9-11-2014

Request for a New AAFCO Ingredient Definition: for ground *Juniperus pinchotii* and *Juniperus ashei*

DEFINITION: Ground Juniper and/or Parts

Prepared for:
AAFCO, Attn: Ms. Erin Bubb
Miscellaneous Products Investigator
Division of Agronomic & Regional Services
Pennsylvania Depart. of Agric., Bureau of Plant Ind.
2301 N. Cameron Street, Harrisburg, PA 17110-9408

Dear Ms. Bubb:

On behalf of Texas A&M AgriLife Research, Dr. Travis Whitney (Texas A&M AgriLife Research, San Angelo) is requesting that ground whole juniper trees (*Juniperus pinchotii* Sudw. and *Juniperus ashei* J. Buchholz) and/or parts, collectively referred to as "ground juniper", be reviewed, approved as a feed ingredient in ruminant animal diets, and published as an official AAFCO ingredient definition. Based upon available published literature and unpublished results from the AgriLife Research Nutrition Program (San Angelo), it has been concluded at this time, that ground juniper (similar characteristics as "Ground Whole Aspen and/or Parts," which received AAFCO approval in 1980; AAFCO, 2011) is safe for use as a feed ingredient in ruminant animal diets, according to good feeding practices and the intended use as cited in this proposal.

Contact information of requester:
Texas A&M AgriLife Research
Attn: Dr. Travis Whitney, Associate Professor
7887 US Hwy 87N
San Angelo, TX 76901
trwhitney@ag.tamu.edu ph: 325-653-4576 ext. 235

Sincerely,

7-1-2014

Travis Whitney — Date
Associate Professor

cc:
John Walker: Professor and Director, TX A&M AgriLife Research and Extension Center, San Angelo

Abbreviations

ADF	acid detergent fiber
ADG	average daily gain
ADIN	acid detergent insoluble nitrogen
AAFCO	Association of American Feed Control Officials
ALD	approximate lethal dose
AST	aspartate transaminase
BUN	blood urea nitrogen
BW	body weight
CLA	conjugated linoleic acid
CP	crude protein
CSH	cottonseed hulls
CSM	cottonseed meal
CT	condensed tannins
d	day
DDGS	dried distillers grains with solubles
DE	digestible energy
DM	dry matter
DMD	dry matter digestibility
DMI	dry matter intake
FDA	Food and Drug Administration
FEC	fecal egg count
G:F	gain-to-feed ratio
GGT	gamma glutamyltransferase
GI	gastrointestinal
h	hour
HCWT	hot carcass weight
HT	hydrolysable tannin
IGF	insulin-like growth hormone
IVM	ivermectin
mo	month
NDF	neutral detergent fiber
NDIN	neutral detergent insoluble nitrogen
NPN	non-protein nitrogen
NRC	National Research Council
OM	organic matter
RDP	rumen degradable protein
RUP	rumen undegradable protein
SBM	soybean meal
SEM	greatest standard error of the mean
SFA	saturated fatty acid
spp.	species
SUN	serum urea nitrogen
tIVDMD	true *in vitro* dry matter disappearance
TMR	total mixed ration
VFA	volatile fatty acids
VO	volatile oil
vs.	versus
wk	week
wt	weight
wt/vol	weight/volume
wt/wt	weight/weight
yr	year

DEFINITION: Ground Juniper and/or Parts

TABLE OF CONTENTS

Figures

Tables

Appendices

1. Summary of Request

This proposal was constructed by Dr. Travis Whitney (Ph.D., Associate Professor, Texas A&M AgriLife Research, San Angelo) who has extensively researched (literature reviews and AgriLife research trials) the chemical characteristics, feeding value, and toxicology of juniper and other feed ingredients with similar physical and chemical characteristics. Dr. John Walker (Ph.D., Professor and Resident Director, Texas A&M AgriLife Research, San Angelo) provides oversight to Dr. Whitney's research program and has reviewed the final draft of this proposal.

Proposal organization:

A detailed description of the proposed ingredient is given in **Section 3** (**Fig. 1 and 2; Tables 1 and 2**) and the manufacturing process is described in **Section 4** (**Fig. 3**). In summary, *Juniperus pinchotii* Sudw. and *J. ashei* J. Buchholz are evergreen coniferous plants that are feed ingredients in ruminant animal diets produced by harvesting and processing the entire above-ground biomass or its parts. Ground juniper is intended to be used as a dietary roughage component in ruminant animal diets in any stage of animal production; roughage is needed by ruminant animals to maintain function and efficiency of the reticulo-rumen. As with many other approved feed ingredients (e.g., cottonseed hulls (**CSH**), peanut hulls, corn stalks, and various hay varieties), ground juniper is not intended to be used as the sole diet of the animal, but mixed with one or more feed ingredients and fed either loose (non-agglomerated) or in pelleted form.

A summary of relevant literature related to the fiber characteristics and feeding value is presented in **Section 8**. **Section 8** not only discusses the use of the proposed ingredient in mixed diets, but the use of non-woody and other woody feed ingredients. These other ingredients are discussed due to similarities in physical and chemical characteristics to *J. pinchotii* and *J. ashei*; this is especially true for other *Juniperus* species and ground aspen. Furthermore, "Ground Whole Aspen and/or Parts" received AAFCO approval in 1980 (AAFCO, 2011). Thus, discussing trials that used aspen in ruminant diets is important because it sets a precedent for the use of juniper.

A safety assessment (non-ruminant, ruminant, and the animal products consumed by humans) is presented in **Section 9**. Like thousands of plant species, juniper is known to contain CT and volatile oil (**Section 9.1**). **Section 9.2** briefly addresses effects of CT and volatile oil in non-ruminant animals, even though non-ruminant toxicity of CT and volatile oil is not relevant to ruminant animals due to numerous factors (e.g., ruminal digestion, regurgitation, re-mastication) that are discussed in **Section 9.3**; this is especially true for studies that inject a secondary compound directly into the bloodstream of the animal. Trials that evaluated consumption of juniper by ruminant animals when juniper was or was not mixed with other feed ingredients are discussed in **Section 9.3**. Trials that dosed ruminant animals with juniper or a secondary compound contained within juniper are not directly applicable to the proposed use of *J. pinchotii* and *J. ashei*, but are presented because some of this literature incorrectly attributes toxicity to juniper secondary compounds (CT or volatile oil). Trials involving chemically pre-treated plant material are not applicable to the proposed ingredient; thus, within individual research trials, only diets that contain non-chemically pre-treated plant material are discussed.

An expert opinion letter and Dr. Whitney's vitae are attached as **Appendices A and B**, respectively. **Appendices C to G** contain additional summary tables and detailed reviews of various research trials; some of which are discussed within the main proposal. Copies of all cited literature (except complete books that were referenced as further sources of information) are located on the enclosed CD; other relevant information or specific literature can be made available upon request.

***In Vitro* Nutrient/Fiber Characteristics** (specific data and references are discussed in **Section 8**).

Ground juniper is a fibrous feed ingredient (generally defined by neutral detergent fiber [**NDF**], acid detergent fiber [**ADF**], and lignin). However, aspen (e.g., **Fig. 5**) and numerous approved non-woody feed ingredients (e.g., CSH, peanut hulls, corn stalks, and various hay varieties; e.g., **Fig. 4**) contain similar, to greater concentrations of fiber than ground juniper and are safe for ruminant animals. In addition, even though the proposed ingredient is not a protein source, CP in mature juniper trees have been reported to be similar (3.4 to 3.6% CP; Stewart et al., 2014) to other roughage ingredients such as CSH, peanut hulls, and corn stover containing (2.6 to 6.7% CP). Furthermore, immature juniper trees (< 3 m in height) have less fiber and greater protein (Stewart et al., 2014) than mature juniper trees.

Ground wood from various *Populus* tree species (e.g. quaking aspen) was thoroughly evaluated during the 1970's by researchers from various institutions, e.g. Penn State, University of WI (Dairy Sci. Dept.; Madison, WI), Forest Products Lab (Madison, WI), SD State University (Brookings), and the University of Alberta. These research efforts led to ground aspen being officially approved by Association of American Feed Control Officials (**AAFCO**, 2011); "**Ground Whole Aspen and/or Parts**" received approval in 1980 and is currently the only woody plant that has an AAFCO definition. Thus, even though nutrient characteristics of ground juniper is similar to many non-woody feed ingredients, ground aspen is discussed in detail within this proposal because as previously stated, it sets a precedent for the approval of "Ground Juniper and/or Parts."

When comparing the nutrient and fiber characteristics of mature ground aspen trees to mature ground juniper trees, most reports suggest that juniper is a more "nutritious" feed ingredient. As highlighted within this proposal, mature ground aspen trees have been reported to contain greater concentrations of NDF and ADF, similar lignin, and less CP than ground mature juniper trees (**Fig. 5 and Table 3**).

> **Literature summarized within this proposal supports the conclusion that the <u>nutrient and fiber characteristics</u> of ground juniper do not pose any extraordinary animal health issues, especially when compared to numerous other roughage feed ingredients that are currently approved for ruminant animals.**

Feeding Value (specific data and references are discussed in **Section 8**).

Even though *in vitro* nutrient characteristics of a feed ingredient is important, an evaluation of the feeding value is even more important because it encompasses the effects of using the ingredient on dry matter intake (**DMI)**, digestibility, and growth performance of the animal. Data presented and summarized in this proposal supports the fact that the feeding value of ground juniper is similar to greater than non-woody ingredients such as CSH and various hay varieties. For example, juniper material safely replaced oat hay in lamb feedlot diets (Whitney et al., 2014) and was safely used at up to 54% (DM basis) of a supplement fed to pregnant ewes (Stewart et al., unpublished data). Furthermore, juniper leaves are as digestible as alfalfa hay (67%; Whitney and Muir, 2010). Immature and mature juniper trees have been reported to be up to 50% and 30% digested, respectively (*in vitro*; Stewart et al., 2014); in comparison (using the same *in vitro* procedures), CSH and oat hay have been reported to be 21% (Whitney and Muir, 2010) and 57% (Whitney et al., 2014) digested, respectively.

The safety assessment is further reinforced by numerous research trials dating back to the early 1900's, all of which reported that processed woody plant products (whole trees, limbs/leaves, sawdust) can safely be used in diets fed to ruminant animals; many of these trials are discussed in detail within this proposal. For example, steers fed a diet consisting of 45% ground aspen "gained weight about twice as fast as the alfalfa-fed group" (Unknown, 1975). Satter et al. (1970) concluded that aspen sawdust (at 32% of the diet) was effective as a partial roughage substitute in mixed diets fed to lactating cows; this research was reinforced by trials conducted by Schingoethe et al. (1981). Satter et al. (1973) reported that 30% aspen sawdust did not negatively affect DMI or milk parameters in dairy cattle and they concluded that it was "as effective as 50% long hay to maintain normal luminal [ruminal] acetate-to-propionate ratios." Furthermore, ground aspen bark is currently being safely fed to cattle and sold commercially by 3XM Grinding and Composting (Olathe, CO; www.3xmgrinding.com/aboutus; Lohmeyer, 2013). Ground aspen wood is also currently being sold in mixed feeds (Land O'Lakes Purina Feed®, e.g., Mazuri Browser Breeder; www.mazuri.com/mazuribrowserbreeder-5653.aspx).

Other ground woody plants (e.g., mesquite, oak, pine) are not currently approved by AAFCO or FDA, but are discussed within the proposal because they have been safely used in ruminant animal diets (e.g., Marion et al., 1957; Marion et al., 1959; Ellis, 1969; Cody et al., 1972; Dinius and Williams, 1975; Parker, 1982). For example, Marion et al. (1957, 1959) safely fed steers a growing diet that contained up to 52% ground mesquite wood and Ellis (1969) fed up to 88% ground mesquite in cow diets. Also, oak sawdust is "essentially non-digestible (Dinius and Baumgardt, 1970) and the DM digestibility of ground aspen has been reported to be between 0% to 41%; the lowest DM digestibility for mature ground juniper trees has been 30% (Stewart et al., 2014). Thus, in many of these studies, the feeding value of ground juniper would have potentially been even greater than ground aspen and other woody plants.

Literature summarized within this proposal, supports the conclusion that the <u>feeding value</u> of ground juniper is similar, to greater than numerous other roughage ingredients (non-woody and woody) currently approved for ruminant animals.

Safety Assessment: (specific data and references are discussed in **Section 9**).

Like thousands of plant species, juniper is known to contain condensed tannins [**CT**] and volatile oil (plant secondary compounds; **Section 9.1**). Depending on numerous factors (e.g., chemical structure, concentrations, total intake), CT and volatile oil can have beneficial or adverse effects on the animal (reviewed by Piluzza et al., 2013 and others that are discussed in this proposal). Furthermore, concentrations of CT and volatile oil in juniper material are similar to (at times much less) concentrations in approved feed ingredients, e.g., various algae products, sorghum grain, CSH, lespedeza hay, ground aspen wood, and pose no extraordinary risks to animal health. For example, Chafton (2006) fed lambs Sericea lespedeza hay (10.7% CT, DM basis) resulting in 128 g of CT intake/day (3.74 g CT intake/kg of BW, DM basis) with no reported health problems (visual assessment or negative ADG). Average daily DMI and ADG increased when kid goats were fed a diet containing 75% Sericea lespedeza (6.5% CT, DM basis) vs. 75% bermudagrass hay, resulting in 62 g CT intake/day (3.28 g CT intake/kg of BW; Moore et al., 2008). Most literature reports that juniper contains 5 to 8% CT (DM basis). If juniper contains 5% CT, then a 40-kg goat would have to consume approximately 2,992 g of juniper/d to consume 3.74 g CT/kg of BW [see above for Serecia lespedeza hay intake]), which equals juniper being consumed at 7.46% of BW, which is impossible.

Section 9.3 discusses the safety assessment (for ruminant animals) of the secondary compounds within ground juniper. This section also addresses literature that contradicts the opinion that volatile oil contained within juniper are "toxic" to ruminant animals. Studying this conflicting literature in detail, along with a thorough review of other related literature, has revealed that there is not a single documented case, in a properly designed trial, in which consumption of *J. pinchotii* or *J. ashei* material negatively affected ruminant animal health. This conclusion is supported by published trials and currently unpublished data from Dr. Whitney's Research Program.

It should also be noted that the following are generally recognized as safe (GRAS) by FDA and/or listed as an official AAFCO feed ingredient definition. They are either contained within the proposed feed ingredient or contain one or more of the same chemical constituents as the proposed feed ingredient (FDA: 21CFR582 Subchapter E):

a. Section §582.10 **Spices and other natural seasonings and flavorings**, e.g., basil, clover, ginger, oregano, sage contain volatile oil [p. 465 in AAFCO, 2011].
b. Section §582.2 **Essential oils, oleoresins (solvent-free), and natural extractives (including distillates)**, e.g., cascarilla bark, cassia bark, hickory bark, citrus and orange peels, basil, hickory bark, juniper berries, sage, tea, coffee [p. 466 in AAFCO, 2011].
c. Section §582.30 **Natural substances used in conjunction with spices and other natural seasonings and flavorings**, e.g., brown and red algae [p. 468 in AAFCO, 2011].
d. Section §582.40 **Natural extractives used in conjunction with spices, seasonings, and flavorings**, e.g., brown and red algae [p. 468 in AAFCO, 2011];
e. Section §582.60 **Synthetic flavoring substances and adjuvants**, e.g., limonene [p. 468 in AAFCO, 2011].
f. Section §582.6033 **Subpart G – Sequestrants**, e.g., citric acid [p. 465 in AAFCO, 2011].

It is concluded at this time, that consumption of ground juniper (thus, CT and volatile oil contained within juniper) does not pose any extraordinary risks to animal health. This conclusion is based upon the following:

1. A thorough review of the literature. For example, Estell et al. (1998) stated, "Monoterpenes are typically toxic to insects but safe for consumption by mammals (Rice and Coats, 1994). Because many terpenes are classified as "Generally Recognized as Safe" and are natural plant products that are abundant and easily synthesized (Rice and Coats, 1994), they are potential candidates for use in manipulating feeding patterns of browsing herbivores.";
2. Personal experiences with feeding diets containing ground juniper to ruminant animals. No negative animal health issues (visual appraisal) related to feeding ground juniper material in mixed diets have been observed by Dr. Whitney in any Texas A&M AgriLife research trials. Average daily DMI and ADG has at times, been less when animals consumed diets with juniper vs. without juniper. However, (1) reduced animal growth performance has been mainly attributed to fiber characteristics of ground juniper and not a result of post-ingestive adverse health effects due to CT or volatile oil and (2) ADG has never been negative (animals have always remained in a positive energy balance);
3. Numerous approved feed ingredients contain equal to greater concentrations of CT (e.g., "60.44 Ground Whole Aspen and/or Parts") or volatile oil than what has been reported in the proposed ingredient and have similar physical and feeding characteristics (particularly: "60.44 Ground Whole Aspen and/or Parts," CSH, and various varieties of straw);
4. The ALD of various terpenes for non-ruminants are reported to be much greater than what an animal would actually consume on a g/kg of BW basis; this is especially true when comparing ALD in non-ruminants with ruminants, because of rumen microbial terpene digestibility and terpenes being further volatilized as ruminants regurgitate, remasticate, and re-swallow feed;

5. Condensed tannins are poorly (or not at all) absorbed in chickens (Jimenez-Ramsey et al., 1994) and sheep (Terrill et al., 1994) and thus, the biological activity of CT is reduced or eliminated;
6. The proposed ingredient could actually be considered safer than many approved feed ingredients because it is does contain common secondary compounds that pose significant animal health issues such as gossypol (e.g. cottonseed products), coumarin (e.g. sweat clover), saponins (e.g. alfalfa), and nitrates (e.g. oat hay, alfalfa, sudangrass, sweetclover).

Safety Assessment: Humans (specific data and references are discussed in **Section 9.4**).

A thorough review of the literature revealed that maximum probable consumption of meat or milk products from animals that have consumed diets containing ground juniper (details presented in **Sect. 9.4**) does not pose any known or extraordinary risk to the human consumer of those products. This conclusion is based upon the following:

1. Condensed tannins are poorly (or not at all) absorbed in chickens (Jimenez-Ramsey et al., 1994) or sheep (Terrill et al., 1994) and as discussed in a review by Manach et al. (2005), polymeric CT are not absorbed through the human GI tract; thus it would be expected that accumulation in the muscle be "minuscule.";
2. Volatile oil consumed by the ruminant animal is digested to various extents, eructated, bio-transformed, and excreted before they can be deposited into edible animal products (e.g., Welch and Pederson, 1981; Cluff et al., 1982; White et al., 1982; Chizzola et al., 2004; Broudiscou, et al., 2007; Malecky et al., 2009; Malecky and Broudiscou, 2009). Serrano et al. (2007) stated, "The daily [volatile oil] dose administered … did not result in a drastic enrichment [of terpenes] in their [calves] tissues…";
3. There is no evidence that ground juniper material (or its secondary compounds) is carcinogenic or mutagenic in humans. The International Agency for Research on Cancer lists CT as a Group 3 carcinogen, which is "not classifiable as to its carcinogenicity to humans" and "evidence for carcinogenicity is inadequate in humans and inadequate or limited in experimental animals." Further, Manach et al. (2005) published a thorough review on the bioavailability of CT, which supports the fact that consumption of products from animals that have consumed diets with CT poses no known health risk to humans;
4. Humans commonly consume products that are derived from animals that have consumed growing forages and various approved feed ingredients that contain equal to greater concentrations of CT and volatile oil than what has been reported in ground juniper;
5. Human's commonly consume volatile oil constituents (e.g., terpenes) and CT on a daily basis in a wide variety of non-animal food products that are considered safe by FDA, e.g. orange, lemon, mandarin, lime, grapefruit, and wine (Bernhard and Marr, 1960; Williams and Elliot, 1997; Bagchi et al., 2000; Mateo and Jiménez, 2000; Sanchez-Moreno et al., 2003; USDA, 2004);
6. The following food additives are permitted by the FDA for "direct addition to food for human consumption" and are either contained within the proposed feed ingredient or contain 1 or more of the same chemical constituents as the proposed feed ingredient (in **21CFR172**):
    a. Section §172.210 **Coatings on fresh citrus fruit**, e.g., wood rosin (CT and volatile oil).
    b. Section §172.510 **Natural flavoring substances and natural substances using in conjunction with flavors**, e.g., althea root (CT), blackberry bark (CT), white cedar (volatile oil), balsam fir (CT and volatile oil), camphor tree, white oak chips (CT and volatile oil).

    c. Section §172.515 **Synthetic flavoring substances and adjuvants**, e.g., borneol, bornyl acetate, camphene, camphor, cedarwood oil terpenes, citronellol, alpha pinene, alpha terpinene, terpinolene.

7. The following are "substances generally recognized as safe" (GRAS) for human consumption and are either contained within the proposed feed ingredient or contain one or more of the same chemical constituents as the proposed feed ingredient (in **21CFR182**):
    a. Section §182.10 **Spices and other natural seasonings and flavorings,** e.g., basil, clover, ginger, oregano, sage.
    b. Section §182.2 **Essential oils, oleoresins (solvent-free), and natural extractives (including distillates)**, e.g., cascarilla bark, cassia bark, hickory bark, citrus orange products such as peels, basil, hickory bark, juniper berries, sage, tea, coffee.
    c. Section §182.60 **Synthetic flavoring substances and adjuvants**, e.g., limonene.
    d. Section §182.1033 **Subpart B – General purpose food additive**, e.g., citric acid.
8. Even though this proposal is not claiming that ground juniper can be used in ruminant diets to increase human health, it should also be noted that numerous studies and reviews have reported that volatile oil (e.g., terpenes) and CT are either beneficial or have potential to be beneficial to human health (e.g., Dillard and German, 2000; Parr and Bowell, 2000; Mittal, et al., 2003; Crozier et al., 2006; Humphrey and Beale, 2006; Lans et al., 2007; Bhalla et al., 2013). Volatile oils have also been reported to enhance shelf life of meat products by reducing aerobic bacteria counts (Viuda-Martos et al., 2010; Hyldgaard et al., 2012).

***Proposal Conclusion*:**

It should be noted that FDA defines "safe" as (Title 21CFR170.3i):

> **"Safe or safety means that there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance. Safety may be determined by scientific procedures or by general recognition of safety."**

After a comprehensive review, there is reasonable certainty that ground juniper (*Juniperus pinchotii* and *J. ashei*) is "safe" for ruminant animals and safe for humans who consume the animal products. However, as with any feed ingredient (especially high-fiber roughages), care should be taken to ensure that total daily nutrient intake is, at the least, meeting basic maintenance requirements of the animal.

The expectation is that AAFCO and FDA will concur that the information presented within this proposal fully supports the claim that ground juniper (*Juniperus pinchotii* and *J. ashei*) is safe for use as a feed ingredient in ruminant animal diets; thus, published as an official AAFCO feed ingredient definition. If a final conclusion is reached by AAFCO or FDA in which ground juniper is not recognized as being safe for ruminant animals, either due to fiber characteristics or secondary compounds (volatile oil or CT), then thousands of other feed ingredients (from rangeland plants to harvested forages and feeds) with similar chemical and physical characteristics would have to be *needlessly* re-evaluated, re-classified, and re-submitted through the FDA Food Additive Petition process.

2. Proposed Definition

Ground Juniper and/or Parts is to be used as a dietary roughage component for ruminant animals and should not be used as the sole diet of the animal. It consists of the aerial portion of juniper (*Juniperus pinchotii* and *Juniperus ashei*) plants. This ingredient consists of the entire plant or any combination of its parts (leaves, berries, branches, bark, and trunk). Any part below ground level is excluded to avoid dirt and rock contamination. The ingredient should be ground through a screen with holes not greater than 5/8" (1.59 cm) in diameter. If ash > 12%, the words "sand and/or dirt" must appear in the product name. If the ground juniper is mechanically dried, it should be labeled as "Dehydrated Ground Redberry/Blueberry Juniper.

