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Intake of supplemental deer pellets containing ground blueberry juniper by wild pigs

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Abstract: Supplemental feeding of cervid species such as white-tailed deer (*Odocoileus virginianus*; deer) is now a common management practice in the United States. Supplemental feeding can be costly and more expensive when supplements are consumed by non-target species such as wild pigs (*Sus scrofa*; pigs). From May 13 to June 17, 2015, we evaluated the effects of using ground blueberry juniper (*Juniperus ashei)* or cottonseed (*Gossypium* spp.) hulls as a roughage ingredient in a supplemental deer pellet to deter pig consumption at the Texas A&M AgriLife Research Center in San Angelo, Texas, USA. We analyzed dry matter intake, growth performance, *in vitro* digestibility and fermentation, and blood serum chemistry of pigs using a 2 × 2 factorial study design that included 3 feeding periods. Pigs were assigned to 1 of 4 supplement diets (n = 5 pigs/supplement) or to a commercially available swine diet (BASAL; n = 4 pigs). Animals assigned to supplement diets were also offered BASAL based on percentage of body weight (BW) during each period. Supplement diets differed by roughage source and percentage of roughage: cottonseed hulls 20%, cottonseed hulls 40%, blueberry juniper 20%, or blueberry juniper 40%. During each period, the amount of supplement and BASAL diet offered to animals assigned to a supplement was fed as a percentage of BW; period 1 (day 0 to 17) = 5% supplement diet and 5% BASAL diet, period 2 (day 18 to 26) = 5% supplement diet and 2% BASAL diet, period 3 (day 27 to 34) = 5% supplement diet and 5% BASAL diet. Animals assigned to only BASAL were offered the same amount of feed as a percent of BW as supplement animals during each period. We observed a roughage × period interaction (P = 0.03) for supplement dry matter intake g/day and a roughage × period interaction (P < 0.09) for total dry matter intake as a percentage of BW. No differences were observed within period. No other variables had percent roughage x period differences. Ground blueberry juniper was indigestible by pigs; the *in vitro* digestibility of dry matter and gross energy was <1%. Greater blood serum alanine aminotransferase (P = 0.07) in pigs consuming experimental supplement diets suggested the possibility of liver damage. Our findings suggest that there does not appear to be a benefit of using ground juniper as a roughage source to reduce consumption of supplemental deer feed by pigs.

Key words: cottonseed, *Gossypium* spp., juniper, *Juniperus ashei, Odocoileus virginianus*, supplemental feeding, *Sus scrofa*, Texas, white-tailed deer, wild pigs

The wild pig (*Sus scrofa;* pig) population is >6 million individuals across 35 states in the United States (U.S. Department of Agriculture 2017). With the increase in the size and distribution of the pig populations, competition with native wildlife species for natural resources and supplemental feeding has increased (Lambert and Demarais 2001).

Supplemental feeding is used in some regions of the United States to enhance the nutritional intake of cervids to meet maintenance and production requirements when forage quality, abundance, or both is reduced. A well-managed supplemental feeding program can be successful in increasing antler size, body mass, *in utero* productivity, and fawn survival (Ozoga and Verme 1982, Bartoskewitz et al. 2003). But where white-tailed deer (*Odocoileus virginianus*; deer) and pigs interact, deer will often avoid feed sites when pigs are present (Barrett 1982). Loss accrued from non-target species consumption, such as by wild pigs, can be substantial (Bach 1998).

Figure 1. Wild pig (*Sus scrofa*) holding facility at the AgriLife Research Center, San Angelo, Texas, USA. We conducted research from May to June 2015 to evaluate the inclusion of ground blueberry juniper (*Juniperus ashei*) as a deterrent to wild pig consumption of supplemental pellets for white-tailed deer (*Odocoileus virginianus*).

Whole cottonseed (*Gossypium* spp.) has been used as a supplemental food for deer because it is not palatable to non-target species (Cooper 2006, Taylor et al. 2013). Cottonseed contains gossypol, which is a plant secondary metabolite that can be toxic to monogastric animals but is more tolerated by ruminant animals (Reiser and Fu 1962).

Junipers (*Juniperus* spp.) are native, invasive, woody shrubs that occur on millions of hectares of rangelands throughout the United States (Ansley et al. 2006). Juniper produces plant secondary metabolites in the form of condensed tannins and essential oils, specifically monoterpenes (Palo and Robbins 1991). Accumulations of secondary metabolites can reduce palatability, although junipers are utilized by both white-tailed deer and mule deer (*O. hemionus*). Winter diets of mule deer can contain 70–90% junipers (Palo and Robbins 1991). Monoterpenes may also limit intake due to conditioned aversions resulting from biological activity, negative feedback after consumption, and antimicrobial properties that vary by concentration and class (Oh et al. 1967, 1968; Vourc'h et al. 2002).

Zhai et al. (2018) reviewed effects of essential oils on poultry and pigs and found that research on feed intake was equivocal and research on olfactory effects of essential oils was scarce. Several studies indicated depressed intake rates or near complete refusals and other studies indicated that low concentrations of some essential oils can be tolerated and beneficial as a feed additive (Schöne et al. 2006, Windisch et al. 2008, Michiels et al. 2009).

Physiological mechanisms, including inactivation, degradation, and excretion are used by herbivores to deal with plant defenses (Palo and Robbins 1991). The ability to tolerate secondary compounds decreases in herbivores from specialist browsers to grazers. Monogastric herbivores are often less tolerant than ruminants (Huang et al. 2018). Condensed tannins can negatively affect animal production by reducing intake, protein and dry matter digestibility, and to a lesser extent carbohydrate and cell wall digestibility (Robbins et al. 1987*a*, *b*). Mechanisms to detoxify these metabolites can potentially cause damage to the liver and other organs. In ruminant diets, though, recent studies have shown successful utilization of several species of ground juniper as a roughage ingredient. When utilized in total mixed diets of sheep (*Ovis aries*), goats (*Capra aegagrus hircus*), and cattle (*Bos taurus*), no negative impacts to animal health were documented (Whitney et al. 2017, Glasscock et al. 2018, Whitney et al. 2019).

