF digestibility coefficients across treatments with DCP included at rates of 0, 23, 46, and 65% in goat kids. Miron et al. (2002) observed an increase in NDF digestibility in Holstein cows when comparing diets containing approximately 10 and 20% DCP. In addition, the authors noted an increase in DM and CP digestibility. Sudweeks et al. (1975) saw chewing times increase with DCP over corn or soybean meal. Subsequently, eating times for citrus diets were slower. This could promote a greater production of saliva and an enhanced buffering effect in the rumen. In the current study, no difference was calculated between treatments for the observed NEg versus expected NEg when the starter diet was fed; however, a linear increase was seen in the observed energetic efficiency of the diet when the intermediate diet was fed in favor of increasing levels of DCP inclusion. These observations may have been potentially confounded by differing lengths of time in which cattle potentially capitalized on compensatory gain effects due to differing total energy intake differences between treatments because of the decreased intake level of the DCP-fed cattle. Nevertheless, although not definitive, it would collectively appear that energy value estimations of DCP may need to be altered depending on the level of roughage in the diet. The N source and concentration in the diet may also be an important consideration

(Kim et al., 2007). Independent of interactions between roughage levels and DCP, it is apparent that the NEg and NEm values presented by the NRC (2000) are overestimated relative to the performance results seen herein. As is typical with many byproducts from the food production industry, widespread differences between DCP nutrient densities appear to exist between sources.

In the current study, no relationship between morbidity and DCP was observed; however, it is hypothesized that citrus byproducts may have a positive immunomodulatory role. Citrus contains several essential oils, including limonene, linalool, and citrulline, which are known to have an antimicrobial effect. Callaway et al. (2008) demonstrated that DCP reduced the growth of these pathogens when introduced to pure cultures of *E. coli* 0157:H7 and *Salmonella* Typhimurium and in fermentation with ruminal microorganisms in vitro. These results have been verified in vivo with sheep (Callaway et al., 2011a,b). It is possible that the antipathogenic characteristics of DCP may not be limited to the gastrointestinal tract and may be systemic. Additionally, citrus is relatively concentrated in ascorbic acid, which is a critical water-soluble antioxidant in mammals (Sauberlich, 1994). Cummins and Brunner (1991) noted that young dairy calves that were housed in calf hutches versus pens displayed lower plasma vitamin C concentrations. Calves fed vitamin C had lower clinical signs for diarrhea (Cummins and Brunner, 1989). This may also correspond to stress during the receiving period. Accompanying data on the concentration of cytokines following administration of LPS are reported in Burdick et al. (2012) on the cattle herein. In short, there was a treatment effect on serum cytokine concentrations indicating that the level of interferon-γ, interleukin-6, and tumor necrosis factor-α in heifers increased as the level of DCP inclusion increased. From an applied standpoint, the altered acute phase response of the heifers coincided with a reduction in BW loss following the LPS challenge in the data herein. This is interesting considering that DMI tended to decrease as DCP supplementation increased following the LPS challenge. This relationship might be partly explained by a decrease in rumen outflow with increasing levels of citrus pulp as observed by Barrios-Urdaneta et al. (2003). Pelleted citrus pulp is reported to have an effective bulk density ratio of 0.715 during immediate hydration, thereby giving DCP a high water-holding capacity of 4.3 kg/kg of DM (Giger-Reverdin et al., 2002). No differences were detected for morbidity between treatments during the 56-d feeding period. In a review by Duff and Galyean (2007), the authors concluded that the ability to modify immune function and bovine respiratory disease morbidity rates through dietary manipulations is inconsistent. Total energy intake in the diet is critical because the activated immune system is energy demanding. Future studies will need to achieve an equivalent energy con-

sumption to unequivocally evaluate whether DCP has antioxidant capabilities, which can make an applied difference in the diet of stressed receiving cattle.

There are limited reports of the effects of citrus byproducts on the modification of blood metabolites and none following an LPS challenge. Given that the analyzed CP levels of DCP were remarkably lower than anticipated (4.70% CP on a DM basis) by the NRC (2000) and therefore lower than the substituted corn, it was to be expected that SUN concentrations were reduced in the DCP-fed cattle both before and following the LPS challenge. In addition, some ammonia-producing microorganisms have been shown to be sensitive to essential oils, slowing the production of ammonia in the rumen (McIntosh et al., 2003), which may have further exaggerated these results. In the current study, blood glucose concentrations were not different before the LPS challenge but afterward, glucose levels were greater in the CON cattle. Simultaneously, NEFA concentrations were elevated in a progressing order with DCP supplementation. These shifts in mobilization of energy sources in response to an immune challenge may also correspond to shifts in the production of acetate:proprionate during normal fermentation of DCP. Numerous scientists have documented elevated acetate concentrations during ruminal fermentation as the inclusion rate of citrus byproducts is increased in conjunction with diminishing levels of starch-rich feedstuffs (Pinzon and Wing, 1976; Vijchulata et al., 1980; Wing et al., 1988; Ben-Ghedalia et al., 1989; Barrios-Urdaneta et al., 2003). Collectively, it would appear that cattle fed DCP either mobilize energy sources with differing preferences in response to an immune challenge or, as a consequence of altered ruminal metabolism, have different energy sources available to select from.

Conclusion

Dehydrated citrus pulp is used in citrus-producing regions as a least cost formulation energy feedstuff. Given the carbohydrate composition of DCP, it has a reduced risk of ruminal acidosis in replacement of corn, which may be especially advantageous in receiving cattle while transitioning to a finishing diet. However, our results indicated that lightweight receiving cattle struggle to accept DCP due to palatability-related factors. A linear decrease in DMI and, in turn, a linear decrease in ADG and G:F were observed during the entire 56-d receiving period as DCP was increased up to concentrations of 20%. Nevertheless, the intensity of DMI suppression was reduced over time as cattle became increasingly acclimated. No impact on morbidity was observed during the trial, but acute weight loss was decreased for DCP-fed cattle following an LPS challenge. Additionally, blood metabolites were altered in response to an immune challenge. In general, DCP-fed

cattle had elevated concentrations of NEFA and lower concentrations of glucose. Additional research targeted at alternative strategies to improve the palatability of DCP in lightweight cattle is needed to potentially capitalize on economic and health benefits associated with DCP when substituting corn in receiving diets.

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