Suggested IFN categories:

a. Ground redberry/blueberry juniper
b. Ground redberry/blueberry juniper, dehydrated

Please note:
A maximum inclusion level is not proposed for ground redberry/blueberry juniper because:

(1) Maximum inclusion level for any ingredient is based upon dietary requirements of the animal, known chemical composition of that ingredient, and either the known or calculated chemical composition of the daily diet;
(2) Many roughage ingredients (e.g., aspen wood, corn stalks, various varieties of low-quality hay and seed hulls) currently being fed to ruminant animals can also reduce average daily DMI and growth performance, and even though they have no maximum inclusion level in their AFFCO or GRAS definition, they too are not recommended to be the sole diet of the animal;
(3) Many approved ingredients currently being fed to ruminant animals contain similar, to greater concentrations of volatile oil and/or CT as ground juniper, but do not have, nor warrant, a maximum inclusion level in their approved definition.

3. Description of Ingredient

Physical descriptions of *Juniperus pinchotii* Sudw. (common names: redberry and Pinchot juniper) and *J. ashei* Buchholz. (common names: ashe, cedar, mountain cedar, post cedar, Ozark white cedar, Mexican juniper) are depicted in **Figures 1 and 2**, respectively. Within this proposal, *J. pinchotii* is referred to as "redberry juniper" and *J. ashei* is referred to as "blueberry juniper." Both species are from the Family *Cupressaceae* and Genus *Juniperus* and are dioecious evergreen plants. Kingdom (Plantae); Subkingdom (Tracheobionta; vascular plants); Superdivision (Spermatophyta; seed plants); Division (Confierophyta; conifers); Class (Pinopsida); Order (Pinales); Family (Cupressaceae; cypress family); Genus (*Juniperus*).

Redberry juniper has red to copper-brown colored berries and leaves that excrete a white exudate and generally have multiple basal stems at ground level. Blueberry juniper has blue berries and leaves that do not excrete the white exudate and generally have multiple stems arising from a single basal truck.

Figure 1. Visual identification of redberry juniper (http://essmextension.tamu.edu/plants/plant/redberry-juniper-pinchot-juniper)

Figure 2. Visual identification of blueberry juniper (http://essmextension.tamu.edu/plants/plant/ashe-juniper-blueberry-juniper)

The ground juniper ingredient is produced by processing the aerial portion of the tree. This material consists of the entire tree or any combination of its parts (leaves, branches, bark, and trunk). Juniper can be fed fresh (approximately 50 to 75% DM) or naturally or mechanically dried. If this ingredient is mechanically dried, then "dehydrated" should be included in the name. Processed material can be subjected to steam, pressure, or both, but at no time (before, during, or after the manufacturing process) is it chemically treated (e.g., sulfuric acid).

Chemical composition and DM digestibility of juniper trees (and its various parts) from published literature and unpublished data (from Dr. Whitney's TX A&M AgriLife Nutrition laboratory) are reported in **Tables 1 and 2**, and a summary of these tables is presented below for convenience. Neutral detergent fiber (**NDF**; mainly hemicellulose + cellulose + lignin), acid detergent fiber (**ADF**; mainly lignin + cellulose), and lignin are used to describe fiber content and assist in defining feeding value. To an extent, NDF is related to gut fill and ADF is related to digestibility. Hemicellulose and cellulose are digested to various extents by rumen microbes, but lignin is highly indigestible.

Entire juniper tree: harvested, processed, and allowed to air-dry for approximately 3 d (Stewart et al., 2014); reported as percentage of DM.

a. DM (freshly harvested trees), % = 65 to 69
b. CP, % = 3.4 to 4.7
c. NDIN, % = 1.3 to 1.6
d. NDF, % = 50 to 67
e. ADF, % = 41 to 56
f. Ash, % = 4.3 to 5.9
g. Lignin, % = 21 to 30
h. Ca, % = 1.25 to 1.95
i. P, % = 0.03 to 0.05
j. Mg, % = 0.04 to 0.12
k. K, % = 0.16 to 0.31
l. Na, % = < 0.01 to 0.02
m. S, % = 0.05 to 0.07
n. Fe, mg/kg = 102 to 195
o. Zn, mg/kg = 4.7 to 13
p. Cu, mg/kg = 1 to 2
q. Mn, mg/kg = 13.4 to 23.9
r. Mo, mg/kg = < 1 to 0.5
s. Ti, mg/kg = < 12.5
t. Co, mg/kg = < 0.5
u. Cd, mg/kg = < 0.3
v. Cr, mg/kg = < 1
w. B, mg/kg = 7.7 to 9.5
x. Ba, mg/kg = 16.3 to 34.8
y. As, mg/kg = < 2.5
z. Sb, mg/kg = < 5
aa. Al, mg/kg = 120 to 146
bb. Pb, mg/kg = < 2.5
cc. Hg, mg/kg = < 10
dd. Se, mg/kg = < 10
ee. tIVDMD, % = 30 to 50

ff. CT, % = 4.7 to 8.4
gg. Volatile oil, % = 0.3 to 0.6
hh. Labdane, % = < 0.14
- isocupressic acid (**ICA**) , % = < 0.05
- imbricatoloic acid, % = < 0.02
- dihydroagathic acid, % = < 0.06
- agathic acid, % = < 0.11

Juniper tree parts: juniper leaves and stems < 3.6 cm diameter; mechanically dried at 28°C for 4 h (Whitney et al., 2014) or at 55°C for 96 h (Whitney et al., 2010); reported as percentage of DM.

a. DM (fresh material), % = 66
b. DM (mechanically dried for 4 h), % = 94
c. CP, % = 6.6 to 7.6
d. NDF, % = 39 to 44
e. ADF, % = 37 to 42
f. Crude fat = 7
g. Ash, % = 3.2 to 5.5
h. Lignin, % = 17.6
i. Ca, % = 1.1 to 1.8
j. P, % = 0.06 to 0.07
k. Mg, % = 0.12 to 0.2
l. K, % = 0.41 to 0.43
m. Na, % = < 0.01
n. S, % = 0.09
o. Fe, mg/kg = 164 to 253
p. Zn, mg/kg = 11.5 to 14.4
q. Cu, mg/kg = 2.3 to 2.5
r. Mn, mg/kg = 19.6 to 20.3
s. Mo, mg/kg = < 1
t. Ti, mg/kg = < 12.5
u. Co, mg/kg = < 0.5
v. Cd, mg/kg = < 0.3
w. Cr, mg/kg = < 1
x. B, mg/kg = 12.2 to 12.8
y. Ba, mg/kg = 31 to 61
z. As, mg/kg = < 2.5
aa. Sb, mg/kg = < 5
bb. Al, mg/kg = 196 to 280
cc. Pb, mg/kg = < 2.5
dd. Hg, mg/kg = < 10
ee. Se, mg/kg = < 10
ff. tIVDMD, % = 55
gg. CT (mechanically dried), % = 5.6 to 6
hh. CT (fresh material), % = 7.3
ii. Volatile oil (mechanically dried), % = 0.43 to 2.5
jj. Volatile oil (fresh material), % = 3.7 to 4.6

Juniper wood: fresh (not dried) with no leaves. Reported as percentage of DM.

b. Volatile oil, % = 0.21 to 4.9

Juniper tree leaves. Air-dried for 14 to 30 d or collected fresh and immediately frozen. Reported as percentage of DM.

a. DM (fresh material), % = 46 to 56
b. DM (air-dried for ~ 25 d), % = 94.5
c. CP, % = 6 to 9
d. NDF, % = 34 to 40
e. ADF, % = 24 to 31
f. Crude fat = 8.7
g. Ash, % = 4 to 6
h. Ca, % = 1.46
i. P, % = 0.07 to 0.17
j. Mg, % = 0.15
k. K, % = 0.49
l. S, % = 0.07
m. Fe, mg/kg = 602
n. Zn, mg/kg = 15
o. Cu, mg/kg = 2
p. tIVDMD, % = 57 to 72
q. CT (air dried), % = 5.5
r. CT (fresh material), % = 9 to 10
s. Volatile oil (air dried), % = 0.5 to 2.2
t. Volatile oil (fresh material), % = 0.9 to 2.2

Table 1. Chemical composition (% DM basis) and digestibility of redberry (RED) and blueberry (BLUE) juniper trees and parts[1]

	Mature Trees[2]		Immature Trees[2]		Small Stems/Leaves		Leaves Only		
Item[3]	RED	BLUE	RED	BLUE	Dry[4]	Fresh[5]	RED Dry[6]	RED Fresh[7]	BLUE Fresh[7]
DM, fresh material, %	69.1[a]	65.4[a]	68.1[a]	64.9[a]		66.1[h]		46 to 56[j]	52[c]
DM, dried material, %					93.5[f]; 93.8[h]		94.5[b]		
CP, %	3.6[a]	3.4[a]	4.7[a]	4.1[a]	7.6[f]; 6.6[h]	7.2[h]	7.1[b]	7.4[e]; 6 to 9[j]	6.5[d]; 6.5[c]
NDF, %	66.9[a]	67.4[a]	50.1[a]	54.4[a]	39.9[f]; 39.3[h]	43.8[h]	37.8[b]	40[e]	34.3[d]
ADF, %	56.2[a]	55.5[a]	40.7[a]	44.2[a]	36.7[f]; 38.3[h]	42.2[h]	31.2 [b]	28.2[e]	24[d]
NDIN, %	1.4[a]	1.3[a]	1.5[a]	1.6[a]					
ADICP, %					1.5[f]				
ADICP/CP, %					26.3[f]				
Crude fat, %					7.0[f]		8.7[b]		
Lignin, %	25[a]	29.8[a]	21.1[a]	29.4[a]	17.6[f]				
tIVDMD, %	30[a]	30[a]	49.7[a]	43.6[a]	55[f]		67[b]	65[e]; 57 to 66 [j]	71.6[d]
Minerals									
Ash, %	4.3[a]	4.7[a]	5.7[a]	5.9[a]	5.5[f]; 3.2[h]	3.5[h]	5.3[b]	4 to 6[j]	
Ca, %	1.49[a]	1.57[a]	1.78[a]	1.95[a]	1.6[f]		1.46[b]		
Ca, %	1.25[a]	1.57[a]	1.69[a]	1.89[a]	1.1 to 1.8[f]				
P, %	0.04[a]	0.04[a]	0.05[a]	0.05[a]	0.06[f]		0.07[b]	0.09 to 0.17[j]	0.08[c]
P, %	0.03[a]	0.03[a]	0.04[a]	0.04[a]	0.07[f]				
Mg, %	0.08[a]	0.05[a]	0.12[a]	0.08[a]			0.15[b]		
Mg, %	0.07[a]	0.04[a]	0.11[a]	0.07[a]	0.12 to 0.2[f]				
K, %	0.23[a]	0.25[a]	0.31[a]	0.3[a]			0.49[b]		
K, %	0.16[a]	0.19[a]	0.26[a]	0.23[a]	0.41 to 0.43[f]				
Na, %	0.02[a]	0.02[a]	0.02[a]	0.02[a]			0.02[b]		
Na, %	< 0.005[a]	< 0.005[a]	< 0.005[a]	< 0.005[a]	< 0.01[f]				
S, %	0.05[a]	0.05[a]	0.07[a]	0.07[a]	0.09[f]				
Fe, ppm	195[a]	102[a]	163[a]	145[a]			602[b]		
Fe, ppm	118[a]	114[a]	129[a]	98[a]	164 to 253[f]				
Zn, ppm	9[a]	11[a]	13[a]	10[a]			15[b]		
Zn, ppm	4.7[a]	4.8[a]	9.7[a]	6.9[a]	11.5 to 14.4[f]				
Cu, ppm	2[a]	1[a]	2[a]	2[a]			2[b]		
Cu, ppm	1.9[a]	2[a]	1.9[a]	2[a]	2.3 to 2.5[f]				
Mn, ppm	16[a]	13[a]	21[a]	22[a]			28[b]		
Mn, ppm	13.3[a]	13.1[a]	18.9[a]	23.9 [a]	19.6 to 20.3[f]				
Mo, ppm	0.5[a]	< 0.1[a]	0.5[a]	< 0.1[a]			< 1[b]		
Mo, ppm	< 1[a]	< 1[a]	< 1[a]	< 1[a]	< 1[f]				
Ti, ppm	< 12.5[a]	< 12.5[a]	< 12.5[a]	< 12.5[a]	< 12.5[f]				
Co, ppm	< 0.5[a]	< 0.5[a]	< 0.5[a]	< 0.5[a]	< 0.5[f]				
Cd, ppm	< 0.3[a]	< 0.3[a]	< 0.3[a]	< 0.3[a]	< 0.3[f]				
Cr, ppm	< 1[a]	< 1[a]	< 1[a]	< 1[a]	< 1[f]				
B, ppm	7.8[a]	7.7[a]	9.5[a]	9.4[a]	12.2 to 12.8[f]				
Ba, ppm	16.7[a]	16.3[a]	34.8[a]	18.6[a]	31 to 61[f]				
As, ppm	< 2.5[a]	< 2.5[a]	< 2.5[a]	< 2.5[a]	< 2.5[f]				

Sb, ppm	< 5[a]	< 5[a]	< 5[a]	< 5[a]	< 5[f]
Al, ppm	129[a]	135[a]	146[a]	120[a]	196 to 280[f]
Pb, ppm	< 2.5[a]	< 2.5[a]	< 2.5[a]	< 2.5[a]	< 2.5[f]
Hg, ppm	< 10[a]	< 10[a]	< 10[a]	< 10[a]	< 10[f]
Se, ppm	< 10[a]	< 10[a]	< 10[a]	< 10[a]	< 10[f]

[1]Source of data: [a] = Stewart et al. (2014); [b] = Whitney and Muir (2010); [c] = NRC (2007); [d] = Adams et al. (2013a); [e] = Adams et al. (2013b); [f] = Whitney et al. (2014); [h] = Whitney et al. (2010); [i] = T. R. Whitney (unpublished data); [j] = Huston (1981).

[2]Mature trees were > 3 m in height; Immature trees were 0.5 to 1.8 m in height.

[3]DM of fresh material= dry matter content of material not air or mechanically dried; DM of dried material= dry matter material after being air or mechanically dried; NDIN = neutral detergent insoluble nitrogen; ADICP = acid detergent insoluble crude protein (CP); NDF = neutral detergent fiber; ADF = acid detergent fiber; tIVDMD = 48-h true *in vitro* dry matter digestibility.

[4]Dry = redberry juniper leaves and stems (< 3.6 cm diameter) chipped, dried at 28°C for 4 h (Whitney et al., 2014) or dried at 55°C for 96 h (Whitney et al., 2010).

[5]Fresh = redberry juniper leaves and stems (< 3.6 cm diameter) chipped fresh, frozen, and chopped (Whitney et al., 2010).

[6]Red Dry = leaves collected from air-dried redberry juniper branches (Whitney and Muir, 2010).

[7]Red fresh and Blue fresh = leaves collected from live redberry or blueberry juniper branches, respectively.

Table 2. Secondary compound composition (% DM basis) of redberry (RED) and blueberry (BLUE) juniper trees and parts[1]

Item[3]	Mature Trees[2] RED	Mature Trees[2] BLUE	Immature Trees[2] RED	Immature Trees[2] BLUE	Redberry Parts Dry[4]	Redberry Parts Fresh[5]	Wood Only, Fresh RED	Wood Only, Fresh BLUE	Leaves RED Dry[6]	Leaves RED Fresh[7]	Leaves BLUE Fresh[8]
CT, % of DM											
Extractable CT	3.1[a]	2.7[a]	5.5[a]	4[a];	3.6[f]; 3.35[u]	6.1[g]			4.12[c]	6.84[e]	6.3[d]
Protein-bound CT	1.1[a]	2.2[a]	1.6[a]	2.7[a];	1.9[f]; 1.77[u]	1.1[g]			0.96[c]	3.4[e]	2.54[d]
Fiber-bound CT	0.59[a]	0.82[a]	1.2[a]	1.3[a];	0.5[f]; 0.54[u]	0.1[g]			0.38[c]	0[e]	0.21[d]
Total CT	4.7[a]	5.7[a]	8.4[a]	8[a];	6[f]; 5.66[u]	7.3[g]			5.46[c]	10.24[e]	9.06[d]
Total oil, % of DM	0.46[a]	0.4[a]	0.6[a]	0.3[a]	2.5[g]; 0.43[f]; 0.99[r]	3.7[g]; 4.6[g]	0.21[q]	4[q]; 4.9[q]	0.52[c]; 2.2[c];1.1[o]	0.94 to 1.08[e]; 1.5[o]; 1.96[s]; 1.83[t]	2.18 to 3.46[d]; 1.5[s]; 2.15[t]
Volatile oil, mg oil/g DM											
Tricyclene					0.21[g]	0.19[g]; 0.22[g]			0[c]; 0.1[c]	0.03[e]; 0.39[t]	0.76[d]; 0.27[t]
α-Thujene					0.41[g]	0.8[g]; 0.84[g]			0.06[c]; 0.3[c]	0.08[e]	
α -Pinene					0.5[g]	0.96[g]; 0.84[g]			0.08[c]; 0.36[c]	0.11[e]; 1.04[s]; 0.49[t]	0.32[d]; 0.74[s]; 0.57[t]
Camphene					0.24[g]	0.28[g]; 0.27[g]			0.03[c]; 0.12[c]	0.05[e]; 0.48[s]; 0.34[t]	0.69[d]; 1.38[s]; 0.35[t]
Sabinene					6.6[g]	8.74[g]; 9.84[g]			0.69[c]; 3.89[c]	2.27[e]; 3.39[t]	*t*[d]; 5.44[t]
Myrcene					1.09[g]	1.61[g]; 1.9[g]			0.09[c]; 0.53[c]	0.29[e]; 1.78[s]; 0.9[t]	0.42[d]; 0.68[s]; 0.84[t]
3-Carene					0.07[g]	1.44[g]; 0.17[g]			0.00[c]; 0.1[c]	0.01[e]	0.1[d]
α -Terpinene					0.67[g]	1.42[g]; 2.14[g]			0.16[c]; 0.56[c]	0.18[e]	*t* [d]
Limonene					1.61[g]	2.15[g]; 2.74[g]			0.21[c]; 0.92[c]	0.3[e]; 2.4[s]; 2.19[t]	1.84[d]; 2.56[s]; 2.44[t]
γ-Terpinene					1.1[g]	2.25[g]; 3.12[g]			0.23[c]; 0.9[c]	0.3[e]; 0.79[t]	0.14[d]; 0.87[t]
cis-Sabinene hydrate					0.17[g]	0.19[g]; 0.3[g]			0.03[c]; 0.23[c]	0.14[e]	
Terpinolene					1.02[g]	0.94[g]; 1.07[g]			0.1[c]; 0.37[c]	0.14[e]	0.13[d]
trans-Sabinene hydrate					0.18[g]	0.15[g]; 0.32[g]			0.02[c]; 0.21[c]	0.09[e]	
Camphor					4.68[g]	5.17[g]; 6.82[g]			2.6[c]; 10.9[c]	2.3[e]; 3.2[s]; 4.93[t]	10.07[d]; 6.2[s]; 5.88[t]
Camphene hydrate					0.17[g]	0.16[g]; 0.2[g]			0.06[c]; 0.32[c]	0.07[e]	0.24[d]
Citronellal					0.12[g]	0.29[g]; 0.21[g]				0.09[e]	*t* [d]; 0.67[t]
Borneol					0.19[g]	0.02[g]; 0.12[g]			0.05[c]; 0.31[c]	0.06[e]; 0.19[t]	0.39[d]; 0.13[t]
Terpin-4-ol					3.09[g]	5.9[g]; 8.66[g]			0.32[c]; 1.04[c]	0.7[e]; 1.53[t]	*t* [d]; 1.43[t]
Citronello									0.01[c]; 0.15[c]	0.45[e]; 0.46[t]	
Bornyl acetate					0.63[g]	0.82[g]; 0.8[g]			0.15[c]; 0.28[c]	0.16[e];1.12[s]; 1.49[t]	2.74[d]; 2.42[s]; 1.46[t]

Elemol	1.21[g]	1.19[g]; 2.42[g]	0.04[c]; 0.1[c]	0.55[e]	0.16[d]
Volatile Oil, % of total oil					
Tricyclene	0.82[g]	0.52[g]; 0.47[g]	0.04[c]; 0.49[c]; 0.2[o]	0.3[o]; 0.4[p]; 2.16[t];	3.47[d]; 2.9[p];1.27[t]
alpha-Thujene	1.63[g]	2.18[g]; 1.8[g]	1.21[c]; 1.34[c]; 0.8[o]	1[o]; 0.5[p];	
alpha-Pinene	1.97[g]	2.6[g]; 1.8[g]	1.51[c]; 1.62[c]; 1.0[o]	1.5[o]; 2[p]; 5.3[s]; 2.69[t];	1.46[d]; 0.6[p]; 4.92[s]; 2.63[t]
Camphene	0.94[g]	0.76[g]; 0.5[g]	0.64[c];0.55[c]; 0.3[o]	0.4[o]; 0.4[p]; 2.4[s]; 1.87[t];	3.21[d]; 2.3[p]; 9.18[s]; 1.65[t]
Sabinene	26[g]	23.8[g]; 21.3[g]	13.3[c]; 17.5[c]; 27.1[o]	27.5[o]; 22[p]; 18.54[t]	*t*[d]; 25.3[t]
Myrcene	4.3[g]	4.4[g];4.1[g]	1.80[c]; 2.39[c]; 2.9[o]	2.8[o]; 2[p]; 9.1[s]; 4.94[t]	1.87[d]; 0.8[p]; 4.5[s]; 3.92[t]
3-Carene	0.27[g]	3.9[g];0.37[g]	0.10[c]; 0.46[c]; t[o]	t[o]; 0.5[p]	0.64[d]
alpha-Terpinene	4.33[g]	6.12[g];6.76[g]	3.11[c]; 2.52[c]; 2.5[o]	2.6[o]; 1.3[p]	*t*[d]; t[p]
Limonene	6.34[g]	5.85[g];5.93[g]	4.03[c]; 4.15[c]; 3.3[o]	3.1[o]; 3.6[p]; 12.2[s];11.96[t]	8.3*t*[d]; 4.6[p]; 17[s]; 11.3[t]
gamma-Terpinene	4.3[g]	6.1[g];6.76[g]	4.43[c]; 4.06[c]; 3.9[o]	4.1[o]; 2.2[p]; 4.3[t]	0.59[d]; 0.3[p]; 4[t]
cis-Sabinene hydrate	0.66[g]	0.52[g];0.65[g]	0.67[c]; 1.04[c]; 1.5[o]	1.6[o]; 1.2[p]	
Terpinolene	1.04[g]	2.56[g];2.32[g]	1.92[c]; 1.68[c]; 1.4[o]	1.7[o]; 0.8[p]	0.59[d]; 0.3[p]
trans-Sabinene hydrate	0.69[g]	0.41[g];0.7[g]	0.40[c]; 0.93[c]; 1.5[o]	1.8[o]; 1[p]	
Camphor	18.5[g]	14.1[g];14.8[g]	50.3[c]; 49.3[c]; 26[o]	22.7[o]; 31.4[p]; 16.4[s]; 27[t]	46.8[d]; 64.9[p]; 41.2[s]; 27.3[t]
Camphene hydrate	0.69[g]	0.44[g];0.43[g]	1.18[c];1.46[c]; 0.9[o]	0.9[o];0.8[p]	1.3[d]; 1.7[p]
Borneol	0.73[g]	0.05[g];0.26[g]	0.89[c]; 1.4[c]; 1.9[o]	1.4[o]; 1.6[p]; 1.04[t]	1.72[d]; 2[p]; 0.62[t]
Terpin-4-ol	12.2[g]	16.1[g];18.8[g]	6.27[c]; 4.68[c]; 7.5[o]	10.1[o]; 7.5[p]; 8.36[t]	*t*[d]; 0.2[p]; 6.9[t]
Citronellol	1.24[g]	0.93[g];0.88[g]	0.27[c]; 0.67[c]; 4[o]	3.7[o];3[p]; 2.54[t]	3.12[t]
Bornyl acetate	2.5[g]	2.2[g];1.7[g]	2.94[c]; 1.27[c]; 2.5[o]	3.6[o]; 7.1[p]; 5.7[s]; 8.14[t]	11.87[d]; 9.2[p]; 16.1[s]; 6.8[t]
Elemol	4.97[g]	3.2[g];5.2[g]	0.80[c]; 0.47[c]; 3[o]	2.9[o]; 2.6[p]	0.73[d]

[1]Source of data: [a] = Stewart et al. (2014); [c] = Whitney and Muir (2010); [d] = Adams et al. (2013a); [e] = Adams et al. (2013b); [f] = Whitney et al. (2014): [g] = Whitney et al. (2010); [o] = Adams (2010); [p] = Adams (2011); [q] = Adams (1987); [r] = T. R. Whitney (unpublished data); [s] = Riddle et al. (1996); [t] = Owens et al. (1998); [u] = Whitney et al. (2013).