The objective of our research was to evaluate the inclusion of ground blueberry juniper (*J. ashei*) in supplemental pellets for deer to prevent or decrease pig consumption. We investigated consumption rates by wild pigs and *in vitro* digestibility of supplemental pellets. We also evaluated blood serum chemistry. Our hypothesis was that the inclusion of ground blueberry juniper as a roughage ingredient in supplemental pellets for deer would deter consumption by pigs. The results of this study may be beneficial in helping to decrease economic losses due to non-target species consumption when supplemental feeding.

Study area

We conducted the study from May 13 to June 17, 2015 at the San Angelo Research Center, San Angelo, Texas, USA. The center is operated by Texas A&M AgriLife Research and focuses on technology and strategies to improve the management of range livestock and wildlife and the dissemination of that knowledge to the people of West Texas. A portion of the center's feedlot facilities were inspected and approved by the Texas Animal Health Commission as a pig holding facility (Figure 1).

Figure 2. An individual pen for a wild pig (*Sus scrofa*) in the holding facility at the AgriLife Research Center, San Angelo, Texas, USA. We conducted research from May to June 2015 to evaluate the inclusion of ground blueberry juniper (*Juniperus ashei*) as a deterrent to wild pig consumption of supplemental pellets for white-tailed deer (*Odocoileus virginianus*).

Methods

Animal management

To conduct this experiment, we live-captured 24 pigs in 5 groups during spring 2015 in Menard and Sutton counties, Texas. After capture, each group was transported to the holding facility at Texas A&M AgriLife Research Center. Animals were randomly assigned to individual pens (2.44 × 2.97 m) with an automatic watering system and feed bunk where they were housed for the duration of the study (Figure 2). Upon arrival, pigs were fed a mixture of whole corn (*Zea mays*) and a commercially available 18% crude protein (CP) pig grower pellet (Ring Leader Pig Grower 18% Grower Ration for Pigs, Angelo Pellets, Inc., San Angelo, Texas). The grower pellet was also used as the basal (BASAL) diet for pigs during the duration of the study.

Six days prior to the start of the study, the pigs were transitioned to strictly the grower pellet. We collected fecal samples, collected upon arrival, that were subsequently analyzed. Coccidia and strongyloide parasites were detected in the samples. Thus, we treated all pigs with Ivermectin (Bimeda-MTC Animal Health Inc., Cambridge, Ontario, Canada) and Sulmet® (Huvepharam Incorporated, Peachtree City, Georgia, USA).

We recorded pig sex and body weight (BW) prior to the start of the study. We grouped pigs by gender, stratified by BW, and randomly assigned pigs to a supplement diet (n = 5/diet). Supplement diets were pelleted and varied only by the roughage source and percentage of roughage: 20% cottonseed hulls (CSH20), 40% cottonseed hulls (CSH40), 20% blueberry juniper (JUN20), or 40% blueberry juniper (JUN40). The remaining 4 animals received the pelleted grower diet for pigs (BASAL; 18% CP) for the duration of the study. Due to the variation in composition of BASAL and supplement diets, BASAL was not included in the statistical analysis of intake and growth performance. The BASAL was included in the statistical analysis of blood serum chemistry and lab analysis of chemical composition and digestibility of diets.

Pigs were fed twice daily at 0800 and 1600 hours. During period 1 (day 0 to 17), pigs were fed their assigned supplement diet at 5% of BW and BASAL diet at 5% of BW. During period 2 (day 18 to 26), pigs continued to receive 5% of BW of supplement diet but were restricted to 2% of BW of BASAL. During period 3 (day 27 to 34), pigs continued to receive 5% of BW of supplement diet and BASAL was increased to 5% of BW. The BASAL was increased during period 3 to determine if the restriction of BASAL during period 2, when pigs may have been forced to consume the supplement diets to meet their nutritional needs, caused a negative feedback from the supplement diets. A negative feedback would cause pigs to avoid the supplement diets when they could again meet their nutritional needs with the increased BASAL diet. Pig BW was recorded on day 0, 18, 27, and 34. Average daily dry matter intake (DMI) was determined between days that BW was recorded.

On day 35, at the completion of the feeding trial, all pigs were euthanized using a captive bolt followed immediately by exsanguination. Blood serum was collected from all pigs, and a necropsy documenting organ condition was performed on 2 pigs from each of the BASAL and JUN40 groups and 1 pig from each of the JUN20 and CSH40 groups.

Sample collection and measurements

Woody plant harvesting and feed processing, collection, and analysis. We harvested the above ground biomass from mature blueberry juniper and chipped (Vermeer, X1500, Pella, Iowa, USA) in September 2014 and dried the biomass to approximately 93% dry matter (DM). Chipped juniper was ground in a hammermill to pass through a 4.76-mm sieve, then bagged

Table 1. Chemical composition and digestibility (% dry matter basis) of cottonseed (*Gossypium* spp.) hulls, sorghum (*Sorghum bicolor*) grain, dried distillers grains with solubles, and ground blueberry juniper (*Juniperus ashei*) used in supplement diets fed to wild pigs (*Sus scrofa*), AgriLife Research Center, San Angelo, Texas, USA, May to June 2015.

	Ingredient[b]			
Item[a]	CSH	Sorghum grain	DDGS	Juniper
Nutrient composition				
DM, %	92.3	92.6	91.8	93.8
CP, %	3.5	11.9	30.4	2.8
ADICP, %	3.2	1.5	1.3	1.6
NDF, %	85.2	7.0	30.4	65.0
ADF, %	62.1	5.3	12.9	52.1
Lignin, %	16.4	0.9	2.9	21.2
Crude fat, %	0.6	3.1	8.7	3.2
Ash, %	3.6	3.6	4.7	4.8
Ca, %	0.12	0.04	0.07	1.53
P, %	0.04	0.21	0.88	0.04
S, %	0.06	0.14	0.93	0.04
K, %	0.99	0.34	1.33	0.16
Mg, %	0.14	0.12	0.38	0.04
Na, %	0.01	0.01	0.31	0.01
Fe, ppm	33	48	85	98
Zn, ppm	5	20	64	9
Cu, ppm	3	3	8	2
CT, %				
Extractable	1.4	0	-	3.2
Protein-bound	1.8	0	-	2.3
Fiber-bound	0.2	0	-	0.0
Total	3.4	0	-	5.5

[a]DM = dry matter; CP = crude protein; ADICP = acid detergent insoluble CP; NDF = neutral detergent fiber; ADF = acid detergent fiber; CT = condensed tannins.
[b]CSH = cottonseed hulls; Juniper = ground blueberry juniper; DDGS = corn dried distillers grains with solubles produced from corn ethanol production (POET, Sioux Falls, South Dakota, USA).

and stored under cover. Subsamples were dried to constant weight in a forced-air oven at 103° C to determine DM concentration.

We evaluated the nutritive characteristics of juniper using random subsamples that were mechanically dried and hammermilled (4.76-mm screen). We collected subsamples of cottonseed hulls (CSH), sorghum (*Sorghum bicolor*) grain, and distiller's dried grains with solubles (DDGS) and combined for analysis (Table 1). Three random subsamples of diets were collected during each period, combined, and analyzed. We dried these samples at 55° C in a forced-air oven (Model 630, NAPCO®, Portland, Oregon, USA) for 48 hours, ground through a 1-mm screen (Wiley Mill, Arthur H. Thomas Co., Philadelphia, Pennsylvania, USA) and stored at –20° C. We analyzed nitrogen with a standard method (Method 990.03; Association of Official Analytical Chemists International [AOAC] 2006) and crude protein (CP) calculated as 6.25 × N. We analyzed neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to procedures of Van Soest et al. (1991), which were modified for an Ankom 2000 Fiber Analyzer (Ankom

Technology Corporation Fairport, New York, USA) using α-amylase and Na sulfite. We used standard methods to analyze ash (Method 942.05; AOAC 2006) and minerals, the latter by a Thermo Jarrell Ash IRIS Advantage HX Inductively Coupled Plasma Radial Spectrometer (Thermo Instrument 137 Systems Incorporated, Waltham, Massachusetts, USA). We analyzed fatty acid composition according to the procedures of Archibeque et al. (2005). We assayed condensed tannins in the juniper, cottonseed hulls, and sorghum grain for soluble, protein-bound, and fiber-bound fractions (Table 1) by methods described by Terrill et al. (1992); samples were oven dried and standards prepared for each individual ingredient as recommended by Wolfe et al. (2008).

In vitro dry matter digestibility and gas production. To determine DM disappearance and gas production, we ground 3 random subsamples of each of the treatment diets, BASAL diet, and juniper samples to pass through a 1-mm screen. We used a modified 3-step enzymatic and microbial fermentation procedure (Boisen and Fernández 1997, Bindelle et al. 2007). Two grams of each substrate were added to a flask with a phosphate buffer solution (100 ml, 0.1 *M* 7:1) and an HCL solution (40 ml, 0.2 *M*). We adjusted the pH to 2.0, and 2 ml of 5 mg/ml chloramphenicol (Sigma C-0378) solution (dissolved in ethanol) was added to prevent bacterial growth during hydrolysis. We added 4 ml of 100 mg/ml fresh porcine pepsin (Sigma P-7000) and samples underwent gentle agitation, hand shaken for 5 seconds every 15 minutes, for 2 hours in a water-bath at 39° C. After 2 hours, 40 ml of 0.2 *M* phosphate buffer and 20 ml of a 0.6 *M* NaOH were added, and the pH was adjusted to 6.8 with 1 *M* HCL or 1 *M* NaOH. Porcine pancreatin (4 ml, 100 g/L pancreatin, Sigma P-1750) solution (dissolved in 0.2 *M* phosphate buffer) was added and agitation continued for an additional 4 hours.

Using a nylon cloth (50-**µm** pore size, Ankom Technology Corp., Macedon, New York), we filtered the residues, washed them with distilled water, ethanol (2 × 20 ml 95% ethanol), and acetone (2 x 20 ml 99.5% acetone), dried them for 72 hours at 55°C, and weighed them to determine *in vitro* dry matter digestibility (IVDMD). We took the average of 2 replicates and if the difference was >5%, hydrolysis was repeated. We pooled the replicates for each sample, and hydrolyzed residue was used to conducted *in vitro* fermentation and characterization.

We assessed fermentation rate *in vitro* using a cumulative gas production technique adapted to pigs by Bindelle et al. (2007). We incubated residues in a 39°C water-bath under agitation (50 rpm) in a 125-ml glass bottle with pig fecal inoculum and 30-ml buffer solution containing macro- and micro-minerals (Menke and Steingass 1988). Donor pigs of fecal inoculum were obtained from the Prairie Swine Center herd (Saskatoon, Saskatchewan, Canada) and fed a standard commercial diet devoid of antibiotics. We collected fecal samples from the rectum, placed them in an air-tight syringe, and kept them in a water-bath at 39°C. The feces were diluted 20 times in buffer solution and filtered through a 250-µm screen to prepare the inoculum. We transferred the inoculum into bottles with fermentation substrates, sealed them with a rubber stopper, and placed them for incubation. An anaerobic environment was maintained from the time of inoculum preparation until incubation by flushing with CO_2 gas. We used a pressure transducer (GP:50, SIN-54978, Grand Island, New York; Mauricio et al. 1999), fitted with a digital data tracker (Tracker 211, Intertechnology Incorporated, Ontario, Canada) to measure gas accumulation at 2, 5, 8, 12, 16, 20, 24, 30, 36, 48, and 72 hours.