[2]Mature trees were > 3 m in height; Immature trees were 0.5 to 1.8 m in height.

[3]CT = condensed tannins

[4]Dry = redberry juniper leaves and stems (< 3.6 cm diameter) chipped, dried at 28°C for 4 h (Whitney et al., 2014) or dried at 55°C for 96 h (Whitney et al., 2010).

[5]Fresh = redberry juniper leaves and stems (< 3.6 cm diameter) chipped fresh, frozen, and chopped (Whitney et al., 2010). Wood material only; no leaves (Adams, 1987).

[6]Red Dry = leaves collected from air-dried redberry juniper branches (Whitney and Muir, 2010); leaves allowed to air dry by storing at 21°C for 2 week (Adams, 2010).

[7]Red fresh and Blue fresh = leaves collected from live redberry or blueberry juniper branches, respectively.

4. Manufacturing Process

The aerial portion of the entire tree or its aerial parts is harvested by a variety of methods (e.g., **Fig. 3**). The material is then chipped and hammermilled through a screen with holes not greater than 5/8" (1.59 cm) in diameter. At any processing stage, the juniper material can remain "as-is" (fresh) or naturally or mechanically dried (dehydrated) and stored according to good management practices.

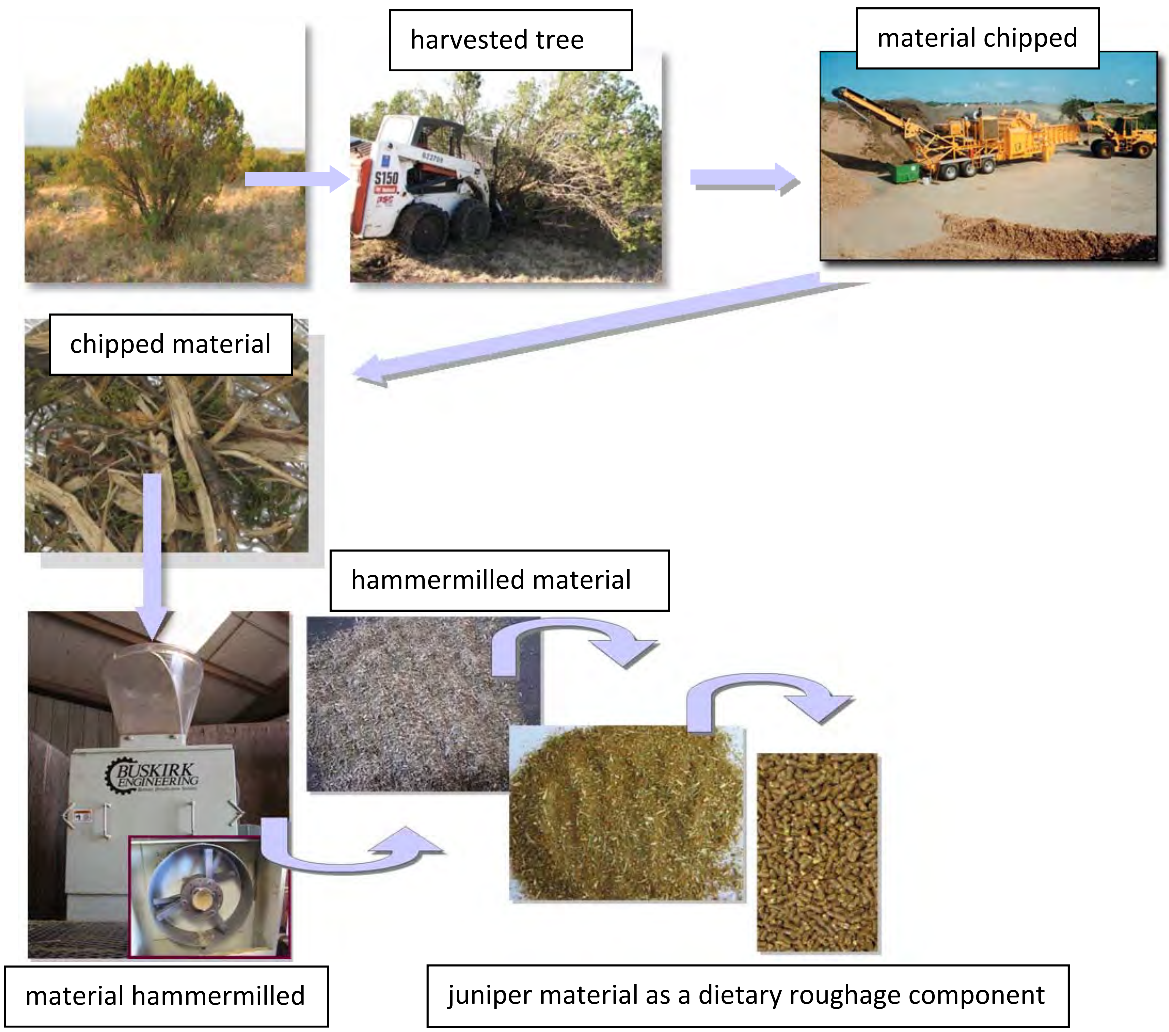


Figure 3. Manufacturing process. Whole tree or its parts are chipped, hammermilled, mixed with other feed ingredients and fed either loose (non-agglomerated) or pelleted.

5. Purpose

The ground juniper ingredient is intended to be used as a dietary roughage component in ruminant animal diets in any stage of animal production. It is intended to be used to either substitute or complement traditional roughage sources such as hay and various varieties of seed hulls in total mixed rations (**TMR**). Ground juniper is not intended to be used as the sole diet of the animal, but mixed with other feed (at least 1 other ingredient) and fed either loose (non-agglomerated) or in pellet form.

6. Use Limitations

The ground juniper ingredient is not intended to be used as the sole diet of the animal or used in diets for monogastric animals (e.g. hogs and birds) or hind-gut fermenters (e.g., horses, woodrats). As with any roughage feed ingredient (e.g. CSH, ground stalks, ground aspen), care should be taken to ensure that total daily nutrient intake is, at the least, meeting the animal's basic maintenance requirements.

7. Prior Sanctioned Use and Historical Regulation

There is no prior sanctioned use or historical regulation of ground *Juniperus* plant species in ruminant animal diets. However, another woody product, "ground whole aspen and/or parts," received AAFCO approval and was adopted as an approved feed ingredient in 1980 (see definition below); thus, sets a precedent for the proposed ground juniper and/or parts.

AAFCO definition (p. 425, 2011 edition):

> "Ground Whole Aspen and/or Parts is generally recognized as a feed ingredient in cattle diets when used in accordance with good nutritional practices. Ground whole aspen (*Populus tremuloides* Michiz and *Populus gradidentata*) is composed of the entire tree including leaves, branches, trunk, and bark. Ground aspen parts may also include leaves, branches, trunk, and bark. Roots and stumps are excluded to avoid contamination of dirt and rocks in the product. (Proposed 1979, Adopted, 1980)"

IFN 1-30-183 Aspen quaking/Aspen large toothed aerial part ground
IFN 1-12-241 Aspen aerial part ground

8. Safety Assessment: Fiber Characteristics and Feeding Value

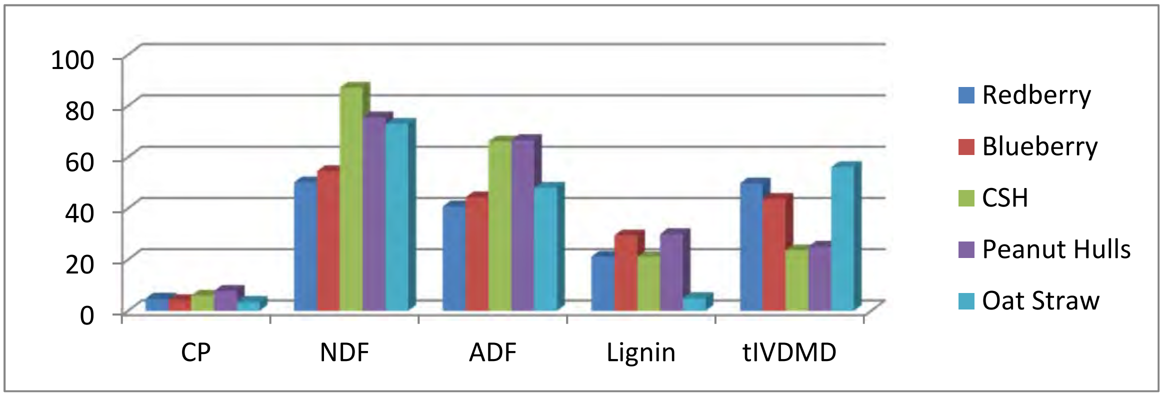
NOTE: Details of each trial summarized in this section (and other supportive research trials) can be found in the Appendices.

Fiber Characteristics

Ground juniper is a fibrous feed ingredient, but a thorough literature review reveals that aspen (**Fig. 5; Table 3)** and numerous common non-woody roughage feed ingredients currently exist that are safely fed to ruminant animals, some of which contain similar, to greater concentrations of NDF, ADF, and/or lignin than ground juniper (**Fig. 4; Tables 4 and 5**). For example, mature ground juniper trees have been reported to contain 50 to 67% NDF, 41 to 56% ADF, and 21 to 30% lignin (DM basis; **Table 1**). In comparison, on a DM basis, cottonseed hulls (**CSH**) have been reported to contain up to 88% NDF, 70% ADF, and 23% lignin; peanut hulls reported to contain up to 77% NDF, 68% ADF, and 30% lignin; and oat hay reported to contain up to 63% NDF, 39% ADF, and 6.4% lignin (**Table 4**).

Mature ground juniper trees have been reported to be 30% to 50% digested (*in vitro*; Stewart et al., 2014) and using the same *in vitro* procedures, CSH and oat hay have been reported to be 21% (Whitney and Muir, 2010) and 57% (Whitney et al., 2014) digested, respectively. Digestibility of peanut hulls have been reported to be between 16 to 25% (Barton et al., 1974) and digestibility of oat straw between 40 and 59% (Goto et al., 2000; Kafilzadeh et al., 2012). **Figure 4** compares CP, fiber, and digestibility of redberry and blueberry juniper trees (at mature or immature stages of growth) to traditional non-woody roughage ingredients and ground aspen.

a. Immature juniper trees



b. Mature juniper trees

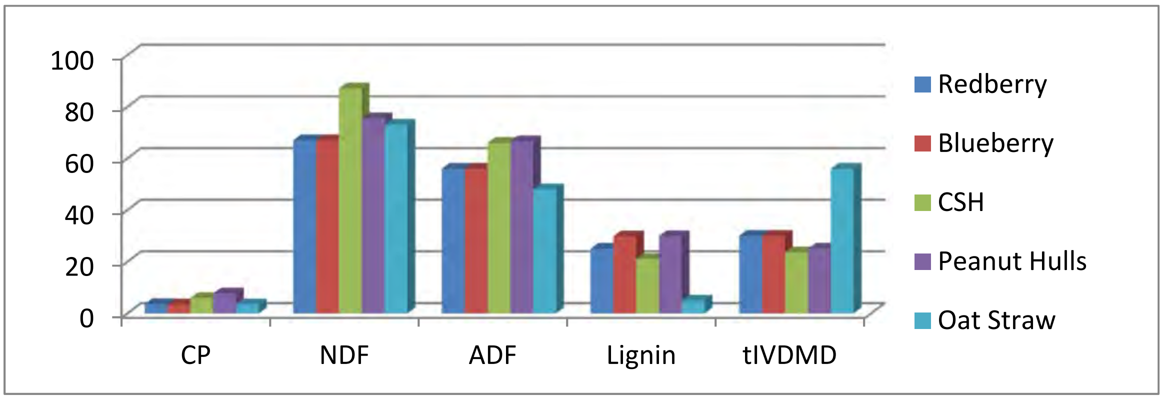


Figure 4. Nutritional composition (% of DM) and true 48-h *in vitro* dry matter digestibility (tIVDMD) of immature (a) and mature (b) redberry and blueberry juniper, and cottonseed hulls (CSH), peanut hulls, and oat straw. CP = crude protein; NDF and ADF = neutral and acid detergent fiber digestibility, respectively.

Ground aspen tree material is the only woody plant feed ingredient officially approved by Association of American Feed Control Officials (AAFCO, 2011); "**Ground Whole Aspen and/or Parts**" received AAFCO approval in 1980. When comparing NDF, ADF, lignin, and CP concentrations in mature ground aspen trees to mature ground juniper trees, most reports suggest that ground juniper is a more "nutritious" roughage feed ingredient. Mature ground aspen trees and sawdust have been reported to contain up to 80% NDF, 67% ADF, 21.1% lignin, and 1.9% CP (DM basis; **Table 3**). Ground aspen wood and sawdust has been reported to be 0% to 41% digested (DM basis; **Table 3**). **Figure 5** compares key nutrients and digestibility of mature ground redberry and blueberry juniper trees to ground aspen material.

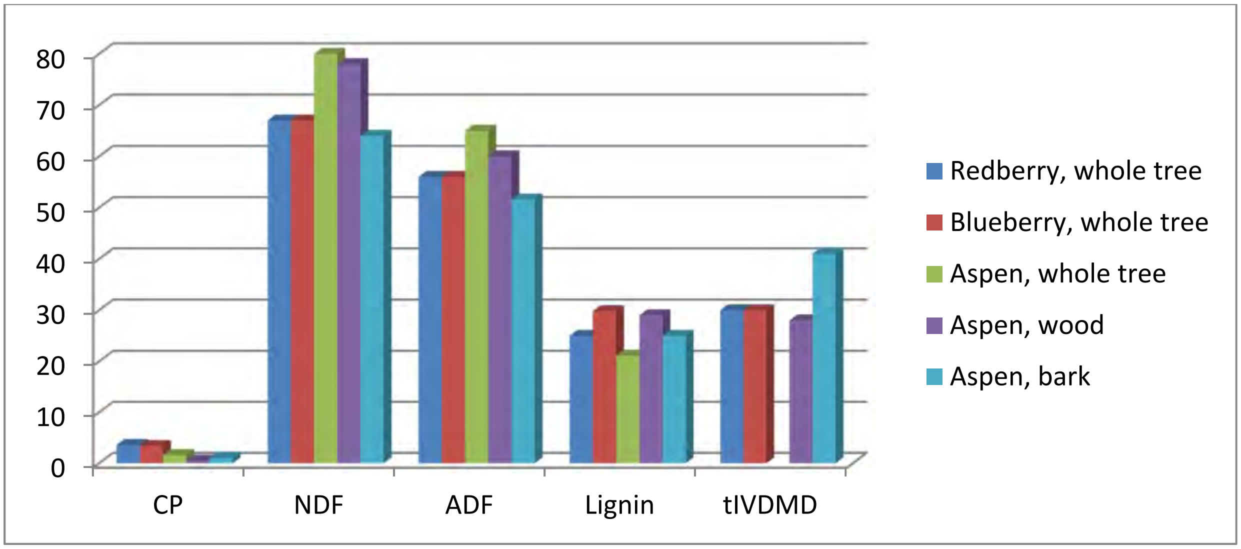


Figure 5. Nutritional composition (% of DM) and true 48-h *in vitro* dry matter digestibility (**tIVDMD**) of mature redberry and blueberry juniper trees, and ground aspen. CP = crude protein; NDF and ADF = neutral and acid detergent fiber digestibility, respectively.

Table 3. Chemical composition (% DM basis) and digestibility of aspen material[1]

Item[2]	Aspen, Whole Tree	Aspen, Leaves[3]	Aspen, Wood[4]	Aspen, Bark[5]	Aspen Sawdust
DM, %	40.8[q]	85[a]; 56.1[nn]		75 to 85[ee]	93[d]
CP, %	1.2[q]; 1.9[mm]	17.2[a]; 7.4[b]; 9.3 to 14.4[i]; 14.4 to 18[j]; 12.6[w]; 13.4 to 18.7[z]; 9.4 to 12[aa];13 to 17[bb]; 5.4[gg]; 6.2[nn]; 17.8[qq]; 30[ss]	0.51[y]	< 3[l]; 1.5[ee]	0.72[d]; < 2[n]
NDF, %	80[mm]	37.4 to 43[z]; 25.6[nn]; 39[qq]; 17.9[ss]		64[o]	79[d]
ADF, %	65[q];65[mm]	25.7 to 26.6[z]; 21.7[nn]; 26[qq]; 12.4[ss]		51.6[o]	67.4[d]; 60[n]
ADICP, g/kg DM		6.2[b]			
Crude fat, %		8.8[nn]; 2.6[ss]	1.08[y]	5 to 10[l]; 7[ee]	1.1[d]
Lignin, %	21.1[dd]; 16.9[mm]	8.3 to 12.4[z]; 7.9[nn]; 11[qq]	26.9 to 38.2[k]; 20[p]; 8.4[y]; 16.3 to 22.4[tt]	29[ee]; 23[o]	20.2[d]; 16 to 22[n]; 20 to 24[pp]
tIVDMD, %					
Digestibility, other methods, %		27.8[b]; 60 to 65.4[z]; 49[nn]	20 to 23[c]; 33[k]; 31 to 37[ff]	50[l]; 50[m]; ;30[n]; 26 to 30[o]; 50[p]	19.4[d]; 28 to 41[m]; 33, 35, 37, 40[p]; 0; 4 to 38[pp]
Minerals					
Ash, %	3.2[q]; 0.5[dd]; 2.2[mm]	9[a]; 1.4[nn]; 7.5[ss]	0.8[p]; 0.2[y]; 0.7 to 5[tt]	4.3[v];4.5[ee]	0.51[d]; < 1 to 10[n]; 1[p]
Ca, %			0.18[p]	1.09[v]	
P, %			0.003[p]	0.035[v]	
Mg, %			0.03[p]	0.06[v]	
K, %			0.06[p]	0.22[v] 0.2 [ee]	
Na, %			< 0.01[p]	0.16[v]	
S, %	< 0.01[v]		< 0.01[p]	< 0.01[v]	
Fe, ppm			35[p]	194[v]	
Zn, ppm			19[p]	68[v]	
Cu, ppm	4.9[v]		6[p]	21[v]	
Mn, ppm			10[p]	36[v]	
Al, ppm			16[p]	140[v]	
Ba, ppm			19[p]	85[v]	
Cd, ppm	0.28[v]				
Cr, ppm			0.3[p]	2[v]	
Pb, ppm	1.45[v]				
Hg, ppm	< 0.05[v]				

[1]Source of data: [a] = NRC (2007); [b] = Bas et al. (1985), used linear regression to determine digestibility; [c] = Bender et al. (1970); [d] = Myung and Kennelly (1992); [i] = Erwin et al. (2001); [j] = Mansfield et al. (1999); [k] = DeMartini and Wyman (2011); [l] = Millett et al. (1970); [m] = Mellenberger et al. (1971); [n] = NRC (1983, p. 77, 78, 229); [o] = Gharib et al. (1975) ; [p] = Baker et al. (1975); [q] = Singh (1978); [v] = A. J. Baker, Forest Products Lab, Madison, Wis., personal communication (1978; in NRC, 1983, p. 241); [w] = Dickson and Larson, 1976; [y] = Shaw et al (2009); [z] = McWilliam et al. (2005); [aa] = Stevens et al (2007); [bb] = Stevens and Lindroth (2005); [cc] = Garleb et al. (1988); [dd] = Xu and Tschirner (2012); [ff] = Mathison et al. (1986); [gg] = Bailey et al. (2007); [mm] = Schingoethe, et al (1981); [nn] = Ullrey et al. (1972); [pp] = Feist et al. (1970); [qq] = McWilliam (2004); [rr] = Häikiö et al. (2009); [ss] = NRC (2003); [tt] = Micko (1987, in Peterson and Peterson, 1992)

[2]DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; NDIN = neutral detergent insoluble nitrogen; ADICP = acid detergent insoluble CP; tIVDMD = 48-hour true *in vitro* dry matter digestibility; Digestibility = methods other than tIVDMD used to determine digestibility.

[3]Aspen leaves; data is from sun-cured leaves (NRC, 2007), regression (Bas et al., 1985), fresh leaves from growing trees (Erwin et al., 2001), or fresh leaves from growing trees in the laboratory (Mansfield et al., 1999). Leaves and stems < 3 mm in diameter (McWilliam et al., 2005).

[4]Aspen wood does not include any leaf material.