Blood serum collection and analysis. We collected a 10-ml blood sample from each pig after dispatch, via a jugular sample using a non-heparinized vacutainer collection tube (serum separator tube, gel, and clot activator; Becton Dickenson, Franklin Lakes, New Jersey, USA). Blood was allowed to clot before being centrifuged (Beckman Coulter TJ6 refrigerated centrifuge, Fullerton, California, USA) at 970 × g for 25 minutes at 4°C. Serum was removed and frozen at –20°C until analyzed. The Texas A&M Veterinary Diagnostic Laboratory, Amarillo, Texas, analyzed serum chemistry using an Olympus AU400E analyzer (Olympus America Inc., Center Valley, Pennsylvania).

Necropsy. We conducted a post-mortem evaluation on 2 pigs from each of the BASAL and JUN40 groups and 1 pig from each of the JUN20 and CSH40 groups. We examined the liver, lungs, spleen, kidneys, heart, and digestive tract for lesions, abrasions, ulcers, or any signs

Table 2. Effects of roughage ingredient and percentage of roughage on wild pig (*Sus scrofa*) supplement, basal, and total diet dry matter intake[a], AgriLife Research Center, San Angelo, Texas, USA, May to June 2015.

	Diet[b]							
	CSH		JUN			P-value[b]		
Item[c]	20	40	20	40	SEM[d]	P	R × P	PR × P
Supplement DMI, g/day						<0.001	0.03	0.12
Day 0 to 19	121.1	108.0	76.3	31.0	78.0			
Day 20 to 28	491.1	400.6	297.0	175.8	140.2			
Day 29 to 35	9.7	0.6	37.3	13.5	52.5			
Day 0 to 35	207.3	169.7	136.9	73.4	71.3			
Supplement DMI, % of BW						<0.001	0.40	0.91
Day 0 to 19	1.04	0.97	0.66	0.31	0.49			
Day 20 to 28	2.36	1.90	1.46	1.13	0.60			
Day 29 to 35	0.09	0.04	0.17	0.09	0.09			
Day 0 to 35	1.16	0.97	0.76	0.51	0.37			
Basal DMI, g/day						<0.001	0.60	0.62
Day 0 to 19	814.1	807.8	755.9	706.6	103.9			
Day 20 to 28	44.5	524.1	451.1	423.1	66.8			
Day 29 to 35	906.0	1,042.0	925.6	781.8	118.4			
Day 0 to 35	721.2	791.3	710.5	637.1	92.5			
Basal DMI, % of BW						0.86	0.99	0.99
Day 0 to 19	4.64	4.58	4.84	4.67	0.24			
Day 20 to 28	1.83	2.02	1.98	2.01	0.06			
Day 29 to 35	3.28	3.85	3.90	3.80	0.20			
Day 0 to 35	3.25	3.48	3.57	3.49	0.14			
Total DMI, g/day						0.04	0.41	0.77
Day 0 to 19	943.2	957.0	864.7	771.7	145.4			
Day 20 to 28	942.5	965.9	780.5	633.1	184.0			
Day 0 to 35	936.2	1,002.2	879.9	744.8	154.4			
Total DMI, % of BW						<0.001	0.09	0.58
Day 0 to 19	5.7	5.5	5.5	5.0	0.6			
Day 20 to 28	4.2	3.9	3.5	3.2	0.6			
Day 29 to 35	3.4	3.9	4.0	3.9	0.3			
Day 0 to 35	4.4	4.4	4.3	4.0	0.5			

[a]During period 1 (day 0 to 19), pigs were fed 5% of BW supplement diet and 5% of BW BASAL diet. Period 2 (day 18 to 26), pigs were fed 5% of BW supplement diet and 2% of BW BASAL diet. Period 3 (day 27 to 34), pigs were fed 5% of BW supplement diet and 5% of BW BASAL diet.
[b]P = period; R = roughage source; PR = percentage of roughage.
[c]DMI = dry matter intake; BW = body weight; Total DMI = day 19, 28, 35, and overall.
[d]SEM = greatest standard error of the means.

that would indicate aversion to the supplement diets. The data are reported in Appendix A. The experimental protocol was approved by the Texas A&M University Institutional Animal Care and Use Committee (# 2014-021A).

Statistical analysis

We analyzed pig supplement, BASAL, and total diet dry matter intake (daily and as a percentage of BW) using the PROC GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, North Carolina, USA) as a 2 × 2 factorial with a model that included type of roughage, percentage of roughage, period, and all interactions; gender was also included as a fixed effect to account for males and females within each treatment group. Day and group (5 groups of pigs arrived on different days) were random effects and individual pig was the subject. Pig dry matter intake (as a percentage of BW) and gain:feed were analyzed using a beta distribution.

We analyzed blood serum parameters using the PROC GLIMMIX procedure of SAS, but with a model that included type of roughage, percentage of roughage, and the interaction, along with gender. Group was the random effect and individual pig was the subject. Lognormal distributions were used for blood serum creatinine and aspartate aminotransferase, which did not have normal distributions; data were post-processed to the original scale. We assessed collinearity with pair-wise correlations using the Pearson correlation procedure of SAS.

We reported the results as least squares means with greatest standard errors. We evaluated differences in least squares means using the DIFF procedure of SAS with a SIMULATE adjustment. When an interaction was observed ($P < 0.10$), effects were evaluated within that interaction. All growth performance data are presented by period; each feeding period was unique in the total percentage of BASAL and supplement diets that were fed.

Results and discussion

Treatment chemical and physical composition and digestibility

Ground juniper had less CP and NDF, and more lignin compared to CSH (Table 1). Similar CP and lignin percentages for ground blueberry juniper were reported by Stewart et al. (2015). Crude fat was greater for ground juniper compared to CSH, although crude fat in juniper also includes volatile oil that is not nutritious (Cook et al. 1952). Crude protein in the treatment diets was similar for CSH20 and JUN20 and approximately 2.5% greater in CSH40 compared to JUN40 (Table 2). Although NDF was approximately 20% less for ground juniper compared to CSH, within the mixed diets, NDF was only 0.1% different between CSH20 and JUN20 and 1.3% different between CSH40 and JUN40. Total condensed tannin concentration was 2.1% greater in ground juniper compared to CSH.

We did not analyze for total volatile terpene oil. Owens et al. (1998) collected fresh blueberry juniper from trees in central Texas and reported monoterpenoid concentration of 9.16 mg/g, fresh weight. Volatile oil from blueberry juniper consists of 65 compounds: 48% monoterpenes, 38% sesquiterpenes, and 14% diterpenes (Stewart et al. 2015). Air drying and mechanically drying juniper can reduce total oil concentration (Adams 2010, Whitney et al. 2014). Dried juniper was reported to contain <1.1% terpene oil (DM basis; Stewart et al. 2015).

When comparing JUN40 to BASAL, data for fatty acid composition of treatment diets showed greater levels of eicosadienoic, palmitic, palmitoleic, stearic, and oleic acid for JUN40. Linoleic acid was approximately 30% less for JUN40 compared to BASAL and all other diets. Deficiency in essential linoleic acid can lead to liver and kidney degradation, frequent infections, poor wound healing, reproductive failure, and cardiovascular disease (Connor 1999, Bradley and Lord 2001). Elevated levels of palmitic acid can be related to essential fatty acid deficiency (Bradley and Lord 2001).

Animal dry matter intake

We observed a period effect ($P < 0.001$) for supplement dry matter intake as a percentage of BW. Consumption of supplement diets increased during period 2 for all treatment groups when the BASAL diet was restricted. Although animals were fed the same percentage of BASAL and supplement diets in periods 1 and 3, consumption of supplement diets in period 3 was lower for all treatment groups compared to consumption in period 1. There tended to be ($P < 0.09$) a roughage × period interaction for total dry matter intake as a percent-

Table 3. Effects of roughage ingredient and percentage of roughage on wild pig (*Sus scrofa*) total growth performance[a], AgriLife Research Center, San Angelo, Texas, USA, May to June 2015.

	Diet[b]								
		CSH		JUN			P-value[c]		
Item[d]	BASAL	20	40	20	40	SEM[e]	P	R × P	PR × P
BW, kg							<0.001	0.008	0.06
Day 0	20.9	18.9	19.8	16.8	16.3	3.2			
Day 19	24.6	25.3	26.2	22.6	20.7	3.7			
Day 28	26.3	28.5	28.7	24.7	21.7	3.9			
Day 35	28.2	30.5	32.4	28.2	24.8	4.2			
ADG							0.004	0.06	0.08
Day 0 to 19	197.0	320.8	335.4	322.7	273.5	65.2			
Day 20 to 28	189.1	339.3	267.5	248.9	155.1	67.5			
Day 29 to 35	275.5	271.6	527.8	522.6	485.0	88.3			
Day 0 to 35	220.5	310.6	376.9	364.7	304.5	64.2			
G:F							<0.001	0.005	0.01
Day 0 to 19	0.27	0.35	0.38	0.38	0.33	0.03			
Day 20 to 28	0.24	0.37	0.30	0.34	0.24	0.06			
Day 29 to 35	0.38	0.30	0.51	0.56	0.60	0.06			
Day 0 to 35	0.30	0.34	0.39	0.43	0.39	0.04			

[a]During Period 1 (day 0 to 19), pigs were fed 5% of BW supplement diet and 5% of BW BASAL diet. Period 2 (day 18 to 26), pigs were fed 5% of BW supplement diet and 2% of BW BASAL diet. Period 3 (day 27 to 34), pigs were fed 5% of BW supplement diet and 5% of BW BASAL diet.
[b]Supplement diets were pelleted and ingredient composition only differed by roughage source, either cottonseed (*Gossypium* spp.) hulls (CSH) or ground blueberry juniper (*Juniperus ashei*; JUN). *J. ashei* (entire above-ground biomass) was chipped, dried, and hammermilled to pass a 4.76-mm sieve. BASAL = Ring Leader Pig Grower 18%, manufactured by Angelo Pellets, Inc., San Angelo, Texas.
[c]P = period; R = roughage source; PR = percentage of roughage.
[d]BW = body weight; ADG = average daily gain; G:F = gain to feed.
[e]SEM = greatest standard error of the means.

age of BW. No interactions were observed for percentage of roughage × period; this may have been indicative of the small sample size and variability in pig BW since intake rates of supplement diets below 0.5% BW were observed in multiple animals during each period—period 1: CSH20 (n = 1), CSH40 (n = 1), JUN20 (n = 4), JUN40 (n = 3); period 2: CSH40 (n = 1), CSH40 (n = 0), JUN20 (n = 0), JUN40 (n = 1); period 3: CSH20 (n = 3), CSH40 (n = 3), JUN20 (n = 3), and JUN40 (n = 4). Cappai et al. (2010) reported that growing pigs fed a 70% ripe whole acorn shreds diet had a hyperacute swelling of the parotid gland within 24 hours, followed by increased production of proline-rich proteins within 7 days, indicating a reaction to the protein binding capacity of hydrolysable tannins. Salivary tannin-binding proteins tend to precipitate only tannins commonly found in an animal's diet. If pigs fed JUN20 and JUN40 reacted similarly to hydrolysable tannins in juniper, they may have adapted quickly.

Animal performance

Traditionally, condensed tannins have been considered anti-nutritional for monogastric species with negative impacts on performance, dry matter intake, and digestibility (Butler 1989). In the present study, average initial BW at day 0 was 17 kg (Table 3). However, due to variability in capturing wild animals, individual pig BW ranged from 9.53–32.66 kg. There tended to be a percentage of roughage × period (P < 0.06) interaction for pig BW. We also observed

Table 4. Ingredient and chemical composition (% dry matter basis) and digestibility of supplement and BASAL diets feed to wild pigs (*Sus scrofa*), AgriLife Research Center, San Angelo, Texas, USA, May to June 2015.