Table 4. Chemical composition (% DM basis) and digestibility of traditional ruminant animal feed ingredients[1]

Item[2]	Cottonseed Hulls	Peanut Hulls	Oat Hay	Oat Straw	Corn Stover, Stalks	Lespedeza Hay	Alfalfa Hay
DM, %	90[a]; 95.7[s];89.6[cc]; 89.9 and 90.6[mm]; 90.4[nn]	91[a]; 90.5[u]	90[a]; 92.9[h]	91[a]; 89.4[b]; 95.4[c]; 92.1[e]; 87 to 91[t]	80[a]	92 to 93.5[jj];	92[mm]; 90.9[nn]
CP, %	5.5[a]; 6.6[g];3.9 to 5.4[s]; 6.7[cc];4.2[nn]	7[a]; 8.4[u]	10[a]; 12.6[h]	4[a]; 8.4[b]; 2.4 to 4.8[c]; 3.13[e]; 92.1[e]; 1.7[f]; 2.6 to 3.2[t]	5[a]; 3.7[r]	11.8 to 13.2[ii]; 13.1[ll]	17[nn]
NDF, %	87[a]; 80[g]; 84[cc]; 85.4 and 87.6 and 86.5[mm]; 88.3[nn]	74[a]; 77[u]	63[a]; 58[h]	73[a]; 77[b]; 62.6 to 70.8[c]	70[a]; 70.8[r]		48.8[nn]
ADF, %	68[a]; 69.5[g]; 64.5[s]; 62[cc]; 65.3[nn]	65[a]; 68.3[u]	39[a]; 35.3[h]	48[a]; 44[b]; 43.7 to 53.3[c]	44[a]; 47.1[r]		38.7[nn]
ADICP, %			0.8[h]				
ADICP/CP, %			6.8[h]				
Crude fat, %	1.9[a]; 0.9[g]; 1 to 2.8[s];	1.5[a]; 1.8[u]	2.3[a]; 2.1[h]	2.3[a]; 2[e]; 1.4[f]; 1.9[t]	1.3[a]	2.8 to 3.2[ii]	3.4[nn]
Lignin, %	21.8[s]; 21.3[cc]; 20.1[mm]; 19 and 22.9[mm]; 23[oo]	29.9[u]; 31.4[oo]	6.4[h]	4.9[b]; 5.2 to 9.2[c]	10.5[r]; 17.2[hh]	16 to 23[ii]; 14[ll]	
tIVDMD, %	20.8[g] ; 21 and 26 and 16[mm]		57.4[h]				
Digestibility, other methods, %	34.3[cc]; 31[oo]	15.9 to 25[d]; 4[oo]		58.7[b]; 40 to 54[c]; 52[f]; 54 to 58[t]	51[r]	43 to 58[ii]; 45.1[ll]	
Minerals							
Ash, %	3[a]; 2.8[g]; 3.2[s];2.9[nn]	5[a]; 3.6[u]	8[a]; 6[h]	8[a]; 7.7[e]; 7.7[f]; 7.5[t]	7[a]; 4.7[r]	5.4 [ii]	7.8[nn]
Ca, %	0.15[a]; 0.18[g]; 0.2[mm]; 0.15[nn]	0.2[a]	0.4[a]; 0.3[h]; 0.36[h]	0.24[a]; 0.31[f]	0.35[a]; 0.28[r]		1.22[mm]; 1.19[nn]
P, %	0.08[a]; 0.1[g]; 0.08[mm]; 0.09[nn]	0.07[a]	0.27[a]; 0.14[h]; 0.2[h]	0.07[a]; 0.1[f]	0.19[a]; 0.1[r]		0.29[mm]; 0.24[nn]
Mg, %	0.2[g]; 0.2[mm]; 0.14[nn]		0.12[h]	2.4[a]; 0.05[f]	0.23[r]		0.37[mm]; 0.27[nn]
K, %	1.1[a]; 1.14[g]; 0.88[nn]	0.9[a]	1.6[a]		1.1[a]; 1.9[r]		1.56[nn]
Na, %	0.02[g]; 0.02[nn]			0.22[a]	0.11[r]		0.07[nn]
S, %	0.05[a]; 0.09[g]; 1[mm]; 0.08[nn]		0.21[a];0.2[h]		0.14[a]		0.33[mm]; 0.27[nn]
Fe, ppm	58[g]; 108[mm]; 131[nn]		137[h]		0.02[r]		424[mm]; 155[nn]
Zn, ppm	10[a]; 14[g]; 22[nn]		0.28[a]	6[a]	22[a]; 170[r]		26[nn]
Cu, ppm	5[g]; 4.9[mm]; 13.3[nn]		6.7[h]				7.6[mm]; 9.9[nn]
Mn, ppm	17[g]; 15.7[mm]		86.9[h]				36.4[mm]; 42[nn]
Mo, ppm	0.4[g]; < 1[mm]; 0.02[nn]		< 1[h]				< 1[mm]; 0.23[nn]
Co, ppm	< 0.5[mm]		< 0.5[h]				< 0.5[mm]
Cd, ppm	< 0.3[mm]		< 0.3[h]				< 0.3[mm]
B, ppm	14.6[mm]		4.3[h]				31.3[mm]
Ba, ppm	4[mm]		12.2[h]				37.6[mm]
As, ppm	< 2.5[mm]		< 2.5[h]				< 2.5[mm]
Sb, ppm	< 5[mm]		< 5[h]				< 5[mm]
Al, ppm	60.3[mm]		116[h]				251.2[mm]
Cr, ppm	< 1[mm]		< 1[h]				< 1[mm]
Pb, ppm	< 2.5[mm]		< 2.5[h]				< 2.5[mm]
Hg, ppm	< 10[mm]		< 10[h]				< 10[mm]

[1]Source of data: [a] = NRC (2007); [b] = Goto et al., 2000; [c] = Kafilzadeh et al., 2012; [d] = Barton et al., 1974; [e] = Dowe, 1947; [f] = Fishwick et al. 1974; [g] = Whitney and Muir (2010); [h] = Whitney et al. (2014); [r] = Vetter (1973, *in NRC 1983, p. 234*); [s] = NRC (1983, *p. 235: Brown et al. (1977), Hale et al. (1969), and*

Heinemann (1976); [t] =Anderson (1978, *in NRC 1983, p. 236*), Rexen (1977, *in NRC 1983, p. 236*), Horton and Steacy (1979, *in NRC 1983, p. 236*) [u] = Utley and McCormick (1972 *in NRC 1983, p. 236*); [cc] = Garleb et al. (1988); [hh] = Wingren (2005); [ii] = Swanson and Herman (1944); [jj] = Rusoff et al. (1946); [ll] = Hawkins (1955); [mm] = Whitney, T. R. (mid- to late-bloom; *unpublished data from TX A&M AgriLife Nutrition Laboratory, San Angelo*) ; [nn] = NRC (2000; alfalfa hay, full bloom); [oo] = Van Soest, 1969 (in Van Soest, 1982).

[2]DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; NDIN = neutral detergent insoluble nitrogen; ADICP = acid detergent insoluble CP; tIVDMD = 48-hour true *in vitro* dry matter digestibility; Digestibility = methods other than tIVDMD used to determine digestibility.

Table 4, continued. Chemical composition (% DM basis) and digestibility of traditional ruminant animal feed ingredients[1]

Item[2]	Corncobs	Barley Straw
DM, %	96.6[a]	94[a]
CP, %	3.9[a]	2.6[a]
NDF, %	91.3[a]	84.9[a]
Digestibility, other methods, %	54.2[a]	35.4[a]
Minerals		
Ash, %		
Ca, %	0.6[a]	0.5[a]
P, %	0.2[a]	0.2[a]
Mg, %	0.1[a]	0.2[a]

[1]Source of data: [a] = Ndlovu and Buchanan-Smith, 1985.

[2]DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; Digestibility = methods other than 48-hour true *in vitro* dry matter digestibility.

Feeding Trials

Note: The most relevant research trials are discussed in this section. Additional information and more specific details (e.g., specific equations and the data that were used in the calculations) of these trials, along with other published literature, are located in the Appendices.

Research trials dating back to the early 1900's have shown that ground woody products can successfully be used as a roughage ingredient in livestock diets. Maynard (1920; Cornell Univ. Experiment Station) published a review related to the use of feed in Germany during wartime. In this review, the author discusses that "unconsidered or unused" feeds were found to be useful. Other notable comments include: (1) "According to experiments… cellulose from pine wood was found digestible in large measure and its use in quantities up to two and a quarter pounds per horse/d, together with 6 pounds of oats and 3 to 6 pounds of hay was advocated" and (2) "… it must be noted that the unusual materials employed were resorted to by necessity and not from any belief that they were superior to, or even as satisfactory as the rations normally used."

During the 1920's, the WI Agricultural Experiment Station, USDA Forest Product's Laboratory (Madison, WI), USDA experiment station (Beltsville, MD), USDA Bureau of Animal Industry, and the MA Agricultural Experiment Station (Amherst, MA) began evaluating the use of raw and pre-treated ground wood material as a feed ingredient (Sherrard and Blanco, 1921; Morrison et al., 1922; Archibald, 1926). These initial trials evaluated various feeding programs to establish nutritive values for various types of sawdust. Authors evaluated performance and health of sheep and cattle, and milk production from dairy cattle. Research using ground wood declined during the 1930s, but the number of studies that evaluated the use of foliage (leaves and small twigs) increased; this is especially true in the Soviet Union where they termed the foliage material, "Muka" (NRC, 1983). In addition, between 1920 to 1960, numerous trials evaluated the use of chemically pre-treated wood as livestock feed (reviewed by Ellis, 1969).

During the 1950's, interest in utilizing the entire tree as livestock feed was once again raised after the Texas Agricultural Experiment Station (now TX A&M AgriLife Research) reported that ground mesquite wood could be successfully used in cattle diets (Marion et al., 1959). Marion et al. (1957, 1959) reported that calves fed a diet of 52% ground mesquite (mixed with concentrates) consumed approximately 2.57 kg of mesquite/d (**12.6 g/kg of BW**) without any negative health effects. **Table 5** shows that the ground mesquite had greater CP and similar total fiber vs. CSH. During Trial 1, steers were fed a diet with up to 46% mesquite, resulting in a maximum daily mesquite intake of 4.28 kg (**14.6 g/kg of BW**); no negative health effects were observed. During Trial 2, steers were fed a diet with up to 50.6% mesquite, resulting in a maximum daily mesquite intake of 6.85 kg (**17.3 g/kg of BW**); no negative health effects. In conclusion, these authors stated "No ill effects resulted from feeding the ground wood."

Table 5. Chemical composition (DM basis) of ground mesquite wood and cottonseed hulls

Feed	CP, %	Fat, %	NFE[1], %	Fiber, %	Ash, %	P, %	Carotene, ppm
Ground mesquite wood	6.3	0.8	37.5	51.5	3.8	0.06	26
Cottonseed hulls	4.5	1.0	52.5	52.5	3	0.03	0

[1]NFE = nitrogen-free extract = 100 – (CP, fat, water, ash, and fiber).

During the late 1960's and throughout the 1970's and 1980's, Texas Tech University (Lubbock) evaluated the use of non-treated and pre-treated ground mesquite trees in steer and cow diets (Ellis, 1969; Parker, 1982). Ellis (1969) reported in a preliminary trial (no control group) that ground 6-yr old mesquite trees (with leaves) were processed, mixed with other ingredients and fed to cows. Maximum consumption of ground mesquite was 7.26 kg/d. Results suggested that the cows "were not on a high enough nutritional plane to support milk production." Authors report negative health in some of the cows, but state that they do not know if these effects were due to the diet or state that "the death did not appear to be related to the ration" or "such weight loss is recognized as normal for cows being wintered on the range." Authors also report that "data indicate that the ration containing mesquite was reasonably adequate for maintenance. The sharp weight loss post parturition suggests that the wood was inadequate as a major component of the ration for suckling cows."

Additional notes related to Ellis (1969):

- The entire mesquite tree (including leaves) was ground and fed; thus, it should be noted that mesquite leaves (~ 5% of the daily diet) have been shown to reduce intake and ADG in sheep (Baptista and Launchbaugh, 2001).
- Assuming that DM of the concentrate mixture, dried mesquite, and molasses was 90%, 94%, and 75%, respectively, cows initially were fed diets containing approximately 10% mesquite. Quantity of mesquite was gradually increased until cows were consuming approximately 88% mesquite.
- No adverse effects on animal health were reported. However, because of the small numbers of cows "in these two trials, no definite conclusion can be drawn as to the feasibility of maintaining a large herd on mesquite."

Numerous other trials evaluated the use of other tree species and various sawdust varieties. For example, Cody et al. (1972) fed calves (2 wk old and older) mixed diets containing 10, 15, 25, 35 and 45% shortleaf southern pine sawdust for up to 20 mo. Certain experimental groups were observed for performance; other groups were slaughtered after specific feeding periods. Gross and microscopic pathologic examinations of GI sections and major visceral organs were conducted. Results indicated that rations containing saw dust did not physically injure the GI lining nor was "any toxic effect apparent." Twenty-five percent sawdust appeared to be the most desirable level; higher levels occasionally induced impaction of digesta. In regards to the entire study, authors' concluded, "Histological examination revealed no tissue destruction or penetration of the GI mucosa, at any SD level."

Additional notes (Cody et al., 1972):

Trial 1:

- There was only 1 animal/treatment, thus data could not be analyzed statistically. Calves were fed individually from 6 wk to 10 mo of age. As a percentage of total consumption, average sawdust consumption was approximately 26% (DM basis). "Forty-five % sawdust depressed intake… Therefore, the percentage of sawdust fed to animal D was reduced to 35%." No gross lesions were attributed to the sawdust, but a calf fed a control diet (no sawdust) had a liver abscess.
- The calf fed 35% sawdust "displayed moderate abdominal distention, although clinical signs of impaction were not apparent. At slaughter, this animal's rumen contents were firmer and

appeared less moist than those from calves not fed sawdust and this animal had an enlarged omasum, “containing an accumulation of sawdust.”

Trial 2:

- “Consumption of concentrate mixtures containing 15% sawdust was comparable with that of rations containing no sawdust. No adverse effects of sawdust on health were noted.

Preliminary trials:

The authors used 10 calves (6 wk to 8 mo of age) and 1 mature rumen-fistulated cow. “With rations containing 25% sawdust, rumen and reticular mucosa appeared to remain normal; that percentage of sawdust did not induce rumino-reticular or omasal compaction, nor did it obstruct muscle sphincters. At 35% sawdust, rumen distention was noted; however, anorexia was observed in only one animal receiving this sawdust level. Postmortem examination revealed ruminal and omasal impaction in each of four calves receiving 35% sawdust as the only roughage. Anorexia was attributed to impaction of the pyloric sphincter. When 2.3 kg of baled bromegrass hay was fed daily to each of six heifers receiving a pelleted concentrate containing 35% sawdust, rumen distention was not apparent nor was health or appetite noticeably impaired. This ration was fed up to and during gestation. No apparent adverse effect on parturition was noted.”

ASPEN

Ground wood from various *Populus* tree species (e.g., quaking aspen) was thoroughly evaluated during the 1970’s by researchers from various institutions, e.g. Penn State, University of WI (Dairy Sci. Dept.; Madison, WI), Forest Products Lab (Madison, WI), SD State University (Brookings), and the University of Alberta. These research efforts led to the approval of ground aspen as a feed ingredient by AAFCO in 1980 (AAFCO, 2011). At one time, ground aspen feed was commercially available from a wood processing mill. Currently, ground aspen bark is being used and sold commercially (3XM Grinding and Composting, Olathe, CO; www.3xmgrinding.com/aboutus; Lohmeyer, 2013) and ground aspen wood is being sold in mixed feeds (Land O’Lakes Purina Feed®, e.g., Mazuri Browser Breeder; www.mazuri.com/mazuribrowserbreeder-5653.aspx).

The Billings Gazette (Unknown, 1975) reported that the SD Department of Game, Fish, and Parks and SD State University collaborated and successfully (no reports of negative health issues) fed steers a diet consisting of 45% ground aspen. Results indicated that “aspen-fed cattle gained weight about twice as fast as the alfalfa-fed group” and that cattle on a pervious trial were “tasty and tender.” Satter et al. (1970) fed lactating cows a mixed diet containing 32% aspen and reported: (1) aspen sawdust (consumed up to 4.8 kg/d or 7.6 g/kg of BW) was effective as a partial roughage substitute in a high-grain dairy ration; (2) if less dietary aspen would be equally as effective in complete pelleted dairy rations, aspen sawdust could become an attractive roughage substitute in areas where hay is expensive and difficult to obtain; and (3) no adverse effects on animal health. Satter et al. (1973) reported that a mixed diet containing 30% aspen sawdust did not negatively affect DMI or milk parameters in dairy cattle and concluded that it was “as effective as 50% long hay to maintain normal luminal [ruminal] acetate-to-propionate ratios.” In this trial, cows consumed up to 5.32 kg of aspen/d (8.38 g/kg of BW).

Research by Satter et al. (1970, 1973) was reinforced by Schingoethe et al. (1981) who reported that 30% aspen pellets (made from whole aspen trees) in a mixed diet, could be safely fed to lactating dairy cows. These cows safely (no adverse effects on animal health) consumed approximately 5.82 kg of aspen/d (9.77 g/kg of BW) and the authors reported that the aspen diet vs. control diet, increased ruminal pH and did not affect milk production. Authors also state that "the amount of fiber in the aspen ration may limit feed intake due to gut fill during peak of lactation. For this reason feeding more than 30% of the total ration DM as aspen would not be recommended, and even 30% … might be too high for cows in early lactation." *Dr. Whitney's comment*: This statement does not seem warranted, considering that total DMI and milk production were similar for cows fed diets with or without (control) aspen wood.

Mathison et al. (1986) fed sheep and cattle diet containing 42% ground aspen and 58% hay. Apparent digestibility of the aspen in the diet was approximately 25 to 37% and the cattle consumed approximately 2.52 kg of aspen/d (6.3 g/kg of BW). Authors report that the cattle consumed a total of 6 kg of the hay and aspen mixture/d, which was "77% of the intake … when only hay was fed; sheep ate "61% as much of the aspen-hay mixture" vs. hay alone. No adverse effects on animal health were reported and the authors concluded, "… the feeding value of unprocessed aspen in ruminant diets is less than 75% of the feeding value of straw."

JUNIPER

Very little research related to the use of woody products as animal feed ingredients was done between 1980 and 2007. However, in 2008, a TX A&M AgriLife Research (San Angelo) trial evaluated effects of replacing CSH with air-dried redberry juniper leaves in Rambouillet lamb feedlot diets (Whitney and Muir, 2010). In this trial, a maximum of 30% juniper leaves was included in a mixed diet that fed for 28 d; lambs were transitioned onto a mixed diet containing 15% juniper for an additional 49 d. Maximum daily juniper leaf consumption was 357 g (11.9 g of juniper/kg of BW). No negative effects on animal health were reported based upon visual assessment, but replacing 50% of the CSH with redberry juniper leaves increased lamb performance, compared to diets containing CSH or juniper as the sole roughage source. Authors concluded, "Results indicate that air-dried redberry juniper leaves can effectively be used as a roughage source and can replace all of the CSH in lamb feedlot rations, but may reduce intake and consecutively growth at greater inclusion levels. Authors also suggested that secondary compounds and their interactions with nutrients should be considered when evaluating the nutrient requirements of the animal and its rumen microbial populations. Utilization of juniper as a roughage source could provide ranchers with a readily available on-site feed resource and possibly lessen the negative impact of this undesirable invasive brush species…"

Results from Whitney and Muir (2010) led to a trial in which mixed diets containing ground juniper leaves and small stems were fed to lambs (Whitney et al., 2014). Whitney et al. (2014) evaluated effects of using ground redberry juniper (leaves and stems) in Rambouillet wether lamb (n = 45) feedlot diets on growth, blood serum, fecal, and wool characteristics. In a randomized design study with 2 feeding periods (Period 1 = 64% concentrate diet, 35 d; Period 2 = 85% concentrate diet, 56 d), lambs were individually pen-fed isonitrogenous corn DDGS-based diets where 0% (0JUN), 33% (33JUN), 66% (66JUN), or 100% (100JUN) of the oat hay was replaced by juniper (**Table 6**). During Period 1, lambs consumed approximately 367 g of juniper/d (12.2 g/kg of BW; **Table 7**). During Period 2, lambs consumed approximately 207 g of juniper/d (4.73 g/kg of BW; **Table 7**). Serum urea N (**SUN**; 18 to 31 mg/dL; **Table 8**) increased quadratically ($P = 0.01$) and fecal N increased linearly ($P = 0.004$), which was partially be attributed to greater dietary urea and CT intake. Most wool characteristics were not affected, but wool growth/kg of BW decreased quadratically (*P* = 0.04) as percentage of juniper increased in the diet. Overall, results indicated that replacing all of the ground oat hay with ground juniper in lamb growing and finishing diets is not detrimental to animal performance or health. The authors concluded:

> "… Results indicate that ground juniper leaves and stems can effectively replace all of the oat hay in corn DDGS-based growing and finishing diets without negatively affecting animal health [evaluated by visual assessment], performance, or wool characteristics. However, using a combination of juniper and oat hay during the growing period (Period 1; high roughage diet) increased growth performance and reduced total feedlot costs as compared to using juniper or oat hay as the sole roughage source."

Table 6. Ingredient, chemical composition (% DM basis), and digestibility of treatment diets

	Diet[1]							
	Period 1				Period 2			
Item[2]	0JUN	33JUN	66JUN	100JUN	0JUN	33JUN	66JUN	100JUN
Ground juniper	–	12.0	24.0	36.0	–	5.0	10.0	15.0
Oat hay	36.0	24.0	12.0	–	15.0	10.0	5.0	–
Dried distillers grains	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Cottonseed meal	–	–	–	–	–	–	–	–
Sorghum grain	14.15	14.55	14.93	15.32	35.05	35.19	35.31	35.43
Molasses	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Limestone	2.75	2.2	1.67	1.13	2.85	2.62	2.40	2.19
Ammonium chloride	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Salt	0.9	0.9	0.9	.9	0.9	0.9	0.9	0.9
Mineral premix	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Urea	–	0.15	0.30	0.45	–	0.09	0.19	0.28
Nutrient Composition, %								
DM	91.8	91.9	91.9	92.0	91.0	91.7	91.8	91.8
CP	22.8	22.9	22.5	22.0	23.3	24.1	23.2	23.9
ADICP	0.7	0.8	1.0	1.1	0.7	1.3	1.8	1.7
NDF	33.8	32.9	30.7	29.7	28.0	27.2	26.1	24.2
ADF	17.2	18.1	18.0	19.0	13.8	14.6	15.3	13.9
Crude fat	7.0	7.6	8.0	8.2	7.5	8.0	7.9	8.2
Lignin	3.8	5.3	5.6	7.7	2.5	3.5	5.2	5.2
Ca	1.2	1.1	1.1	1.0	1.4	1.4	1.5	1.3
P	0.6	0.6	0.6	0.6	0.62	0.67	0.66	0.68
Ca:P	2.5	1.8	1.8	1.7	2.3	2.1	2.3	1.9
Ash	10.1	8.7	8.4	7.7	10.2	9.7	9.7	7.5
Volatile oil, %	0.04	0.11	0.07	0.11	0.04	0.03	0.03	0.03
True IVDMD, %	74.5	73.8	71.9	72.2	81.1	81.5	80.0	79.6

[1]Treatment diets were isonitrogenous, non-agglomerated feedlot growing rations containing ground juniper that replaced 0% (0JUN), 33% (33JUN), 66% (66JUN), or 100% (100JUN) of the ground oat hay. During Period 1 (d 0 to 35), lambs were fed a 64% concentrate ration. Lambs were transitioned over 4 d into Period 2 (d 36 to 91) onto an 85% concentrate ration.

[2]ADICP = acid detergent insoluble CP; true IVDMD = true 48-h *in vitro* dry matter digestibility.

Table 7. Effects of replacing oat hay with ground juniper on lamb performance

	Diet[1]					*P*-value[2]	
Item/d[3]	0JUN	33JUN	66JUN	100JUN	SEM[4]	Linear	Quadratic
Period 1							
DMI, kg; overall	0.96	1.38	1.30	1.02	0.07	0.73	<0.001
ADG, kg; overall	0.14	0.22	0.22	0.13	0.02	0.41	<0.001
G:F, kg/kg; overall	0.15	0.16	0.17	0.12	0.01	0.17	0.007
Period 2							
DMI, kg/d; d 42 to 91	1.28	1.34	1.45	1.38	0.06	0.19	0.31
ADG, kg; d 42 to 91	0.24	0.25	0.25	0.28	0.01	0.03	0.29
G:F, kg/kg; overall	0.19	0.18	0.18	0.20	0.01	0.41	0.06
Entire trial, d 0 to 91							
DMI	1.16	1.34	1.39	1.24	0.06	0.27	0.004
ADG	0.20	0.24	0.24	0.22	0.01	0.30	0.02
G:F	0.18	0.18	0.17	0.18	0.01	0.87	0.68
BW, kg; final shorn	43.5	46.1	46.6	43.8	1.1	0.74	0.01

[1]Treatment diets were isonitrogenous and non-agglomerated, and contained ground juniper that replaced 0% (0JUN), 33% (33JUN), 66% (66JUN), or 100% (100JUN) of the ground oat hay.