	Diet				
Item[a]	BASAL	CSH20	CSH40	JUN20	JUN40
Cottonseed hulls		20	40		
Ground juniper		-	-	20	40
DDGS		20	13.4	20	13.4
Ground sorghum grain		40	26.6	40	26.6
Cottonseed meal		14	14	14	14
Molasses, cane		3	3	3	3
Salt		0.5	0.5	0.5	0.5
Mineral premix		1.5	1.5	1.5	1.5
Pellet binder		1.0	1.0	1.0	1.0
Nutrient composition, %					
DM	92	91.4	91.3	91.4	91.4
CP	23.9	19.3	18.1	19.9	16.6
aNDF	10.2	28.6	37.3	28.5	36
ADF	5.9	18.7	29.1	17.5	27.6
Ca	2.06	0.54	0.60	0.75	0.84
P	0.83	0.56	0.56	0.64	0.50
Ca:P	1.7	1.0	1.1	1.2	1.7
Ash	8.91	5.38	5.88	6.13	5.15
Fatty Acid, %					
Myristic (14:0)	0.32	0.28	0.47	0.26	0.44
Myristoleic (14:1)	0.02	0.00	0.00	0.03	0.11
Palmitic (16:0)	20.93	17.96	19.49	19.22	32.84
Palmitoleic (16:1)	1.17	0.46	0.50	0.69	6.32
Stearic (18:0)	2.04	1.48	1.81	1.77	5.84
Oleic (18:1cis-9)	29.16	28.37	28.44	28.80	34.92
Linoleic (18:2)	44.05	49.41	46.41	46.87	13.58
α-Linolenic (18:3)	1.58	1.53	1.61	1.72	0.69
Arachidic (20:0)	0.00	0.00	0.00	0.00	0.13
Eicosadienoic (20:2)	0.00	0.03	0.10	0.00	3.56
Arachidonic (20:4)	0.00	0.00	0.16	0.00	0.00
Docosenoic (22:1)	0.32	0.08	0.16	0.00	0.15
Eicosapentaenoic (20:5)	0.41	0.28	0.54	0.63	0.96
Lignoceric (24:0)	0.00	0.11	0.30	0.00	0.45
Digestibility, %					
IVDMD	71.7	46.9	49.1	48.5	58.9
IVDGE	73.1	42.1	45.8	44.1	55.4
IVFDM	79.2	53.8	46	55.6	42.8
Gas residue at 72 hours	207.2	169.4	117.3	164.0	100.7

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SCFA					
Acetic	2.48	2.01	1.64	1.8	1.27
Propionic	1.13	0.97	0.78	0.96	0.56
Iso-butiric	0.05	0.05	0.03	0.04	0.02
Butyric	0.53	0.38	0.21	0.39	0.15
Iso-valeric	0.05	0.04	0.02	0.04	0.01
Valeric	0.05	0.02	0.02	0.01	0.00
Total SCFA	4.29	3.47	2.71	3.24	2.03

[a]DDGS = corn dried distillers grains with solubles were a byproduct of corn ethanol production (POET, Sioux Falls, South Dakota, USA). Mineral premix = Ca, Zinc methionine hydroxy analogue chelate, manganese methionine hydroxy analogue chelate, copper methionine hydroxy analogue chelate, $CoCO_3$, vitamins B_7, a supplement, and roughage products (Nutra Blend, LLC, Neosho, Missouri, USA). CP = crude protein. aNDF = a-amylase used in neutral detergent fiber (NDF) procedure no Na sulfite. ADF = acid detergent fiber. IVDMD = *in vitro* dry matter (DM) disappearance from gastric and small intestinal hydrolysis; IVDGE = *in vitro* DM digestibility of gross energy from gastric and small intestinal hydrolysis; IVFDM = *in vitro* DM disappearance from large intestine fermentation. Gas residue at 72 hours = ml/g DM substrate. SCFA = short chain fatty acids produced during fermentation, mmol/g DM.

roughage × period ($P < 0.008$) and a period ($P < 0.001$) effect. Within supplement diets, all pigs gained weight during each period but on average, pigs fed JUN40 gained less weight during each period than all other supplement diets except for pigs consuming CSH20 on day 35. On average, all animals consuming supplement diets gained more weight during period 1 than during period 3, though total amount of treatment and BASAL diet offered was equal. This could have indicated an aversion to the supplement diets after restriction of the BASAL diet during period 2.

Condensed tannins have a characteristic bitter taste that can result in reduced dry matter intake, and responses can range from seconds to days, depending on the amount ingested, concentration, and toxicity (Barboza et al. 2009). Growing pigs fed acacia (*Acacia tortilis*) leaf meal-based diets at inclusion levels of 0–150 g/kg DM showed increased average daily feed intake and ADG before decreases were observed (Ndou et al. 2015). At low levels of inclusion, tannins and fiber may have bound with amino acids and energy, and pigs may have consumed more feed to meet amino acid and energy requirements (Ndou et al. 2015). Pig gain to feed decreased linearly with increasing levels of inclusion, and feed intake and ADG was constrained if levels of inclusion exceed 66.9 and 64.8 g/kg DM, respectively.

There tended to be an interaction for percentage roughage × period ($P = 0.08$), and roughage × period ($P = 0.06$) for ADG (Table 4). A period effect was also observed for ADG ($P = 0.004$). At the end of period 2 (day 28), there was an increase in ADG for CSH20 and a small decrease in ADG for BASAL. Larger decreases in ADG were recorded for CSH40, JUN20, and JUN40, which were expected due to BASAL diets being limited to 2% of BW. Average daily gain increased for these groups during period 3 (day 35) when the BASAL diet returned to 5% of BW.

An interaction between percentage of roughage × period ($P = 0.01$) and roughage × period ($P = 0.005$) was observed for gain to feed. A period effect ($P < 0.001$) was also overserved. As also observed during period 2 for ADG, there was a decrease in gain to feed for BASAL, CSH40, JUN20, and JUN40 but an increase in gain to feed for CSH20. Pig gain to feed also increased during period 3 (day 35) as the BASAL diet was returned to 5% of BW. Although exact ages could not be determined, 2 pigs fed BASAL and 1 pig fed CSH20 appeared to be mature animals and remaining juveniles. The BASAL group ($n = 4$ animals) included 2 mature animals, which may have been reflected in overall (day 0 to 35) less ADG and gain to feed. Further research is needed that evaluates pig growth and health during extended feeding periods and using greater concentrations of ground juniper.