[2]Linear and quadratic orthogonal contrasts.

[3]During Period 1 (d 0 to 35), lambs were fed a 64% concentrate ration. Lambs were transitioned over 4 d into Period 2 (d 36 to 91) onto an 85% concentrate ration.

[4]SEM = greatest standard error of the mean.

Table 8. Effects of dried distillers grains with solubles (DDGS) and replacing oat hay with ground juniper on lamb serum urea N, IGF-1, Ca, and P, and fecal P and N during Period 2 (d 36 to 91)

	Diet[1]						*P*-value[2]			
Item[3]	CNTL	0JUN	33JUN	66JUN	100JUN	SEM[4]	CNTL vs. 0JUN	CNTL vs. DDGS	Linear	Quadratic
Blood serum										
Urea N, mg/dL	31	19	22	21	18	1	<0.001	<0.001	0.39	0.01
IGF-1, ng/mL	225	162	195	196	192	17	0.01	0.04	0.24	0.28
Ca, %	9.7	9.2	9.8	10.0	10.3	0.4	0.32	0.68	0.03	0.63
P, %	8.4	8.5	8.6	8.7	8.6	0.4	0.84	0.56	0.73	0.78
Fecal, d 91										
P, %	0.69	0.77	0.84	0.76	0.87	0.06	0.45	0.13	0.42	0.77
N, %	2.9	2.4	2.5	2.5	2.7	0.08	<0.001	<0.001	0.004	0.17

[1]Treatment diets were isonitrogenous and non-agglomerated, and contained ground juniper that replaced 0% (0JUN), 33% (33JUN), 66% (66JUN), or 100% (100JUN) of the ground oat hay. An additional control diet (CNTL) similar to 0JUN, but using sorghum grain vs. DDGS, was used to evaluate any negative effects of using 40% DDGS.

[2]Orthogonal contrasts. CNTL vs. DDGS = CNTL vs. average of DDGS-based diets (0JUN, 33JUN, 66JUN, and 100JUN). Linear and quadratic contrasts of 0JUN, 33JUN, 66JUN, and 100JUN diets.

[3]Lambs were transitioned over 21 d onto their respective diet. During Period 1 (d 0 to 35), lambs were fed a 64% concentrate ration. Lambs were transitioned over 4 d into Period 2 (d 36 to 91) onto an 85% concentrate ration. Blood serum was analyzed on d 77, 84, and 91 and average values are presented since no treatment × d interactions ($P > 0.12$) were observed. Fecal P and N were analyzed only on d 91.

[4]SEM = greatest standard error of the mean.

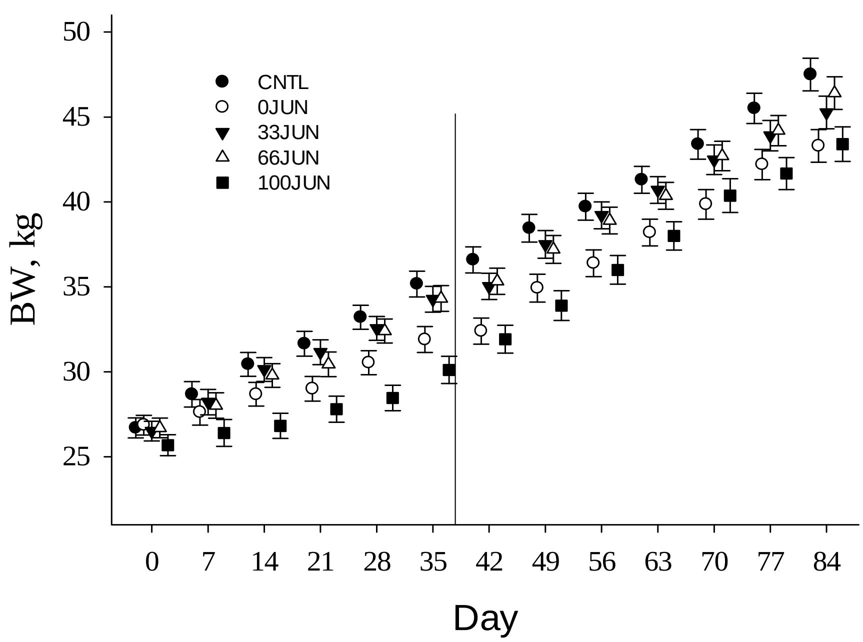


Fig. 6. Effects of replacing oat hay with ground juniper on lamb BW. During Period 1 (d 0 to 35), lambs were fed a 64% concentrate ration. Lambs were transitioned over 4 d into Period 2 (d 36 to 91) onto an 85% concentrate ration.

Data (*has not been statistically analyzed*) from the most recent AgriLife Research trial, suggested that ground redberry juniper trees (entire tree) can be used in pregnant ewe supplements without negatively affecting ewe health (evaluated by visual assessment) or lambing, or lamb birth weight or weaning weight (**Table 10**; Stewart, et al., unpublished data). Approximately 56 d after conception, mature, pregnant Rambouillet ewes (n = 28) were randomly allocated to individual pens, fed a basal hay diet and assigned to a pelleted treatment supplement that contained either 0% (CNTL), 18% (18JUN), 36% (36JUN), or 54% (54JUN) ground juniper (**Table 9**); supplements were fed until lambing. Maximum juniper intake for ewes supplemented with 54JUN was 463.3 g of juniper/d (5.5 g of juniper/kg of BW).

Table 9. Ingredient and chemical composition (DM basis) pelleted treatment supplements

	Supplement			
Item[1]	CNTL	18JUN	36JUN	54JUN
Ground juniper	−	18	36	54
Ground hay	54	36	18	−
DDGS	29.75	29.75	29.75	29.75
Cottonseed meal	3	3	3	3
Sorghum grain	6	6	6	6
Molasses	4	4	4	4
Ammonium chloride	0.75	0.75	0.75	0.75
Salt	1	1	1	1
Mineral premix	1.5	1.5	1.5	1.5
Chemical composition, %				
CP	16	15.3	14.5	13.9
NDF	36	38	41	42
ADF	19	24	30	35
CT	0	0.76	1.51	2.27
Volatile oil	0	0.22	0.41	0.72
tIVDMD, %	75.5	72.5	64.7	50.1

[1]CT calculated based only on CT in the juniper portion in the diet. Ground hay = sorghum sudangrass hay; DDGS = corn dried distillers grains with solubles.

Table 10. Effects of using ground redberry juniper in ewe supplements on DMI, ewe milk quality, lamb survival, birth weight, and growth performance[1]

Item[3]	Supplement Group[2]			
	CNTL	18JUN	36JUN	54JUN
BW of ewe at maximum juniper intake, kg	97.5	94.8	78.5	84.8
Maximum supplement intake, g/d	1,006	992	821	858
Hay intake, g/d	1,241	1,213	1,288	1,526
Total DMI, g/d (basal + supplement)	2,247	2,205	2,109	2,384
Maximum juniper intake, g/d	0	179	296	463
Maximum juniper intake, g/kg of BW	0	1.88	3.77	5.46
CT intake, g/d	0	7.5	12.4	19.5
CT intake, g/kg BW	0	0.08	0.16	0.23
Volatile oil intake, g/d	0	2.18	3.37	6.18
Volatile oil intake, g/kg BW	0	0.02	0.04	0.07
Milk fat, %	3.8	3.3	3.5	3.2
Milk protein, %	3.5	3.5	3.5	3.4
# lambs that died within 14 d of parturition	2	2	0	1
Lamb BW				
0 d after parturition	5.1	5.1	5.0	5.2
14 d after parturition	12	9.7	8.4	11.8
50 d after parturition (weaning)	22.8	21.1	22.0	21.2
Lamb ADG				
0 to 14 d after parturition	0.48	0.33	0.24	0.46
14 to 50 d after parturition	0.30	0.32	0.36	0.28

[1]**Note: Data has not been statistically analyzed or published.**

[2]Ewes fed a basal hay diet and supplemented daily with one of 4 pelleted feeds that contained either 0% (CNTL), 18% (18JUN), 36% (36JUN), or 54% (54JUN) ground juniper.

[3]Intake data represent the day of maximum consumption of supplement by a ewe within each supplement treatment group. Lamb BW and ADG represent only the lambs that were born as single lambs.

In another TX A&M AgriLife Research trial, Whitney et al. (2013) reported that lambs could be fed a mixed, pelleted diet containing 30% ground juniper leaves and stems without negatively affecting animal health; maximum juniper intake was 273 g/d (12.2 g of juniper/kg of BW).

Section 8 Conclusion:

Trials conducted over the past 100 yr that have evaluated the use of tree fodder (leaves and small pliable twigs) and ground wood (whole trees and sawdust from mills), along with numerous research summaries, suggest that ground juniper trees can be safely used in ruminant animal diets. Any negative effects of using ground wood in mixed feeds for ruminant animals are generally attributed to reduced DMI, thus reduced growth performance. In addition, various literature suggests that CT or volatile oil can reduce DMI in a variety of forages and feed ingredients, thus reduced intake of feeds containing ground juniper is not extraordinary.

The literature search revealed only one case of impaction (Cody et al., 1972); however, other roughage feed ingredients can also cause compaction and thus, the proposed ingredient does not pose any extraordinary health issues. It should be noted that feeding ground juniper to ruminant animals (which are observed at least once/day while on trial) has not resulted in any negative health effects (evaluated by visual assessment) in any trials conducted by the TX AgriLife Research Nutrition program, San Angelo. It should also be noted that during these trials, even though DMI and ADG has been reported to be less in lambs fed diets with juniper vs. lambs fed diets without juniper (control), ADG has never been negative (always remained in a positive energy balance).

9. Summary of Safety Assessment: Toxicology

NOTE: Details of each trial summarized in this section, along with other supportive research trials, can be found in the Appendices.

Like thousands of plant species, juniper is known to contain CT and volatile oil (**Section 9.1**). **Section 9.2** briefly addresses effects of CT and volatile oil in non-ruminant animals (e.g. mice), even though non-ruminant toxicity of CT and volatile oil is not relevant to ruminant animals due to numerous factors (e.g., ruminal digestion, regurgitation, re-mastication) that are discussed in **Section 9.3**; this is especially true for toxicology studies that inject a secondary compound directly into the bloodstream of the animal. **Section 9.3** discusses effects of ruminant animals consuming juniper material, thus consuming CT and volatile oil. **Section 9.3** also addresses unsupported claims by a subset of literature, which report that volatile oil contained within juniper is “toxic” to ruminant animals. These authors also commonly made incorrect inferences related to the data and conclusions of their cited references. At times, they also incorrectly attributed reduced DMI to “toxicity” and failed to recognize that: (1) by itself, reduced DMI (vs. control animals) does not define toxicity; (2) other high-fiber feed ingredients (e.g., aspen, CSH, various forage and hay varieties), some of which contain plant secondary compounds, can also reduce DMI at times, but are not considered “toxic”; and (3) toxicity occurs when consumption of the compound exceeds the biotransformation capacity of that compound (Torregrossa et al., 2012), in which these authors (nor their cited references) did not evaluate.

A thorough literature review revealed that there is not a single documented case, in a properly designed trial, in which consumption of *J. pinchotii* or *J. ashei* material (leaves, stems, whole tree) negatively affected ruminant animal health as assessed by visual evaluation of the live animal or internal organs or by blood enzyme analysis). This conclusion is supported by published research trials, some of which were completed by Dr. Whitney’s AgriLife Research Nutrition Program.

Overall, **Section 9** supports the fact that consumption of ground juniper by ruminant animals is safe and poses no extraordinary animal health issues. Estell et al. (1998) stated, “Monoterpenes [contained within volatile oil] are typically toxic to insects but safe for consumption by mammals (Rice and Coats, 1994). These authors also stated, “Because many monoterpenes are classified as “Generally Recognized as Safe” and are natural plant products that are abundant and easily synthesized (Rice and Coats, 1994), they are potential candidates for use in manipulating feeding patterns of browsing herbivores.” Approved feed ingredients currently exist (e.g., sorghum grain, CSH, lespedeza hay, ground aspen wood) that contain similar to greater concentrations of CT than what has been reported in *J. pinchotii* and *J. ashei*. Thus, consumption of CT and volatile oil is a normal occurrence in ruminant animal production. Furthermore, ground juniper could actually be considered safer for the animal than many of the approved feed ingredients, because juniper is not known to contain common secondary compounds that pose significant health issues such as gossypol (e.g., cottonseed products), coumarin (e.g., sweat clover), saponins (e.g., alfalfa), and nitrates (e.g., oat hay, alfalfa, sudangrass, sweetclover).

Multiple databases were searched (from early 1900's to current) for **Section 9**:

1. U.S. National Library of Medicine (PubMed/MEDLINE, TOXNET databases)
2. Carcinogenic Potency Database (CPDB; http://toxnet.nlm.nih.gov/cpdb/chemnameindex.html)
3. Cancerlit
4. Search engines of Texas A&M University Library, Goggle, and various individual journals

***Examples of keywords used during the search*:**

α–pinene, α–terpinene, camphene, camphor, carcinogen, condensed tannins, ellagitannins, essential oil, evergreen, hydrolysable tannins, *Juniperus*, juniper, lignin, limonene, monoterpene, myrcene, phytotoxin, plant secondary compounds, polyphenol, polyphenolics, proanthocyanidins, sabinene, safrole, terpene, terpenoid, toxicosis, trans-sabinene hydrate, volatile oil

9.1 Overview: Secondary compounds

Juniper contains condensed tannins, which are phenolic compounds (also defined as anthocyanidins or proanthocyanidins) and volatile oil (correctly and incorrectly defined as essential oil, monoterpene, terpene, terpenoid). Condensed tannins and volatile oil are defined as plant secondary compounds or secondary metabolites, because they are not directly essential for life, growth, or development of the plant (Fraenkel, 1959; Singleton, 1981; Kutchan and Dixon, 2005). Lignin is also a phenolic compound that is "nearly universal in higher plants" and even though it can be negatively related to digestibility, it is not directly considered to have any toxic effects (Singleton, 1981).

A complete, comprehensive review of the chemistry, metabolism, and function of all plant secondary compounds is not warranted in this proposal. However, secondary compounds have been extensively studied and thorough reviews are available (e.g., Kingsbury, 1964; Singleton, 1981; Hemingway and Laks, 1992; McGarvey and Croteau, 1995; Bravo, 1998; Yoshida et al., 2000; Humphrey and Beale, 2006; Serrano et al., 2009; Makkar et al., 2010; Salminen and Karonen, 2011; Cheynier et al., 2012).

Thousands of secondary compounds are distributed throughout the plant kingdom and certain compounds can be more toxic to herbivores than others, such as hydrolysable tannins (**HT**; in oak leaves: *Quercus* spp.), flavonoids (in mulberry leaves: *Morus alba*), gossypol (in whole cottonseed: *Gossypium* spp.), hymenoxon (in bitterweed: *Hymenoxys odorata*) and coumarin (in sweat clover: *Melilotus* spp.). The prosed ingredient is not known to contain any of these compounds and are therefore, not discussed. A thorough examination of the literature revealed that CT and volatile oil are the only secondary compounds of any significance in *J. pinchotii* and *J. ashei* plants. Hydrolysable tannins (derivatives of gallic acid) are phenolic compounds that are common in many woody plant species and are of particular interest in regards to negatively effecting animal health (Murdiati et al., 1990; Reed, 1995; Waghorn and McNabb, 2003). However, HT are not discussed because coniferous plants (e.g. *Juniperus* spp.) are not known to synthesize HT (Kubitzki and Gottieb, 1984; reviewed by Salminen and Karonen, 2011; Ann Hagerman, Ph.D. and toxicologist, personal communication).

Labdane acids (a diterpene) have been detected in *J. communis* (Feliciano et al., 1991). *Pinus ponderosa* contains labdane acids and its consumption (leaves and/or bark) has been associated with reproductive problems in cattle (Welch et al., 2013). The proposed ingredient (*J. pinchotii* and *J. ashei*) does not contain any significant quantities of labdane acids (< 0.14% of plant DM, Stewart et al., 2014; Kevin Welch and Dale Gardner, Ph.D., Personal Communication, May, 3, 2013). In addition, no reports (published or unpublished) were discovered during a thorough literature review, which would suggest that *J. pinchotii* or *J. ashei* cause any reproductive concerns. In contrast, research trials with goats (Owens et al., 2010) and sheep (Stewart et al., unpublished data) have reported that feeding the proposed ingredient does not negatively affect reproduction, fetal development, or progeny; details presented below.

9.2 Non-ruminant toxicity

A comprehensive review of the toxicity of CT and volatile oil in non-ruminant animals is not warranted in this proposal. This is especially true for toxicology studies that inject a secondary compound directly into the bloodstream. Furthermore, many of the trials evaluating the effects of CT and volatile oil in non-ruminant animals, dose with much greater concentrations than what a ruminant animal could actually consume at a single point in time, during a single meal, or within a given day.

Example 1: The approximate lethal dose (**ALD**) of myrcene has been reported to be 5.06 g/kg BW and 11.4 g/kg BW in mice and rats, respectively (Paumgartten et al., 1990). Maximum concentration of myrcene in redberry juniper leaves was found to be 9.1% of total volatile oil (Riddle et al., 1996; **Table 2**); thus, a ruminant animal would need to consume **56 to 125 g** of volatile oil/kg BW [5.06 g/0.091 and 11.4 g/0.091]. The maximum volatile oil concentration = 4.9% (Adams, 1987; **Table 2**); thus, a ruminant animal would need to consume **1,135 to 2,557 g** of juniper/kg BW within a given day (greater than 114% of the animal's BW), which is impossible.

Example 2: As cited in Wagner (2006) the LD50 oral dose of camphor has been reported to be 1.31 g camphor/kg BW in mice (Gakuho, 1975, "as cited in RTECS, 2005"). Maximum concentration of camphor in redberry juniper leaves was found to be 64.9% of total volatile oil (Adams, 2011; **Table 2**); thus, a ruminant animal would need to consume **2.02 g** of volatile oil/kg BW [1.31 g/0.649]. The maximum volatile oil concentration = 4.9% (Adams, 1987; **Table 2**); thus, a ruminant animal would need to consume **41.2 g juniper**/kg BW [2.0185 g oil/0.049] within a given d, which has never been reported.

Even if concentrations of CT and volatile oil used in non-ruminant toxicology trials represented what a ruminant animal would actually consume in a given meal or within a given day, LD50 or ALD values would not be applicable to ruminant animals because of the complexity of the ruminant system, e.g., microbial digestion, eructation and re-mastication. Refer to **Section 9.3** for a discussion related to effects of animal and rumen physiology on the biological activity of CT and volatile oil.

9.3 Ruminant toxicity

Multiple trials have shown that ground juniper (thus, CT and volatile oil) is safe for consumption by ruminant animals when used according to good feeding practices and the intended use as cited in the enclosed proposal. This conclusion is based upon the following:

1. A thorough review of the literature. For example, Estell et al. (1998) stated, "Monoterpenes are typically toxic to insects but safe for consumption by mammals (Rice and Coats, 1994). Because many terpenes are classified as "Generally Recognized as Safe" and are natural plant products that are abundant and easily synthesized (Rice and Coats, 1994), they are potential candidates for use in manipulating feeding patterns of browsing herbivores.";
2. Personal experiences with feeding diets containing ground juniper to ruminant animals. No negative animal health issues (visual appraisal) related to feeding ground juniper material in mixed diets have been observed by Dr. Whitney in any Texas A&M AgriLife research trials. Within these trials, average daily DMI and ADG has at times been less in animals consuming diets with juniper vs. without juniper. However, (1) reduced animal growth performance (when compared to control animals) has been mainly attributed to fiber characteristics of ground juniper and not a result of post-ingestive adverse health effects due to CT or volatile oil and (2) ADG has never been negative (animals have always remained in a positive energy balance);
3. Numerous approved feed ingredients that contain equal or greater concentrations of CT or volatile oil than what has been reported in the proposed ingredient (e.g., CT in aspen and various varieties of hulls and straw;
4. The ALD of various terpenes are reported to be much greater than what an animal would actually consume; this is especially true when comparing ALD in non-ruminants with ruminants, because of rumen microbial terpene digestibility and terpenes being further volatilized as ruminants regurgitate, remasticate, and re-swallow feed;
5. Volatile oil is rapidly and extensively degraded in the rumen. Cluff et al. (1982) reported that monoterpenoids in the rumen of mule deer were 80% less than what would be expected from the consumption of sagebrush. These authors suggest that *in vitro* trials are not very representative of actual feeding trials; e.g., monoterpenes have been reported to inhibit rumen microorganisms while feeding trials have reported that "big sagebrush is a highly digestible winter forage." Welch and Pederson (1981) reported that the digestibility of big sagebrush was as great as 64.8% and predicted that monoterpenoids are lost from the rumen. These authors also stated, "Apparently 38.5°C, which is close to the normal body temperature …, is sufficient to volatilize monoterpenoids (Moen, 1973)." Furthermore, White et al. (1982) reported that 77% of the monoterpenoids from sagebrush were lost "in the stomach contents of … rabbits…" and that this was due to the volatilization of monoterpenoids during mastication and ingestion. Malecky and Broudiscou (2009) reported that specific terpenes are rapidly and readily degraded by rumen microflora; out of the 9 terpenes studied, 7 terpenes were over 75% digested and 3 were over 90% digested within the first 6 hr of incubation. Numerous other studies reported that terpenes are readily degraded by rumen microorganisms (Chizzola et al., 2004; Broudiscou, et al., 2007; Malecky et al., 2009).
6. Condensed tannins are poorly, to not at all, absorbed in chickens (Jimenez-Ramsey et al., 1994) and sheep (Terrill et al., 1994), thus reducing CT bioavailability in the animal;

Furthermore, the proposed ingredient could actually be considered "safer" than many approved feed ingredients because it is does contain common secondary compounds that pose significant health issues such as gossypol (e.g. cottonseed products), coumarin (e.g. sweat clover), saponins (e.g. alfalfa), and nitrates (e.g. oat hay, alfalfa, sudangrass, sweetclover).

It has been interesting to discover through the intensive review process required for this proposal, that some authors have generally cited previously reported "assumptions" as "facts" when discussing effects of volatile oil on ruminant animal health. This phenomena is unfortunately a common occurrence (Ioannidis, 2005; Vera-Badillo et al., 2013) in research and a combination of many factors: "research bias," "experimental bias," and/or "confirmation bias." Therefore, it appears that "research bias" has led to the unsupported conclusion by some researchers that consuming *J. pinchotii* or *J. ashei* leaves does, as a matter of fact, negatively affects animal health. Therefore, when necessary, results and literature that was incorrectly referenced by these authors to construct their conclusions, are discussed in detail.

In some instances, this subjective trend originated from published trials that made these assumptions even when effects of juniper consumption (or volatile oil drench) on animal health was not directly evaluated or when invalid experimental designs were used, e.g., no control group, no statistical analysis, and/or confounding factors. Even though there is not a single documented case, in a properly designed trial, in which consumption of fresh *J. pinchotii* or *J. ashei* leaves by ruminant animal negatively affects health, a few authors have suggested that the volatile oil contained with juniper leaves is "toxic." However, these authors failed to recognize that:

1. Dosing an animal at a single point in time with a plant secondary compound equal to the amount the animal would consume over the course of a given day, does not represent normal meal patterns, especially in ruminant animals. Further, Boyle and Dearing (2003) stated, "It is possible that the differences among studies are a function of the disparate species examined (woodrats, voles, sheep, …)" and "… it is plausible that the disparate results stem from the use of secondary compounds in foliage versus purified compounds.";
2. Toxicity is not defined solely on a reduction in DMI (when compared to control animals), especially when ADG is positive, when DMI for control animals also declines, when total daily DMI (basal diet + juniper leaves) is not reduced, or when *in vitro* methods are used to evaluate effects of volatile oil but the *in vitro* volatile oil concentrations were much greater than those found in fresh juniper leaves and/or greater than what an animal would ever possibly consume within a given day, especially at a single point in time;
3. "A central concept of toxicology is that effects are dose-dependent; even water can lead to water intoxication when taken in too high a dose" (Wikipedia). Thus, even though juniper volatile oil could "technically" be defined as "toxic," so could all other substances when the dose is in "excess" for a particular biological function;
4. When only a few selected indices "suggest" potential toxicity, this does not confirm toxicity. This is especially true when these indices are not specific to the organ in question (e.g., liver) or when other indices remained within normal physiological limits and/or were either less than or equal to control animals.

Additional problems were discovered during the literature review for this proposal include authors not citing all of the needed information to determine consumption of juniper leaves (and thus, volatile oil) as a percentage of total DMI or on a g/kg of BW basis such as:
(1) animal BW;
(2) ingredient DM;
(3) percentage of volatile oil in the juniper leaves;
(4) basal diet DMI;
(5) if intake data is reported on an as-fed or DM basis.

Literature summary:

Table 14 and Appendices C, D, E, and F report consumption of various feeds that contain CT, volatile oil, or both, when those plants **are mixed** with other feed ingredients.

Table 13 and Appendix G report consumption of various feeds that contain CT, volatile oil, or both, when those plants are **not mixed** with any other feed ingredients.

Dry matter, nutrients, and secondary compound concentrations of juniper and all other feed ingredients were based upon most relevant literature, data from Dr. Whitney's Nutrition Laboratory, or both. When needed to calculate intake (g/kg of BW) NRC (2000; cattle) and NRC (2007; small ruminants) was referenced to determine animal BW and approximate DMI according to information provided by the authors, e.g., 6-wk old goats fed to meet maintenance protein and energy requirements.

In regards to safety assessment, the most relevant trials from those listed in **Tables 13 and 14** and from those discussed in detail in the Appendices are summarized below. No research trials were discovered that evaluated ADL or LD50 values for ground juniper, CT, or volatile oil in ruminant animals. However, in research trials completed by the TX A&M AgriLife Research Nutrition Program, in which ground juniper was mixed with other feed ingredients and fed to ruminant animals, no apparent animal health issues have been directly observed; determined mainly by daily visual appraisal of the animal and ADG. For example (see below and **Appendix C** for more information), Whitney et al. (2014) did not report any negative health effects (daily visual appraisal and negative ADG were positive;) when feedlot lambs consumed mixed diets containing juniper leaves and small stems, which resulted in approximately 22.9 g of CT consumed/day (0.76 g/kg of BW); these lambs also consumed 1.12 grams of volatile oil/day (0.037 g/kg of BW). Whitney et al. (2013) also did not report any negative health effects (daily visual appraisal and negative ADG) when feedlot lambs consumed mixed diets containing juniper leaves and small stems, which resulted in approximately 19.2 g of CT consumed/day (0.86 g/kg of BW); these lambs also consumed 0.55 grams of volatile oil/day (0.02 g/kg of BW). Stewart et al. (unpublished data from Dr. Whitney's Nutrition program) fed supplements containing ground whole redberry juniper trees (up to 54% juniper) to pregnant ewes from day 56 to parturition. Maximum CT intake was 19.5 g/day (0.23 g/kg of BW) and volatile oil intake was 6.18 g/day (0.07 g/kg of BW). In this trial, collective evaluation of ewe ADG, daily DMI of the basal hay diet and juniper-based supplement, and lamb birth weight and ADG, suggested that consumption of juniper (thus, CT and volatile oil) was not detrimental to animal health.

Others have also reported that goats can consume up to 69 g CT/d (1.7 g CT/kg of BW, DM basis) when consuming Sericea lespedeza hay (6.5% CT, DM basis), with no reported health problems (visual assessment or negative ADG; Terrill et al., 2007). Chafton fed lambs Sericea lespedeza hay (10.7% CT, DM basis), resulting in 128 g of CT intake/d (3.74 g CT intake/kg of BW, DM basis) with no reported health problems (visual assessment, or negative ADG). In addition, DMI and ADG increased when kid goats were fed a diet containing 75% Sericea lespedeza (6.5% CT, DM basis) vs. 75% bermudagrass hay, resulting in 62 g CT intake/d (3.28 g CT intake/kg of BW; Moore et al., 2008). Most literature reports that juniper contains 5 to 8% CT (DM basis). If juniper contains 5% CT, then a 40-kg goat would have to consume approximately 2,992 g of juniper/d to consume 3.74 g CT/kg of BW [see above for Serecia lespedeza hay intake]), which equals juniper being consumed at 7.46% of BW, which is impossible. Even if juniper contained the maximum reported concentration of 10.2% CT (DM basis; Adams et al. (2013b), the same 40-kg goat would have to consume approximately 1,466 g of juniper/d to consume 3.74 g CT/kg of BW), which equals juniper being consumed at 3.66% of BW. This is highly unlikely, especially when at least 1 other feed ingredient is mixed with the ground juniper.

Consumption of target plant when mixed with at least one other feed ingredient

Whitney et al., 2014. Effects of using ground redberry juniper and dried distillers grains with solubles in lamb feedlot diets: Growth, blood serum, fecal, and wool characteristics. J. Anim. Sci. 92:1119−1132.

* *This study was completed by Dr. Whitney's TX AgriLife Research Nutrition Program, San Angelo.*

This trial evaluated effects of using ground redberry juniper (*J. pinchotii*: leaves and stems) in Rambouillet wether lamb (n = 45) feedlot diets on growth, blood serum, fecal, and wool characteristics. In a randomized design study with two feeding periods (Period 1 = 64% concentrate diet, 35 d; Period 2 = 85% concentrate diet, 56 d), lambs were individually pen-fed isonitrogenous corn DDGS-based diets where 0% (0JUN), 33% (33JUN), 66% (66JUN), or 100% (100JUN) of the oat hay was replaced by juniper (**Table 6**).

During the spring, redberry juniper branches < 3.6 cm diameter were cut from mature redberry juniper trees. Within 2 d, branches were mechanically chipped and dried and fine-ground to pass a 4.76-mm sieve; oat hay was ground to pass a 6.35-mm sieve. During Period 1, lamb DMI, ADG, and G:F quadratically increased ($P < 0.01$; **Table 7**) as juniper increased in the diet. During Period 2, DMI was similar ($P > 0.19$), ADG increased linearly ($P = 0.03$) and G:F tended to decrease quadratically ($P = 0.06$) as juniper increased in the diet (**Table 7**). Serum urea N (SUN) increased quadratically ($P = 0.01$) and fecal N increased linearly ($P = 0.01$), which was partially be attributed to greater dietary urea and CT intake (**Table 8**). Most wool characteristics were not affected, but wool growth/kg of BW decreased quadratically ($P = 0.04$) as percentage of juniper increased in the diet. A treatment × day interaction was observed for BW ($P = 0.004$; **Fig. 6**) and within the DDGS-based diets, lamb BW increased quadratically ($P < 0.01$) as percentage of juniper incrementally increased in the diet. Results indicated that replacing all of the ground oat hay with ground juniper in lamb growing and finishing diets is not detrimental to animal performance or health (assessed by visual appraisal and ADG).

Conclusions:

"… Results also indicate that ground juniper leaves and stems can effectively replace all of the oat hay in corn DDGS-based growing and finishing diets without negatively affecting animal health, performance, or wool characteristics. However, using a combination of juniper and oat hay during the growing period (Period 1; high roughage diet) increased growth performance and reduced total feedlot costs as compared to using juniper or oat hay as the sole roughage source. The economics of processing, storing, and mixing two roughage sources will need to be considered, but it appears that the most economical feeding regimen in this trial would have been to feed 66JUN during the growing period and then feed 100JUN during the finishing period."

Summary Notes:

- % total CT in ground juniper = 6.0%
- % total CT in ground sorghum grain = 0.55%
- No adverse effects on animal health were reported in either feeding period.

Period 1:

- Values reported below are on a DM basis.
- tIVDMD: ground oat hay = 57.4%; ground juniper = 55%

- Average total daily DMI for lambs fed 100JUN = 1,020 g/d
- Ground juniper daily intake for lambs fed 100JUN =
  367 g of juniper/d = **12.2 g** of juniper/kg BW
- % ground juniper in 100JUN diet = **36%**
- Estimated total CT intake for lambs fed CNTL, 0JUN, 33JUN, 66JUN, and 100JUN =
  3.5, 0.8, 11.0, 19.8, and 22.9 g CT/d, respectively, which =
  0.10, 0.02, 0.32, 0.58, and 0.76 g CT/kg BW, respectively."
- Estimated total volatile oil intake for lambs fed CNTL, 0JUN, 33JUN, 66JUN, or 100JUN =
  0.04, 0.42, 1.56, 0.94, and 1.12 g oil/d, respectively, which =
  0.001, 0.013, 0.046, 0.027, and 0.037 g oil/kg BW (BW at d 35), respectively."

Period 2:
- Total daily DMI for lambs fed 100JUN = 1,380 g/d
- Ground juniper daily intake for lambs fed 100JUN =
  207 g of juniper/d = **4.73 g** of juniper/kg BW
- % ground juniper in diet = **15%**
- Estimated CT intake for lambs fed CNTL, 0JUN, 33JUN, 66JUN, and 100JUN =
  5.58, 2.47, 6.6, 11.5, and 15.1 g CT/d, respectively, which =
  0.118, 0.057, 0.143, 0.247, and 0.345 g CT/kg BW, respectively.

Whitney et al., 2013. Effect of using redberry juniper (*Juniperus pinchotii*) to reduce *Haemonchus contortus* fecal eggs and increase ivermectin efficacy. Vet. Parasitol. 197:182−188.

* *This study was completed (in part) by Dr. Whitney's TX AgriLife Research Nutrition in collaboration with the Agricultural Res. Station (VA State Univ., Petersburg, VA) and the Dept. of Biomedical Sci. and Pathobiology (VA Tech, Blacksburg, VA).*

Paraphrased abstract:

Objective: determine if a redberry juniper-based diet can reduce fecal egg counts (**FEC**) and increase ivermectin (**IVM**) efficacy in IVM-resistant *Haemonchus contortus*. After natural infection was established, cross-bred lambs (n = 64; 6 mo old; BW ~ 22.3 kg) were randomly assigned to pens and fed a pelleted treatment diet (4 pens/treatment and 8 lambs/pen) consisting of traditional feed ingredients mixed with either 30% hay (CNTL) or 30% ground juniper leaves and stems (JUN; **Table 11**). Redberry juniper branches < 3.6 cm diameter were cut from mature redberry juniper trees, chipped, dried, and fine-ground to pass a 4.76-mm sieve. Sorghum sudangrass hay was ground to pass a 6.35-mm sieve. Lambs were fed during two periods: Period 1 (d 0 to 28) and Period 2 (d 28 to 42). On d 28, half of the lambs from each treatment and pen were treated with IVM orally (0.2 mg/kg), creating four treatment groups: lambs fed CNTL or JUN and either not treated (CNTLn, JUNn) or treated (CNTLi, JUNi) with IVM. During Period 1, lambs fed CNTL had greater ($P < 0.001$) ADG than lambs fed JUN (0.09 vs. 0.04 kg/d), which was probably caused by the CNTL diet having greater protein and less ADF, lignin, and CT than the JUN diet. During Period 2, CNTLi lambs had greater ($P < 0.05$) ADG than JUNn and JUNi lambs.

Summary Notes:

- Values reported below are on a DM basis.
- Total average daily DMI for a 22.3-kg goat = 910 g/d
- % ground juniper in diet = **30%**
- Maximum daily juniper intake for lambs fed JUN =
  273 g juniper/d [910 × 0.3] = **12.24 g** juniper/kg BW [273g/22.3 kg BW]
- % total CT in ground sorghum sudangrass hay = 0.91%
- % total CT in juniper material = 5.66%
- % total CT in CNTL diet = 1.06%
- % total CT in JUN diet = 2.11%
- % total volatile oil in JUN diet = 0.06%
- Average CT intake for lambs fed JUN =
  19.2 g/d [910 g total intake × 0.0211] = **0.86 g**/kg BW [19.2g/22.3 kg BW]
- Maximum volatile oil intake for lambs fed JUN =
  0.546 g/d [910 g total intake × 0.0006] = **0.02 g**/kg BW [0.0546g/22.3 kg BW]
- Average daily gain was low, but positive, for lambs in all treatment groups.
- No adverse effects on animal health were reported.

Table 11. Ingredient and chemical composition (DM basis) of sorghum sudangrass hay, dried juniper material, sorghum grain, and treatment supplement

Item, %[2]	Ingredient			Diet[1]	
	Hay	Juniper	Sorghum grain	CNTL	JUN
Ingredient					
Sorghum sudan grass hay				30	0
Redberry juniper				0	30
DDGS				20	20
Cottonseed meal				2	2
Sorghum grain				40.76	41.38
Molasses				3	3
Limestone				1.95	1.06
Ammonia Cl				0.54	0.81
Salt				0.85	0.85
Mineral premix				0.6	0.6
Pellet binder				0.3	0.3
Chemical composition, %					
CP	8.7	7	11.4	19.1	16.8
Crude fat	1.2	7.2	6.4	5.9	6
ADICP	1.4	1.4	1.3	1.8	1.8
NDF	71.2	43.6	9.6	30.2	29.6
ADF	48.5	33.6	4.6	18.2	22.8
ADL	7.3	17	1	5.6	9.1
Ash	14.7	6.2	1.9	8.5	7.3
Ca	0.5	1.8	0.07	0.9	1
P	0.2	0.1	0.5	0.7	0.5
CT, total	0.91	5.66	0.53	1.06	2.11
extractable	0	3.35	0	0	0.71
fiber-bound	0.55	0.54	0.26	0.68	0.68
protein-bound	0.36	1.77	0.27	0.38	0.72
Volatile oil, total		0.43		0.01	0.06

[1]Supplements were pelleted and contained either ground sorghum sudan grass hay (CNTL) or ground redberry juniper leaves and stems (JUN).

[2]Sorghum sudan grass hay = ground to pass a 6.35-mm sieve; redberry juniper = ground leaves and stems, fine-ground to pass a 4.76-mm sieve; DDGS = corn dried distillers grains with solubles; ADICP = acid detergent insoluble crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CT = condensed tannins.

C. Stewart et al. Unpublished data. Effects of using ground redberry juniper in ewe supplements on growth, blood serum, wool characteristics, and on lamb birth and weaning weights.

* *This trial was recently completed by Dr. Whitney's TX AgriLife Research Nutrition Program, San Angelo in collaboration with NM State Univ., Las Cruces; thus,* ***data have not been statistically analyzed or submitted for publication****.*

Approximately 56 d after conception, mature, pregnant Rambouillet ewes (n = 28) were randomly allocated to individual pens, fed a basal Sorghum sudangrass ground hay diet, and assigned to 1 of 4 pelleted treatment supplements that contained either 0% (CNTL), 18% (18JUN), 36% (36JUN), or 54% (54JUN) ground juniper (**Table 9**); supplements were fed until lambing. Mature redberry juniper trees were cut, chipped, and allowed to air-dry under cover for 7 to 12 d to approximately 78% DM. Chipped material was then fine-ground in a hammermill to pass a 4.76-mm sieve. Sorghum sudangrass hay that was used as the basal diet and in the supplements was ground in a hammermill to pass a 6.35-mm sieve.

Summary Notes:

- Values reported below are on a DM basis.
- Maximum juniper intake for ewes supplemented with 54JUN = **463.3 g** juniper/d = **5.5 g** juniper/kg BW
- % total CT in the ground juniper = 4.2%
- % total CT in 54JUN supplement (based solely upon CT in the juniper) = **2.27%**
- Maximum CT consumed/d = **19.5 g** CT/d = **0.23 g** CT/kg BW
- Maximum volatile oil consumed/d = **6.18 g** oil/d = **0.07 g** oil/kg BW
- % ground juniper consumed in relation to total daily DMI = **19.4%**
- No adverse effects on ewe health (daily visual evaluation, change in BC), lambing, lamb birth weight, or lamb weaning weight were observed (**Table 10**).

Consumption of target plant when it is not mixed with any other feed ingredients

When juniper leaves were fed without being mixed with other feed ingredients, the maximum percentage of juniper (as part of the total daily diet) has been reported to be 86.4% (22.2 g/kg of BW; Campbell et al., 2007). In Campbell et al. (2007), goats (37 kg) were fed fresh juniper leaves and a supplement and consumption of volatile oil and CT was estimated to be approximately 0.405 and 1.21 g/kg of BW (DM basis), respectively. These authors did not report any negative animal health issues. However, the validity of some of the conclusions made by these authors is questionable and discussed in detail within this section and **Appendix G**.

In Riddle et al. (1999), 40-kg goats consumed an average of 56.9% fresh juniper leaves (14.1 g/kg of BW) as part of their total daily intake (fed with hay). This amount of juniper intake resulted in volatile oil and CT being consumed at approximately 0.405 and 1.21 g/kg of BW (DM basis), respectively. These authors analyzed DMI, feces, urine, DM digestibility, and N balance and did not report any negative animal health issues. They stated, "Nitrogen balance was… positive for juniper for spring and fall" and that "During fall, DMI of juniper … were significantly lower than alfalfa and CBH." They concluded that "blueberry juniper… foliage can provide nutrients for goats but only as portions of diets." They also stated, "… ashe [blueberry] juniper is of sufficiently high quality (i.e., 50% DMD) to significantly contribute to the diets of grazing animals that have access to other forages and/or supplemental feeds."

Campbell et al. (2010) evaluated the effects of a monoterpene dose (0.27 g/kg of BW) given to female goats. Using various assumptions (explained in detail below), this dose equated to juniper being approximately 59% of the goat's daily DMI, resulting in approximate consumption of 0.27 g of volatile oil/kg of BW and 0.81 g of CT/kg of BW. No adverse effects on animal health were reported. However, the validity of some of conclusions made by these authors is questionable and discussed in detail within this section and **Appendix G**.

Campbell et al., 2007. Effects of supplementation on juniper intake by goats. Rangel. Ecol. Manage. 60:588–595.

Paraphrased Abstract:

The first experiment evaluated the effect on juniper intake of either no supplementation (negative control) or supplementation with corn, alfalfa, or CSM fed at an isonitrogenous CP level of 1.5 g/kg BW for 12 d. Redberry juniper consumption by individually penned goats was measured on d 11 and 12. Each goat received each supplement in a complete 4 × 4 Latin square design. Juniper intake increased for goats supplemented with alfalfa and CSM (*P* = 0.001), but not for those supplemented with corn (*P* = 0.94). A second study investigated the effect of either no supplementation or SBM supplementation on juniper consumption by free grazing goats. Forty goats were assigned to four pasture groups by breed and previous juniper intake, and randomly allocated to either the treatment (supplementation) or control (no supplementation) in a complete block design. Juniper intake was highest for goats supplemented with SBM (*P* = 0.03).

Experiment 1 (Pen Trial):

Feed treatments included a negative control (NC, no supplemental feed), corn (C), alfalfa (A), and CSM. At the target rate, all animals were fed CP to 100% of maintenance protein requirements (NRC 1981). Two breeds and two crossbreeds of goats received 4 feed treatments in a complete 4 × 4 Latin square design with four replications/treatment. Goat breeds (4 animals/breed) were Angora (28.6 kg), Spanish (33.3 kg), Angora × Spanish (29.7 kg), and Spanish × Boer (37.0 kg) for a total of 16 mature (> 2 yr old) nannies. Each trial was 12 d long, with the first 10 d representing a preconditioning period. Each d, supplemental feed was offered from 0800 to 1200 h. Fresh juniper foliage was harvested daily and goats were offered redberry juniper *ad libitum* by attaching branches in each pen.

Experiment 2 (Grazing Trial)

Effects of a SBM supplement, goat breed, and propensity to graze juniper, on juniper intake by free-grazing goats was investigated during a 16-d period in midwinter. Soybean meal was used instead of CSM to prevent gossypol from possibly interacting with other allelochemicals and affecting consumption of juniper. Percentage juniper in the diet of goats for calculating genetic merit was estimated using near-infrared spectroscopy (NIRS) predictions of fecal samples collected when they were free-grazing on juniper-infested pastures. Goats were preconditioned to juniper by grazing on a 16-ha, juniper-infested pasture for a period exceeding 10 d before separating them into 4 pastures. Ten goats were assigned to each pasture by breed. Animals were allocated to either the treatment (supplementation) or control (no supplementation) for 4 d. Goats within a pasture grazed freely together but received supplementation individually. For supplementation, goats were placed in individual stalls at 1000 h for a 3-h period and released back to the pasture. Soybean meal was fed to half the animals at 0.33% BW/d. Supplemental feeding rate was calculated to provide 0.24 g N/kg BW. After 4 d, fecal samples for NIRS estimation of percentage juniper in the diet were collected manually at 1600 h.

Summary:

- It is not apparent if feed intake data is presented on a DM or as-fed basis (not stated by the authors). Compared to other trials related to fresh juniper leaf intake, juniper intake/kg of BW data reported by the authors seems to suggest that it is on an as-fed basis; supported by the fact that these authors have published other literature in which intake is reported on an as-fed basis. However, assuming that authors reported juniper intake on a DM basis results in the greater juniper consumption vs. if they the intake is actually reported on an as-fed basis and is then converted to a DM basis. Thus, the following assumes that the intake data is reported on a DM basis.
- Introduction section: authors state, “Researchers using pen studies measuring juniper intake by … goats have reported maximum intake values of 33.5% (6.7 g/kg BW) of diet composition (Pritz et al., 1997).”
    - It is unclear how 33.5% of the diet was calculated, even if it was incorrectly calculated on an as-fed basis.
- Introduction section: authors state, “Even though juniper species can represent an important part of goat’s diets, the overall intake of juniper tends to be self-limited when juniper consumption is higher than 30% of the diet (Pritz et al., 1997; Bisson et al., 2001; Straka et al., 2004).”
    - The literature does not support this statement.
- Introduction section: authors state, “The restriction in juniper intake appears to be an attempt to regulate consumption of monoterpenes and avoid negative postingestive consequences of monoterpene exposure at higher levels.”
    - The literature does not support this statement; e.g. restricting juniper intake could be due to initial sensory characteristics such as smell, taste, and texture and could be due to CT.

- Introduction section: authors state, "Toxic monoterpenes in juniper deter goat browsing of juniper plants by reducing nutrient assimilation (Riddle et al., 1999)…"
  - The literature related to juniper leaf consumption does not seem to warrant the statement that the monoterpenes in the juniper are "toxic." For example, Riddle et al. (1999) report that N balance was positive when blueberry juniper was consumed and at times, similar to the N balance of goats that consumed coastal bermudagrass hay and no juniper. In addition, Riddle et al. (1999) did not evaluate the correlation of DMI with nutrient assimilation and (2) authors stated that blueberry juniper "is of sufficiently high quality to significantly contribute to the diets of grazing… animals that have access to other forages and/or supplemental feeds."
- No negative effects on animal health were reported.