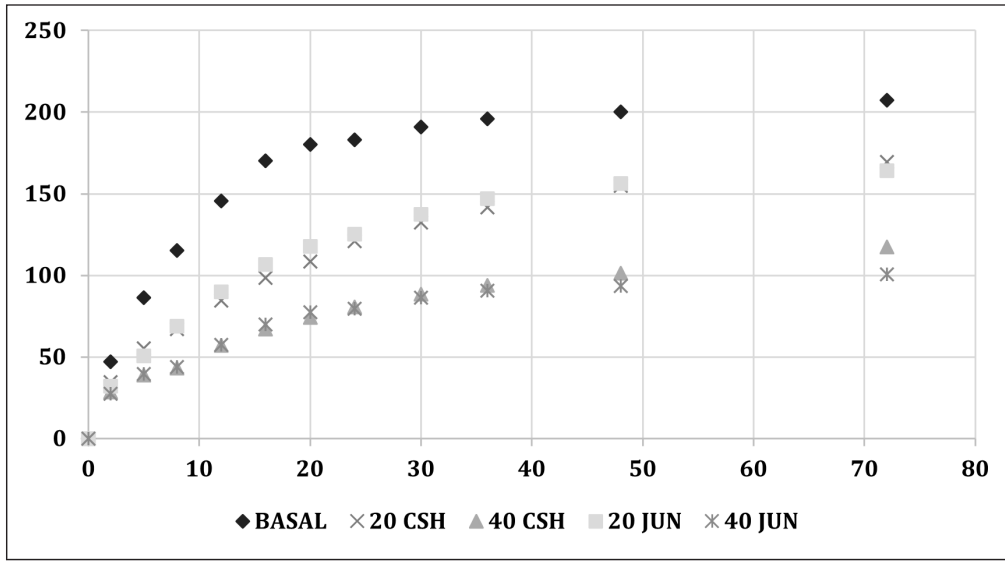

Figure 3. Total gas accumulation, ml/g dry matter residue at 72 hours, by in vitro degradation of supplemental diets fed to wild pigs (*Sus scrofa*), AgriLife Research Center, San Angelo, Texas, USA, May to June 2015. Supplement diets were pelleted and ingredient composition only differed by roughage source; either cottonseed (*Gossypium* spp.) hulls (CSH) or ground blueberry juniper (*Juniperus ashei*; JUN). The juniper (entire above-ground biomass) was chipped, dried, and hammermilled to pass a 4.76-mm sieve. BASAL = Ring Leader Pig Grower 18%, manufactured by Angelo Pellets, Inc., San Angelo, Texas.

In vitro dry matter digestibility and gas production

The mean value for *in vitro* dry matter disappearance and *in vitro* digestibility of gross energy of ground blueberry juniper suggest that it was nearly completely indigestible (Table 4). Similarly, Jha et al. (2011) reported that wood cellulose (Sulka-floc®) was nearly completely indigestible by pigs. Dependent on type and concentration, high fiber diets of low nutritive value have been reported to reduce pig performance (Agyekum and Nyachoti 2017). Pigs lack digestive enzymes that degrade non-starch polysaccharides (Bedford and Schulze 1998). Pre-cecal and total tract nutrient digestibility can be negatively impacted by diets high in fiber. Several mechanisms have been reported on nutrient reduction based on insoluble fiber (non-starch polysaccharides); reduced digesta passage rates; encapsulation of nutrients, which reduces accessibility to digestive enzymes for hydrolysis; increased endogenous intestinal nutrient losses; and high viscosity of the water layer next to the intestinal mucosa (Eastwood and Morris 1992, Wenk 2001, Wilfart et al. 2007, Agyekum and Nyachoti 2017). Although some non-starch polysaccharides are degraded in the hind gut by microbial fermentation, diets high in insoluble fiber may depress fermentation (Noblet and Le Goff 2001, Zijlstra et al. 2012).

In our study, short-chain fatty acid (SCFA), including acetic, propionic, isobutiric, butyric, iso-valeric, and valeric, production was greatest for BASAL. Within supplement diets, diets containing 20% roughage produced more SCFA's than those containing 40% roughage. Also, diets that contained CSH produced more SCFA's than those containing JUN. These results suggest that high-fiber juniper may limit fermentation in the hindgut of the pig. Inclusion of DDGS at 30% in growing pig diets can reduce total tract and hindgut disappearance of dietary fiber and NDF (Urriola and Stein 2010). In a study evaluating true *in vitro* digestibility of dry matter (*tIVDMD)* of mature juniper by sheep, *tIVDMD* was approximately 33% greater than the IVDMD in the current study for pigs (Stewart et al. 2015). In the current trial, digestibility of DM and gross energy were both greater for BASAL compared to all supplement diets. The greatest differences were observed between BASAL and supplement diets containing CSH and the greatest difference in fermentation of dry matter was between BASAL and JUN40. Total gas production at 72 hours (Figure 3) may be related to concentrations of NDF and

Table 5. Effects of roughage ingredient and percentage of roughage on wild pig (*Sus scrofa*) blood serum profile[a], AgriLife Research Center, San Angelo, Texas, USA, May to June 2015.