Trial 1 Results:

- CP of juniper leaves assumed to be = 6.5%
  - e.g. [(0.777 g/kg BW)/(11.953 g/kg BW)*100]
- Goats consumed (on average; authors' Table 3) **11.95** ± 10.2, **10.4** ± 4.7, **6.52** ± 5.5, and **6.39** ± 3.8 g/kg BW of juniper, respectively (assumed to be on a DM basis; not reported by the authors). Thus, **maximum** juniper intake (DM basis) =
  - **22.15 g**/kg BW [11.95 g/kg BW + 10.2] = **819.6 g** of juniper [22.15 g/kg BW × 37 kg BW]
  - Average supplement intake/d = 3.507 g of supplement /kg of BW = [1.473 g/0.42 CP] = 129.8 g of supplement [3.507 g/kg BW × 37 kg average BW]
  - Total DMI/d = [819.6 + 129.8] = 949 g
  - Juniper intake as % of total diet = **86.4%** [819.6 g/949) × 100]
  - Assume redberry juniper volatile oil concentration = 1.83% (Owens et al., 1998): then maximum daily juniper volatile oil consumption =
    15 g/d [819.6 g juniper × 0.0183] = **0.405 g**/kg BW
- Average juniper consumption (DM basis; authors' Table 3) of the CSM treatment =
  - Juniper intake/d = **11.953 g** juniper/kg of BW = **382.5 g** [11.953 g/kg BW × 32 kg BW]
  - Supplement intake/d = 3.507 g supplement/kg BW = [1.473 g/0.42 CP] = 112.23 g [3.507 g/kg BW × 32 kg average BW]
  - Total DMI/d = [382.5 + 112.23] = 497.73
  - Juniper intake as percentage of total diet = **77.3%** [382.5/497.73) × 100]
  - Assume redberry juniper volatile oil concentration = 1.83% (Owens et al., 1998): then maximum daily juniper volatile oil consumption =
    7 **g/d** [382.5 g juniper × 0.0183] = **0.22 g**/kg BW
- Discussion: authors state, "This study showed that protein supplements increased juniper consumption by goats."
  - This conclusion is speculative considering that (1) goats in the negative control group were fed only juniper and (2) goats in the corn group were fed only corn when juniper was offered. In the Methods section, the authors report that "protein sources selected for this study reflected three winter supplements commonly used to correct seasonal forage nutrient deficiencies." In addition, authors conclude the paper with, "The current study was designed to utilize and evaluate typical winter supplementation practices …" However, treatments in Trial 1 do not represent traditional practices and do not represent a supplementation program because they were fed as the sole diet.
- Discussion: Authors discuss high-protein diets affecting detoxification, etc.
  - The study was not designed to evaluate detoxification mechanisms; the main effects of the treatment diets, as discussed by the authors, were probably mainly due to rumen physiology and not toxicology.

- Discussion: authors state, “Monoterpenes have the potential to exacerbate the negative effects of a high starch diet. Oxygenated monoterpenes in sagebrush inhibited cellulolytic bacteria populations in deer (Nagy and Tengerdy, 1968). In goats, VFA profiles of microbial populations before and after dosing with juniper oil shifted, implying a decrease in cellulolytic in favor of saccharolytic species (Straka et al., 2004).”
    - A reduction in cellulolytic and an increase in saccharolytic bacteria would actually be beneficial when consuming a high starch diet. In addition, in a previous publication (Straka et al., 2004, p. 437), the author states, “Juniper consumption within 30% of diet may also result in a favorable shift of VFA production towards lower acetate:propionate ratios, thus improving feed efficiency.”
    - Straka et al. (2004) was a review paper that makes a general inference related to effects of terpenes on microbial species composition; they did not evaluate microbial species composition.
    - Even though Nagy and Tengerdy (1968) make references related to a decrease in certain types of bacteria, they state that “Identification of the microorganisms appearing in the rumen of deer was not the objective of this investigation.” In addition, Nagy and Tengerdy (1968) evaluated the effects of volatile oils and not specific oxygenated monoterpenes.
- Discussion: authors state, “When feed refusals were compared in order to compare gross intake levels, the corn treatment group had the highest level of feed refusal. The most plausible explanation for the decline in feed intake **in this study** was due to attempts by goats to ‘‘correct’’ imbalances in the ruminal environment through their feeding behavior (Cooper et al., 1996).”
    - This seems to contradict the paragraph preceding this statement (within the authors’ article).

Trial 2 Results:

- Greatest level of juniper intake was by supplemented high-consumer goats = **31.4%** ± 2.7% of diet

Riddle et al., 1999. Intake of Ashe juniper and live oak by Angora goats. J. Range Manage. 52:161–165.

Paraphrased Abstract:

Angora mutton goats (BW = 40 kg) were fed diets of either live oak, alfalfa hay, Coastal bermudagrass hay (**CBH**) or female blueberry juniper plus Coastal bermudagrass hay during the spring and fall of 1991 in a digestion and metabolism study. Nitrogen concentration of CBH was nearly equal to that of alfalfa hay; N concentration of the juniper and live oak were much lower than those of the hays and higher in fall than spring. Average DMI and dietary N intake were highest for alfalfa hay, intermediate for CBH, and lower for blueberry juniper and live oak. Goats retained more N when consuming alfalfa and CBH than juniper or live oak during fall, but differences were smaller ($P > 0.10$) during spring. Nitrogen balance was… positive for juniper for spring and fall. During fall, DMI of juniper … were significantly lower than alfalfa and CBH. We conclude that both blueberry juniper… foliage can provide nutrients for goats but only as portions of diets.

Summary (all reported on a DM basis):

- Authors stated (Results/Discussion section; authors' Table 1) that average juniper intake/goat =
  555 g/d, which was part of a total intake of 975 g/d per goat. Thus, juniper intake was approximately **56.9%** of total DMI.
- Maximum daily juniper consumption (authors' Table 1) =
  564 g juniper DM/d = [564 g juniper/40 kg BW] = **14.1** juniper intake/kg BW
- Assuming: 1 mL of oil = 0.825 g; 3% volatile oil in the juniper leaves
  - Average daily volatile oil consumption = [555 × 0.03] = **16.65 g/d** per goat = **0.42** g/kg BW
  - Maximum volatile oil consumption = [564 × 0.03] = **16.92g** per goat = **0.42** g/kg BW
- Summary/Management Implications section, authors stated: "… Pritz et al. (1997) reported that N balance of goats may be negatively affected when substantial amounts of juniper are consumed."
  - As previously discussed in this proposal, "substantial amounts of juniper" were not consumed in the cited study and the negative N balance reported Pritz et al. (1997) could have been due to basal diet consumption, which was not reported.
- Authors also stated: "However, ashe juniper is of sufficiently high quality (i.e., 50% DMD) to significantly contribute to the diets of grazing animals that have access to other forages and/or supplemental feeds."

Campbell et al., 2010. Pharmacokinetic differences in exposure to camphor after intraruminal dosing in selectively bred lines of goats. J. Anim. Sci. 2620−2626.

Paraphrased Abstract and Methods:

A pharmacokinetic dosing study with camphor was used to determine whether selection lines of high-juniper-consuming goats (HJC, n = 12) and low-juniper-consuming goats (LJC, n = 12) differed in disposition kinetics. Post-dosing plasma camphor concentrations were used to examine whether a timed single blood sample collected after intraruminal administration of camphor would be a useful screening test to aid in the identification of HJC. Yearling female goats (n = 24) received a single intraruminal dose of monoterpene cocktail (**0.270 g/kg BW**) containing 4 different monoterpenes that represented their composition previously reported for blueberry juniper. Camphor, the predominant monoterpene in blueberry juniper, was 49.6% of the mix and was the monoterpene analyzed for this study. Blood samples were taken at 15 time points after dosing and camphor was measured in plasma. Maximal plasma concentration of camphor was greater for LJC than HJC (*P* = 0.01). Total systemic exposure (area under the curve) to camphor was 5 times less in HJC goats. Conclusions: 1) HJC goats possess internal mechanisms to reduce bioavailability of camphor, and 2) a blood sample taken at 45 min or at 60 min after intraruminal administration of camphor may be useful for identifying HJC individual animals from within large populations of goats.

- Methods: Authors state, "The dose was chosen to represent the concentration and composition of monoterpenes present in blueberry juniper leaves (Riddle et al., 1996)… The dose was chosen to be biologically relevant to the monoterpene concentration present in a diet of 30% juniper, which is a quantity of intake identified through previous research as eliciting differences in juniper intake between breeding groups (Campbell et al., 2007).

  - Comment: It is unclear how 30% juniper intake was calculated from data presented by Riddle et al. (1996). Others have shown that maximum intake can exceed 56% of DM (Riddle et al., 1999).
  - Comment: Campbell et al. (2007): During Trial 1 (pen trial), intake by breed did not differ and juniper DMI, on a DM basis and as a percentage of the total diet was 77.3%. During Trial 2, maximum juniper intake as percentage of total diet was 31.4%, but breed differences were not apparent.
- Methods: Authors reported that the volatile oil dose "provided (0.270 g/kg BW)."
  - Comment: If the volatile oil content in ***J. ashei*** = 2.15% (DM basis; Owens et al., 1998), then daily consumption of *J. ashei* leaves (as % of DMI) by a 23.64-kg goat would have to be = **296.87 g**/goat [(0.27 g oil/kg BW × 23.64 kg BW)/0.0215] = **12.56 g**/kg BW [296.87/23.64]
    *Assuming total daily DMI of this 23.64-kg goat = 591 g [0.25 BW × 23.64 kg BW × 1000 g], then % juniper in diet = **50.2%** (DM basis) [(296.87/591) × 100]
  - Comment: If volatile oil content in ***J. pinchotii*** = 1.83% (DM basis; Owens et al., 1998), then daily consumption of *J. pinchotii* leaves (as % of DMI) by a 23.64-kg goat would have to be = **348.8 g** [(0.27 g oil/kg BW × 23.64 kg BW)/0.0183] = **14.76 g**/kg BW [348.8/23.64]
    *Assuming total daily DMI of this 23.64-kg goat = 591 g [0.25 BW × 23.64 kg BW × 1000 g], then % juniper in the diet = **59%** (DM basis) [(348.8/591) × 100]
- Discussion: Authors state, "Anti-herbivory properties of monoterpenes in juniper appear to be associated with negative postingestive consequences specifically related to central nervous system triggers for cessation of feeding behavior or satiety.
  - Comment: As reported in this proposal, the link between juniper consumption and **post**-ingestive consequences is not substantiated.
- Discussion: Authors state, "Monoterpenes have been identified as initiating satiety-based feeding cessation in … and domestic sheep (Dziba et al., 2006)
  - Comment: Cessation is defined as "the stopping of an action." Dziba et al. (2006) reported that during the first week of the trial, average feeding time was less for lambs dosed with 1,8-cineole vs. lambs not dosed; however, lambs were only observed for 1 hr immediately after ruminal dosing with 1,8-cineole dose, which does not represent "feeding cessation." During the second week of the trial, DMI was not different among the lambs. In addition, the control animals in Dziba et al. (2006) were not dosed at all (not even with water) because they stated that "we expected no effect of vegetable oil on feeding behavior based on results of Dziba and Provenza (2006). Two issues arise from not dosing the control animals: 1) handling and dosing livestock can affect behavior and feed intake, especially within the first hour of observation and 2) Dziba and Provenza (2006) did not evaluate effects of dosing vs. not dosing with vegetable oil on DMI.
  - Comment: It should also be noted that *J. ashei* and *J. pinchotii* contain very little to no 1,8-cineole (Adams, 2011).
  - Comment: It should also be noted that Dziba et al. (2006) stated that the rumen was dosed with 1,8-cineole (vegetable oil was the carrier), which provided 125 mg of 1,8-cineole/kg BW. However, this dose was actually **156.3 mg** of 1,8-cineole/kg of BW [0.625 mg solution/kg BW × 250 mg of 1,8-cineole/ml solution]. This ruminal dose is between the low and medium doses of 135 and 190 mg of 1,8-cineole/kg of BW evaluated by Dziba and Provenza (2006), which did not result in feeding cessation. Intake of diets dosed with 4 terpene compounds decreased, but intake of diets that did not contain terpenes also decreased.
  - Comment: The "medium dose" evaluated by Dziba and Provenza (2006) resulted in 9.35 g of 1,8-cineole being consumed (within a given day). Assuming that sagebrush leaves contained between 0.3 to 1.1% 1,8-cineole (DM basis; Personius et al., 1987), grazing lambs (BW = 49 kg) would

have to consume 3,117 g to 850 g of sagebrush leaves/d [DM basis; 9.35/0.003 or 9.35/0.011], respectively, which is impossible.

- Discussion: Authors state, "In retrospect, reducing absorption rather than increasing elimination could be a more adaptive response because monoterpenes in juniper cause mild hepatic injury in the form of lipid vacuolation at small dosages (0.18 g oil/kg of BW) and hepatic cellular necrosis at greater dosages (0.36 g oil/kg BW; Straka, 2000).
  - Comment: As reported in this proposal, the Straka (2000) did not use control animals, thus there is no way to determine if juniper or some other factor caused hepatic insult.
- Discussion: Authors state, "Macronutrient intake and BC can affect disposition of phytotoxins in livestock such as …monoterpenes in goats (Campbell et al., 2007; Frost, 2005).
  - Comment: Results from Campbell et al. (2007) do not support this statement because: 1) goats in the negative control group were fed only juniper and 2) goats in the corn treatment group were fed only corn when juniper was offered.
  - Comment: Discussion: Authors discuss high-protein diets affecting detoxification, etc.; however, the study was not designed to evaluate detoxification mechanisms.
- No adverse effects on animal health were reported when dosed with 0.27 g of terpenes/kg of BW.

Table 12. Secondary compound composition of approved ruminant animal feed ingredients[1]

Item	Aspen Leaves[2]	CSH[3]	SL Hay[4]
Extractable CT, %		3.26[a]	
Protein-bound CT, %		2.19[a]	
Fiber-bound CT, %		0.18[a]	
Total CT, %	2.4 to 7.9[h];4.7 to 13.6[i]; 11 to 30[j]; 6 to 20[k]; max of 35%[l]; 7.6[m]; 5.4[n]; 0.7[o]; 5.65[p]; 5 to 22[q]	5.64[a]	4.06[b]; 10.7[r]; 6.5[s,t]; 4.4 to 18.1[u]
Total oil, mg/g DM			
Total oil, % of DM			

[1]Source of data: [a] = Whitney and Muir (2010); [b] = Hawkins (1955); [h] = Erwin et al. (2001); [i] = Mansfield et al., 1999; [j] = Stevens et al. (2007); [k] = Stevens and Lindroth (2005); [l] = Schweitzer et al. (2008; a review paper); [m] = Schimel et al. (1996); [n] = Bailey et al. (2007) ; [o] = McWilliam (2004); [p] = Häikiö et al. (2009); [q] = Kosonen et al. (2012); [r] = Chafton (2006); [s] = Moore et al. (2008); [t] = Terrill et al. (2007); [u] = Terrill et al. (1989).

[2]Aspen leaves; Erwin et al. (2001) evaluated fresh leaves and Bailey et al. (2007) evaluated terminal twigs and leaves.

[3]CSH = cottonseed hulls.

[4]SL = Sericea lespedeza.

Table 13. Maximum intake (DM basis) of various plant species, volatile oil, and CT by ruminant livestock when not mixed with other ingredients[1]

[2]	Animal	BW, kg	Plant	Plant description	Plant, as % of total daily DMI	Max plant DMI, g/d	Max plant DMI, g/kg BW	Max volatile oil intake, g/d	Max volatile oil intake, g/kg BW	Max CT intake, g/d[3]	Max CT intake, g/kg BW[3]
a	Goat	50*	redberry	fresh leaves	-	12.8	0.26*	0.25	0.005*	0.70*	0.01*
	Goat	50*	blueberry	fresh leaves	-	35.4	0.71*	0.53	0.01*	1.93*	0.04*
b	Goat	13.6*	redberry	volatile oil oral dose	-	-	-	2.47	0.181	-	-
		13.6*	redberry	fresh leaves	12.4	48.3	3.55*	0.97*	0.07*	2.64*	0.19*
c	Goat	40	blueberry	fresh leaves	56.9	564	14.1	16.92	0.42	30.79*	0.77*
d	Goat	15	redberry	fresh leaves	20*	45	3	0.82	0.055	2.46*	0.16*
	Goat	29	redberry	fresh leaves	32.6*	87	3	1.59	0.055	4.75*	0.16*
	Goat	15	blueberry	fresh leaves	49.7*	172.5	11.5	3.72	0.248	9.42*	0.63*
	Goat	29	blueberry	fresh leaves	65.6*	333.5	11.5	7.17	0.248	18.2*	0.63*
e	Goat	30	redberry	fresh leaves	19.8*	100	3.35	1.83*	0.061*	5.46*	0.18*
f	Goat	20	redberry	fresh leaves	23.6	94.7	4.74	1.73*	0.087*	5.17*	0.26*
g	Goat	37	redberry	fresh leaves	86.4*	819.6*	22.15*	15*	0.405*	44.75*	1.21*
h	Goat	25.3	redberry	fresh leaves	34.6	240.4	9.5	4.4*	0.23*	13.13*	0.52*
i	Goat	29.2	redberry	fresh leaves	3.48*	18.98	0.65	0.35*	0.012*	1.04*	0.04*
j	Goat	54.1	redberry	fresh leaves	5.26*	54.1	1*	0.24*	0.005*	2.95*	0.06*
k	Goat	23.6	redberry	fresh leaves	9.5*	53.1	2.25	0.97*	0.04*	2.90*	0.12*
l	Lamb	31.8	redberry	fresh leaves	16.93*	87.5	2.75	1.6*	0.05*	4.78*	0.15*
m	Goat	23.6	redberry	volatile oil oral dose	59*	348.8*	14.8	6.38	0.27	19.0*	0.81*

[1]Data marked with * was calculated from the authors' data and other references; not explicitly stated by the authors. All data are reported on a DM basis.

[2]Source of data: [a] = Riddle et al. (1996); [b] = Pritz et al. (1997); [c] = Riddle et al. (1999); [d]= Bisson et al. (2001); [e] = Ellis et al. (2005); [f] = Dunson et al. (2007); [g] = Campbell et al. (2007); [h] = Frost et al. (2008); [i] = Dietz et al. (2010); [j] = Owens et al. (2010); [k] = George et al. (2010); [l] = Anderson et al. (2013); [m] = Campbell et al. (2010).

[3]CT intake was not reported by these authors, thus CT was calculated by assuming that the juniper leaves contained 5.46% CT (DM basis; Whitney and Muir, 2010).

Table 14. Maximum intake (DM basis) of plant species, volatile oil, and CT by ruminant livestock when consuming total mixed diets[1]

[2]	Animal	BW, kg	Plant[3]	Plant description	Plant, as % of total daily DMI	Max plant DMI, g/d	Max plant DMI, g/kg of BW	Max volatile oil intake, g/d	Max volatile oil intake, g/kg BW	Max CT intake, g/d	Max CT intake, g/kg of BW
a	Lamb	30	redberry	ground small stems and leaves	36	367	12.2	1.12	0.037	22.9*	0.76*
b	Lamb	22.3*	redberry	ground small stems and leaves	30	273	12.24*	0.546*	0.02*	19.2*	0.86*
c	Ewe	84.2	redberry	ground tree	19.4	463.3	5.5	6.18	0.07	19.5*	0.23
d	Lamb	47.5	redberry	ground small stems and leaves	50	89	1.87	0.383*	0.008*	4.88*	0.103*
e	Lamb	30	redberry	leaves	30	357	11.9	1.86	0.06	52.4*	1.75*
f	Lamb	55	one-seed	leaves	20.7	200.2	3.64	4.36*	0.079*	11.85*	0.215*
g	Goat	23.5	ERC	leaves	22.6	84	3.57	0.719	0.03		
h	Lamb	25*	*Populus*	sawdust	10	84*	3.36				
i	Cow	635	*Populus*	sawdust	32	4,830	7.6				
j	Cow	635	*Populus*	sawdust	30	5,320	8.38				
k	Steer	378	*Quercus*	sawdust	50	4,500	11.9				
	Steer	252	*Populus*	sawdust	50	1,230	4.9				
l	Cow	595	*Populus*	ground stems/trees	30	5,820	9.77				
m	Cattle	400	*Populus*	ground stems/trees	42	2,520	6.3				
	Sheep	-	*Populus*	ground stems/trees	42	-	-				
n	Calf	204	*Prosopis*	ground stems	52.2	2,570	12.6				
	Steer	293	*Prosopis*	ground stems	46	4,280	14.6				
	Steer	396	*Prosopis*	ground stems	50.6	6,850	17.3				
o	Cow	800*	*Prosopis*	ground stems	88	7,260	9.1				
p	Heifer	270.2	*Pinus*	sawdust	15	989	3.66				

[1]Data marked with * is calculated from data from other references and/or personal experience. All data are reported on a DM basis.

[2]Source of data: [a] = Whitney et al. (2014); [b] = Whitney et al. (2013); [c] = Stewart et al. (unpublished data); [d] = Whitney et al. (2011); [e] = Whitney and Muir (2010); [f] = Giacomini et al. (2006); [g] = Animut et al. (2004); [h] = Dinius et al. (1970); [i] = Satter et al. (1970); [j] = Satter et al. (1973); [k] = Dinius and Williams (1975); [l] = Schingoethe et al. (1981); [m] = Mathison et al. (1986); [n] = Marion (1959); [o] = Ellis (1969); [p] = Slyter and Kamstra (1974).

[3]Redberry = redberry juniper/*J. pinchotii*; one-seed = one-seed juniper/*J. monosperma*; ERC = eastern red cedar.

9.4 Risks related to human consumption of the animal products

A thorough review of the literature showed that the maximum probably consumption of meat or milk products from animals that have consumed diets containing ground juniper (as described in this proposal) does not pose any known or extraordinary risk to the human consumer of those products. This conclusion is based upon the following: (1) humans commonly consume products that are derived from animals that have consumed various approved feed ingredients that contain equal, to greater concentrations of CT and volatile oil than what has been reported in *J. pinchotii* and *J. ashei*; (2) CT and volatile oil consumed by the ruminant animal are rapidly digested, eructated, bio-transformed (e.g. in liver and gut wall), and excreted before they can be deposited into animal products; and (3) the following have GRAS status and are either contained within the proposed feed ingredient or contain 1 or more of the same chemical constituents (see FDA GRAS Sections 582.1 [ex. basil, clover, oregano, sage]; 582.2 [ex. Cascarilla bark, Cassia bark, basil, hickory bark, juniper berries]; 582.6 [ex. limonene]); and 582.1033 [citric acid].

Condensed tannins:

Manach et al. (2005) published a thorough review on the bioavailability of CT, which supports the fact that consumption of products from animals that have consumed diets with CT poses no known health risk to humans. The International Agency for Research on Cancer (IARC) lists CT as a Group 3 carcinogen, which is "not classifiable as to its carcinogenicity to humans" and "evidence for carcinogenicity is inadequate in humans and inadequate or limited in experimental animals." Condensed tannins are poorly (or not at all) absorbed in chickens (Jimenez-Ramsey et al., 1994) or sheep (Terrill et al., 1994) and as discussed in a review by Manach et al. (2005), polymeric CT are not absorbed through the human GI tract; thus it would be expected that accumulation in the muscle be "minuscule." Manach et al. (2005) also state that Spencer et al. (2000), "suggested that polymers could be degraded into monomers during their transit in the stomach. However, Rios et al. (2002) clearly demonstrated that this does not occur in humans…" Furthermore, human's readily consume CT on a daily basis in a wide variety of food products that are considered safe and even beneficial to human health (Manach et al., 2005; McRae and Kennedy, 2011).