	Diet[b]								
		CSH		JUN			P-value[c]		
Item[d]	BASAL	20	40	20	40	SEM[e]	R	PR	R × PR
Glucose, mg/dl	136.5	146.8	142.0	120.4	132.6	11.4	0.14	0.75	0.47
SUN, mg/dl	15.0	13.5	19.2	18.3	16.4	2.7	0.71	0.48	0.18
Creatinine, mg/dl	1.1	0.9	0.9	0.8	0.8	0.1	0.16	0.91	0.80
Bilirubin, mg/dl	0.10	0.15	0.12	0.13	0.15	0.02	0.89	0.67	0.31
Albumin, g/dl	4.3	4.1	4.1	4.3	4.4	0.1	0.17	0.73	0.62
Globulin, g/dl	2.9	2.8	2.5	2.5	2.6	0.3	0.71	0.87	0.46
A:G	1.5	1.6	1.7	1.8	1.7	0.2	0.54	0.99	0.59
TP g/dl	7.2	6.9	6.7	6.7	7.0	0.2	0.75	0.92	0.18
ALT, U/L	36.8	48.8	46.8	40.0	39.0	4.3	0.07	0.73	0.92
AST, U/L	213.9	170.1	117.9	177.1	88.7	45.1	0.64	0.29	0.71
ALP, U/L	142.5	133.5	188.2	178.8	140.9	31.0	0.97	0.73	0.12
CPK, U/L	8,922	8,450	7,590	7,528	5,346	2,110	0.46	0.48	0.76
Ca, mg/dl	12.0	11.4	12.1	13.1	12.6	0.7	0.12	0.92	0.39
P, mg/dl	7.6	8.3	8.1	8.3	8.7	0.7	0.60	0.83	0.60
Cl, mEq/L	106.2	105.3	102.8	102.6	102.2	1.5	0.28	0.30	0.48
Na, mEq/L	147.1	147.1	146.0	144.2	146.9	2.0	0.58	0.61	0.30
K, mEq/L	8.7	8.6	7.0	7.5	7.5	0.6	0.51	0.16	0.17
Na:K ratio	17.2	17.1	21.2	20.3	20.0	1.5	0.50	0.21	0.19

[a]During period 1 (day 0 to 19), pigs were fed 5% of BW treatment diet and 5% of BW BASAL diet. Period 2 (day 18 to 26), pigs were fed 5% of BW treatment diet and 2% of BW BASAL diet. Period 3 (day 27 to 34), pigs were fed 5% of BW treatment diet and 5% of BW BASAL diet.
[b]Treatment diets were pelleted and ingredient composition only differed by roughage source, either cottonseed (*Gossypium* spp.) hulls (CSH) or ground blueberry juniper (*Juniperus ashei*; JUN). The juniper (entire above-ground biomass) was chipped, dried, and hammermilled to pass a 4.76-mm sieve. BASAL = Ring Leader Pig Grower 18%, manufactured by Angelo Pellets, Inc., San Angelo, Texas.
[c]R = roughage source; PR = percentage of roughage.
[d]SUN = Serum urea nitrogen; A:G = albumin:globulin ratio; TP = Total protein; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; ALP = alkaline phosphatase; CPK = Creatine phophokinase.
[e]SEM = greatest standard error of the mean.

ADF. Within mixed diets, NDF and ADF concentrations were greatest in CSH40 and JUN40. Reflective of the fermentation characteristics, total SCFA was approximately 1 mmol/g DM residue to 4 mmol/g DM residue greater for BASAL than supplement diets.

Blood serum profiles

We detected a roughage effect (P = 0.07) on serum alanine aminotransferase (Table 5). Pigs consuming BASAL had the lowest level of serum alanine aminotransferase. Elevated serum alanine aminotransferase, ASP, and alkaline phosphatase are descriptors of liver damage. Research on monogastric species has shown elevated levels of alanine aminotransferase in diets containing tannins and cottonseed. For example, quadratic increase in alanine aminotransferase was observed in domestic growing pigs fed *A. tortilis,* high in condensed tannins, as inclusion increased (Ndou et al. 2015). Average alanine aminotransferase concentra-

tions in wild boars were greater than values for domestic pigs (Harapin et al. 2003). The alanine aminotransferase concentrations in the present trial are also greater than reference ranges for domestic pigs but fall within values reported for wild pigs from 3 Texas locations (Shender et al. 2002). All other serum biochemical ranges also fall within ranges as reported by Shender et al. (2002) and reference intervals established by Casas-Díaz et al. (2015).

Management implications

Pigs continue to be problematic for the agricultural industry and in the management of native wildlife and livestock species. Our findings suggest that there does not appear to be a benefit of using ground juniper as a roughage source to reduce consumption of supplemental feed for deer by pigs and therefore, other strategies may be needed.

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Appendix A. Observations of organ condition recorded during post-mortem necropsy of wild pigs (*Sus scrofa*) fed supplemental diets, AgriLife Research Center, San Angelo, Texas, USA, May to June 2015.

	Diet[a]				
Organ	BASAL	CSH40	CSH20	JUN20	JUN40
Liver	Normal	Normal	-	Normal	Right hemorrhage
					Fluid filled cyst
Lung	Hemorrhagic due to bolt	Normal	-	Normal	Normal
Spleen	Normal	Normal	-	Lesion	Scarring
Kidney	Normal	Normal	-	Normal	Hemorrhagic
Heart	Normal	Normal	-	Normal	Normal
Stomach		Normal	-	Hyperkeratosis	Lesion hyperkeratosis
					Submucosal hemorrhage
					Ulcer

[a]During period 1 (day 0 to 19), pigs were fed 5% of BW supplement diet and 5% of BW BASAL diet. Period 2 (day 18 to 26), pigs were fed 5% of BW supplement diet and 2% of BW BASAL diet. Period 3 (day 27 to 34), pigs were fed 5% of BW supplement diet and 5% of BW BASAL diet. Supplement diets were pelleted and ingredient composition only differed by roughage source, either cottonseed (*Gossypium* spp.) hulls (CSH) or ground blueberry juniper (*Juniperus ashei*; JUN). The juniper (entire above-ground biomass) was chipped, dried, and hammermilled to pass a 4.76-mm sieve.

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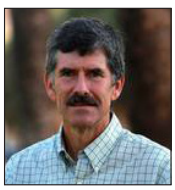

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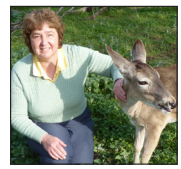

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