Volatile oil:

Cluff et al. (1982) reported that monoterpenoids in the rumen of mule deer were 80% less than what would be expected from the consumption of sagebrush and attribute differences between *in vitro* trials showing that monoterpenes inhibit rumen microorganisms vs. trials which report that "big sagebrush is a highly digestible winter forage." Welch and Pederson (1981) reported that the digestibility of big sagebrush was as great as 64.8% and also predicted that monoterpenoids are lost from the rumen. These authors also reported, "This hypothesis is based on our observations that α-pinene was lost from the *in vitro* digestion tubes and that camphor formed a condensated ring … above the surface of the *in vitro* digestion solution. The force that drove these compounds out of the digestion solution was heat. Apparently 38.5°C, which is close to the normal body temperature of mule deer, is sufficient to volatilize the monoterpenoids (Moen, 1973, *in Welch and Pederson, 1981*)." Furthermore, White et al. (1982) reported that

77% of the monoterpenoids from sagebrush were lost "in the stomach contents of … rabbits…" and that this was due to the volatilization of monoterpenoids during mastication and ingestion.

Malecky and Broudiscou (2009) also reported that specific terpenes are rapidly and readily degraded by rumen microflora; out of the 9 terpenes studied, 7 terpenes were over 75% digested and 3 were over 90% digested within the first 6 hr of incubation. Even though digestibility of terpenes in an *in vitro* system differ greatly than in the animal, numerous studies have reported that terpenes are readily degraded by rumen microorganisms (Chizzola et al., 2004; Broudiscou, et al., 2007; Malecky et al., 2009). Furthermore, the likelihood of volatile oil (terpenes) being deposited in ruminant animal products and consumed by humans is further decreased due to:

(1) The proposed ingredient will more than likely not be fed within 24 hr of harvesting, thus volatile oil concentrations will decline due to air and/or mechanical drying (Utsumi et al., 2006; Adams, 2010; Whitney et al., 2010);
(2) The majority of meat and milk products are cooked or pasteurized, respectively, which will further volatilize terpenes and reduce human consumption;
(3) When consumed by humans, additional terpenes are lost through mastication and digestion.

Ruminant animals regularly consume terpenes while grazing because numerous plant species contain volatile oils, e.g., terpenes (Mariaca et al., 1997; Prache et al., 2005). Even though very little volatile oil constituents are deposited in animal products, it is not uncommon (reviews; Prache et al., 2005). However, terpenes in animal products has not resulted in any known health risks to humans. Terpenes can be transferred into milk (Fernandez et al., 2003; Favaro et al., 2005; Viallon et al., 2000; Tornambé et al., 2006; Poulopoulou et al., 2012; Vasta et al., 2012) and eventually cheese (Dumont and Adda, 1978; Viallon et al., 1999; Caprino et al., 2004); however, at a very low level. Abilleira et al. (2011) reported a maximum of 377 µg/kg (± 393) of raw milk in grazing ewes and that only 9 monoterpenes and 3 sesquiterpenes were identified. In a review by Morand-Fehr et al. (2007) they reported a maximum of 480 µg monoterpenes/kg of milk and 1,200 µg sesquiterpenes/kg of milk.

Serrano et al. (2007) stated, "The daily [volatile oil] dose administered in the present experiment was 5-fold higher…" however, this "did not result in a drastic enrichment [of terpenes] in their tissues…". In a review by Vasta and Priolo (2006), they stated,

> "Terpenoid molecules represent a small percentage of all the volatile compounds of ruminant meat. … their [terpene] presence in ruminant meat or in dairy products can be considered as an indicator of green forage diets. The suggested source of some terpenes (phytane, phyt-1-ene, phyt-2-ene, neophytadiene) is through decomposition of chlorophyll by rumen microorganisms (Body, 1977). A number of studies reported that these terpenoid compounds were present at higher levels in the tissue of animals allowed to graze (Larick et al., 1987; Maruri and Larick, 1992; Suzuky and Bailey, 1985; Young et al., 1997) … Elmore et al. (2002) found phyt-2-ene and 1-phytene at much higher levels in the muscle of cattle fed grass silage, compared to concentrate-fed animals. It is known that some terpenoid compounds (mono- and sesquiterpenes) are directly transferred from grass to animal tissue. Priolo et al. (2004) found that b-cariophyllene detected in lamb perirenal fat could allow the perfect discrimination between animals raised on pasture and those fed a grain-based diet…"

Vasta and Priolo (2006) reviewed numerous publications and reported (authors' Table 1) that maximum concentrations of phyt-1-ene, phyt-2-ene, and neophytadiene detected in subcutaneous fat of ruminant animals (sheep and/or cattle) was reported to be 18.51 ppm (= 0.00185%), 38.4

ppm, and 15.4 ppm respectively. Furthermore, Serrano et al. (2011) stated, "Most of the terpenes for which significant differences were observed between diets were not detected or of low concentration in the fat of calves ..."

Human's consume volatile oil constituents (e.g. terpenes) on a daily basis in a wide variety of food products that are considered safe by FDA, e.g. orange, lemon, mandarin, lime, grapefruit, and wine (Bernhard and Marr., 1960; Bagchi et al., 2000; Mateo and Jiménez, 2000; Sanchez-Moreno et al., 2003; USDA, 2004). Furthermore, even though this proposal is not claiming that ground juniper can be used in ruminant diets to increase human health, it should also be noted that numerous studies and reviews have reported that terpenes and CT are (or have potential to be) beneficial to human health (reviews by: Dillard and German, 2000; Parr and Bowell, 2000; Mittal, et al., 2003; Crozier et al., 2006; Humphrey and Beale, 2006; Lans et al., 2007; Bhalla et al., 2013). In addition, spraying volatile oils onto meat products has been reported to enhance shelf life by reducing aerobic bacteria counts (Viuda-Martos et al., 2010; Hyldgaard et al., 2012).

10. How to ensure the safety of ingredient

As with all feed ingredients, during harvesting, chipping, and transporting the ingredient, care should be taken to:

(1) Ensure that other plant species are not processed along with the juniper material. This requires selective harvesting of the target plant and cleaning equipment of debris;
(2) Ensure that only the aerial portion of the tree is processed to eliminate roots, soil, and rocks;
(3) Ensure that the juniper trees have not been directly or indirectly subjected to herbicide or pesticide treatment. If indirect contact is in doubt, then: a) follow herbicide or pesticide label regarding grazing or animal feeding restrictions; b) do not harvest the juniper trees for use as an animal feed ingredient; and/or c) analyze samples of leaf and/or processed material for potential residues;
(4) Use best management practices to eliminate mold and foreign contamination during storage.

11. Proposed Labeling

Suggested IFN categories:
a. Ground redberry/blueberry juniper
b. Dehydrated ground redberry/blueberry juniper

Product Name[*]**:**	Ground redberry or blueberry juniper
Product Name[*]**:**	Ground redberry or blueberry juniper, dehydrated
Purpose Statement:	To be used as a dietary roughage component of any class of ruminant animal in any stage of animal production.
Guaranteed Analysis[‡]	Ash (maximum), 12%
Feeding Directions:	The ingredient should be ground through a screen with holes not greater than 5/8” (1.59 cm) in diameter.

[*]**Note**: Ground redberry or blueberry juniper should be classified as a “roughage product.”

[‡]If ash > 12%, the words “sand and/or dirt” must appear in the product name. If the ground juniper is mechanically dried, it should be labeled as “Dehydrated Ground Redberry/Blueberry Juniper.”

12. Literature Cited

AAFCO (Association of American Feed Control Officials). 2011. 2011 Official publication. AAFCO.

Abilleira, E., M. Virto, A. I. Nájera, M. Albisu, F. J. Pérez-Elortondo, J. C. Ruiz de Gordoa, M. de Renobales, and L. J. R. Barron. 2011. Effects of seasonal changes in feeding management under part-time grazing on terpene concentrations of ewes' milk. J. Dairy Res. 78:129–135.

Adams, R. P. 2010. Chemosystematics of *Juniperus*: Effects of leaf drying on essential oil composition. Phytologia. 92:186−198.

Adams, R. P. 2011. Junipers of the world: The genus *Juniperus*, 3rd ed. Trafford Publishing, Victoria, Canada.

Adams, R. P. 1987. Yields and seasonal variation of phytochemicals from *Juniperus* species of the United States. Biomass. 12:29−139.

Adams, R. P., J. P. Muir, C. A., Taylor, Jr., and T. R. Whitney. 2013a. Differences in chemical composition between browsed and non-browsed *Juniperus ashei* Buch. trees. Biochem. System. Ecol. 46:73−78.

Adams, R. P., C. A., Taylor, Jr., T. R. Whitney, W. C. Stewart, and J. P. Muir. 2013b. Goats and deer do not use terpenoids to select or avoid browsing on *Juniperus pinchotii* Sudw. trees. Phytologia. 95:238−245.

Anderson, D. C. 1978. Use of cereal residues in beef cattle production systems. J. Anim. Sci. 46:849−861.

Anderson, J. R., C. B. Scott, C. A. Taylor, Jr., C. J. Owens, J. R. Jackson, D. K. Steele, and R. Brantley. 2013. Using experience and supplementation to increase juniper consumption by three different breeds of sheep. Rangel. Ecol. Manage. 66:204−208.

Animut, G., A. L. Goetsch, R. E. Estell, R. C. Merkel, L. J. Dawson, T. Sahlu, and R. Puchala. 2004. Effects of methods of exposure to Eastern red cedar foliage on cedar consumption by Boer crossbred wether goats. Small Rumin. Res. 54:197−212.

Archibald, J. G. 1926. The composition, digestibility, and feeding value of hydrolyzed sawdust. J. Dairy Sci. 9:257−271.

Bagchi, D., M. Bagchi, S. J. Stohs, D. K. Das, S. D. Ray, C. A. Kuszynski, S. S. Joshi, and H. G. Pruess. 2000. Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicology. 148:187–197.

Bailey, J. K., J. A. Schweitzer, B. J. Rehill, D. J. Irschick, T. G. Whitham, and R. L. Lindroth. 2007. Rapid shifts in the chemical composition of aspen forests: an introduced herbivore as an agent of natural selection. Biol. Invasions. 9:715–722.

Baker, A. J., M. A. Millett, and L. D. Satter. 1975. Wood and wood-based residues in animal feeds. Aspen Bibliography. Paper 5153:75−105. http://digitalcommons.usu.edu/aspen_bib/5153

Baptista, R., and K. L. Launchbaugh. 2001. Nutritive value and aversion of honey mesquite leaves to sheep. J. Range Manage. 54:82−88.

Barton, F. E., II., H. E. Amos, W. J. Albrecht, and D. Burdick. 1974. Treating peanut hulls to improve digestibility for ruminants. J. Anim. Sci. 38:860−864.

Bas, F. J., F. R. Ehle, and R. D. Goodrich. 1985. Evaluation of pelleted aspen foliage as a ruminant feedstuff. J. Anim. Sci. 61:1030−1036.

Benchaar, C., S. Calsamiglia, A. V. Chaves, G. R. Fraser, D. Colombatto, T. A. McAllister, and K. A. Beauchemin. 2008. A review of plant-derived essential oils in ruminant nutrition and production. Anim. Feed Sci. Technol. 145:209−228.

Bender, F., D. P. Heaney, and A. Bowden. 1970. Potential of steamed wood as a feed for ruminants. Forest Prod. J. 20:36−41.

Bernhard, R. A., and A. G. Marr. X. 1960. The oxidation of terpenes. I. Mechanisms and reaction products of $_{D}$-Limonene autoxidation. J. Food Sci. 25:517−530.

Bhalla, Y., V. K. Gupta, and V. Jaitak. 2013. Anticancer activity of essential oils: A review. J. Food Sci. Agric. 933643−3653.

Bisson, M. G., C. B. Scott, and C. A. Taylor, Jr. 2001. Activated charcoal and experience affect intake of juniper by goats. J. Range Manage. 54:274–278.
Body, D. R. 1977. Characterization of bovine rumen liquor isoprenoid hydrocarbons with reference to dietary phytol. Lipids. 12:204–207.
Boyle, R., and M. D. Dearing. 2003. Ingestion of juniper foliage reduces metabolic rates in woodrat (*Neotoma*) herbivores. Zoology. 106:151−158.
Bravo, L. 1998. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev. 56:317−333.
Broudiscou, L-P., A. Cornu, and A. Rouzeau. 2007. *In vitro* degradation of 10 mono- and sesquiterpenes of plant origin by caprine rumen microorganisms. J. Sci. Food Agric. 87:1653−1658.
Brown, W. H., F. M. Whiting, B. S. Daboll, R. J. Turner, and J. D. Schuh. 1977. Pelleted and nonpelleted cottonseed hulls for lactating dairy cows. J. Dairy Sci. 60:919−923. *in NRC 1983, p. 235*
Buckley, W. T., and R. M. Tait. 1981. Chronic copper toxicity in lambs: A survey of blood constituent responses. Can. J. Anim. Sci. 61:613−624.
Cal, L., C. Borteiro, A. Benech, E. Rodas, M. N. Abreu, L. C. Cruz, J. R. Gonzalez-Montana. 2009. Histological changes of the liver and metabolic correlates in ewes with pregnancy toxemia. Arq. Bras. Med. Vet. Zootec. 61:306−312.
Campbell, E. J., R. A. Frost, T. K. Mosley, J. C. Mosley, C. J. Lupton, C. A. Taylor, Jr., J. W. Walker, D. F. Waldron, and J. Musser. 2010. Pharmacokinetic differences in exposure to camphor after intraruminal dosing in selectively bred lines of goats. J. Anim. Sci. 88:2620–2626.
Campbell, E. S., C. A. Taylor, Jr., J. W. Walker, C. J. Lupton, D. F. Waldron, and S. Y. Landau. 2007. Effects of supplementation on juniper intake by goats. Rangeland Ecol. Manage. 60:588–595.
Carlson, G. P. 1996. Clinical chemistry tests. Smith, B. P., editor. In: Large animal medicine. Mosby-Year Book, Inc., St Louis MO, pp. 441−469.
Carpino, S., J. Horne, C. Melilli, G. Licita, D. M. Barbano, and P. J. VanSoest. 2004. Contribution of native pasture to sensory properties of Ragusano cheese. J. Dairy Sci. 87:308–315.
Cerutti F., R. Rizzi, M. Faustini, and C. Colombani. 2003. Factors affecting some blood parameters and rectal temperature in cows in the Tropics. In: N. Lacetera, U. Bernabucci, H. H. Khalifa, B. Ronchi, and A. Nardone, editors, Interactions between climate and animal production (EAAP Technical Series, No. 7). Wageningen Acad. Pub., The Netherlands. 123 p.
Chafton, L. A. 2006. The effect of a condensed tannin containing forage, sericea lespedeza, on existing and challenge infections of *Haemonchus contortus* in sheep. MS Thesis, Louisiana State University, Baton Rouge, LA.
Cheeke, P. 1998. Natural Toxicants in Feeds, Forages and Poisonous Plants. Interstate Publ., Danville, IL.
Cheynier, V., P. Sarni-Manchado, and S. Quideau. 2012. Recent advances in polyphenol research, Vol. 3. Wiley-Blackwell Publisher.
Chizzola, R., W. Hochsteiner, and S. Hajek. 2004. GC analysis of essential oils in the rumen fluid after incubation of *Thuja orientalis* twigs in the Rusitec system. Res. Vet. Sci. 76:77−82.
Cluff L. K., B. L. Welch, J. C. Pederson, and J. D. Brotherson. 1982. Concentration of monoterpenoids in the rumen ingesta of wild mule deer. J. Range Manage. 35:192−194.
Cody, R. E., J. L. Morrill, and C. M. Hibbs. 1972. Effect of dietary screened sawdust on health, feed intake, and performance of the bovine. J. Anim. Sci. 35:460−465.
Cooper, S. D. B., I. Kyriazakis, and J. D. Oldham. 1996. The effects of physical form of feed, carbohydrate source, and inclusion of sodium bicarbonate on the diet selections of sheep. J. Anim. Sci. 741240−1251.
Cornelius, C. E. 1989. Liver function. In: J. J. Kaneko, editor, Clinical biochemistry of domestic animals. Academic press, New York. p. 364−397.
Crozier, A. T. Yokota, I. B. Jaganath, S. C. Marks, M. Saltmarsh, and M. N. Clifford. 2006. Chpt. 7: Secondary metabolites in fruits, vegetables, beverages and other plant-based dietary components. In: A. Crozier, M. N. Clifford, and H. Ashihara, editors, Plant secondary metabolites: Occurrence, structure, and role in the human diet. Blackwell Pub., Ames, Iowa.

DeMartini, J. D., and C. E. Wyman. 2011. Changes in composition and sugar release across the annual rings of *Populus* wood and implications on recalcitrance. Biores. Technol. 102:1352−1358.
Dickson, R., and P. R. Larson. 1976. Leaf chemical composition of twenty-one Populus hybrid clones grown under intensive culture. In: Proceedings of the 10th central states forest tree improvement conference. West Lafayette, IN. Purdue University: p. 20−29.
Dietz, T. H., C. B. Scott, C. A. Taylor, Jr., C. J. Owens, E. S. Campbell, and R. Brantley. 2010. Feeding redberry juniper (*Juniperus pinchotii*) at weaning increases juniper consumption by goats on pasture. Rangel. Ecol. Manage. 63:366−372.
Dillard, C. J., and J. B. German. 2000. Phytochemicals: Nutraceuticals and human health. J. Sci. Food Agricul. 80:1744−1756.
Dinius, D. A., and B. R. Baumgardt. 1968. Ration dilution and feed intake in the sheep. J. Anim. Sci. 27:1767.
Dinius, D. A, and B. R. Baumgardt. 1970. Regulation of food intake in ruminants. 6. Influence of caloric density of pelleted rations. J. Dairy Sci. 53:311−316.
Dinius, D. A., A. D. Peterson, T. A. Long, and B. R. Baumgardt. 1970. Intake and digestibility by sheep of rations containing various roughage substitutes. J. Anim. Sci. 30:309–312.
Dinius, D. A., and E. E. Williams. 1975. Sawdust as a diluent for adapting cattle to concentrate diets. J. Anim. Sci. 41:1170−1179.
Distel, R. A., and F. D. Provenza. 1991. Experience early in life affects voluntary intake of blackbrush by goats. J. Chem. Ecol. 17:431–449.
Dowe, T. W. 1947. A study of the digestibility of sorghum silage and oat straw. M.S. Thesis. Kansas State College of Agriculture and Applied Sci.
Dumont, J. P., and J. Adda. 1978. Occurrence of sesquiterpenes in mountain cheese volatiles. J. Agric. Food Chem. 26:364–367.
Dunson, W. T., C. B. Scott, E. S. Campbell, C. A. Taylor, Jr., M. A. Carr, and T. R. Callaway. 2007. Chapter 64: Rumen function and the ability of goats to consume redberry juniper (*Juniperus pinchotii*). In: K. E. Panter, T. L. Wierenga, and J. A. Pfister, editors, poisonous plants: global research and solutions. Wallingford, Oxon, United Kingdom: CABI Publishing. p. 377–385.
Dziba L. E., J. O. Hall, and F. D. Provenza. 2006. Feeding behavior of lambs in relation to kinetics of 1,8-cineole dosed intravenously or into the rumen. J. Chem. Ecol. 32: 391–408.
Dziba, L. E., and F. D. Provenza. 2006. Dietary monoterpene concentrations influence feeding patterns of lambs. Appl. Anim. Behav. Sci. 109:49−57.
Ellis, C. R., R. E. Jones, C. B. Scott, C. A. Taylor, Jr., J. W. Walker, and D. F. Waldron. 2005. Sire influence on juniper consumption by goats. Range. Ecol. Manage. 58:324–328.
Ellis, L. C. 1969. Wintering cows on ground mesquite. M.S. Thesis. TX Tech. Univ., Lubbock.
Elmore, J. S., M. M. Campo, M. Enser, and D. S. Mottram. 2002. Effect of lipid composition on meat-like model systems containing cysteine, ribose and polyunsaturated fatty acids. J. Agric. Food Chem. 50:1126–1132.
El-Sabban, F. F., T. A. Long, and B. R. Baumgardt. 1971. Utilization of oak sawdust as a roughage substitute in beef cattle finishing rations. J. Anim. Sci. 32:749−755.
Erwin E. A., M. G. Turner, R. L. Lindroth, and W. H. Romme. 2001. Secondary plant compounds in seedling and mature aspen (*Populus Tremuloides*) in Yellowstone National Park, Wyoming. Amer. Midland Naturalist. 145:299−308.
Estell, R. E., E. L. Frederickson, D. M. Anderson, K. M. Havstad, and M. D. Remmenga. 2002. Effects of four mono- and sesquiterpenes on consumption of alfalfa pellets by sheep. J. Anim. Sci. 80:3301–3306.
Estell, R. E., E. L. Fredrickson, D. M. Anderson, K. M. Havstad, and M. D. Remmenga. 1998. Relationship of tarbush leaf surface terpene profile with livestock herbivory. J. Chem. Ecol. 24:1−12.
Favaro, G., F. Magno, A. Boaretto, L. Bailoni, and R. Mantovani. 2005. Traceability of asiago mountain cheese: A rapid, low-cost analytical procedure for its identification based on solid-phase microextraction. J. Dairy Sci. 88:3426–3434.

Feist, W. C., A. J. Baker, and H. Tarkow. 1970. Alkali requirements for improving digestibility of hardwoods by rumen micro-organisms. J. Anim. Sci. 30:832–835.
Feliciano, A. S., E. Caballero, B. Del Rey, and I. Sancho. 1991. Diterpene acids from *Juniperus communis* Subsp. *Hemisphaerica*. Phytochemistry. 30:3134−3136.
Fernandez, C., C. Astier, E. Rock, J.-B. Coulon, and J.-L. Berdagué. 2003. Characterization of milk by analysis of its terpene fractions. Intern. J. Food Sci. Technol. 38:445–451.
Fishwick, G., J. Fraser, R. G. Hemingway, J. J. Parkins, and N. S. Ritchie. 1974. The voluntary intake and digestibility of oat straw by pregnant beef cows as influenced by urea and phosphorus supplementation contained in molassed sugar-beet pulp. J. Agric. Sci. 82:427−432.
Foley, W. J., S. McLean, and S. J. Cork. 1995. Consequences of biotransformation of plant secondary metabolites on acid-base metabolism in mammals—a final common pathway? J. Chem. Ecol. 21:721–743.
Fraenkel, G. S. 1959. The raison d'être of secondary plant substances. Science. 129:1466−1470.
Frost, R. 2005. Age and body condition of goats influences consumption of redberry juniper (*Juniperus coahuilensis*) and disposition of four monoterpenes. Ph.D. Dissertation. Univ. Idaho, Moscow.
Frost, R. A., K. L. Launchbaugh, and C. A. Taylor, Jr.. 2008. Age and body condition of goats influences consumption of juniper and monoterpene treated feed. Rangeland Ecol. Manage. 61:48–54.
Gardner, D. R., K. E. Panter, L. F. James, and B. L. Stegelmeir. 1998. Abortifacient effects of lodgepole pine (*Pinus contorta*) and common juniper (*Juniperus communis*) on cattle. Vet. Human Toxicol. 40:260−263.
Garleb, K. A. G. C. Fahey, Jr., S. M. Lewis, M. S. Kerley, and L. Montgomery. 1988. Chemical composition and digestibility of fiber fractions of certain by-product feedstuffs fed to ruminants. J. Anim. Sci. 66:2650−2662.
George, C. H., C. B. Scott, T. R. Whitney, C. J. Owens, B. J. May, and R. Brantely. 2010. Supplements containing escape protein improve redberry juniper intake by goats. Range. Ecol. Manage. 63:655−661.
Gharib, F. H., R. D. Goodrich, J. C. Meiske, and A. M. El Serafy. 1975. Effects of grinding and sodium hydroxide treatment on poplar bark. J. Anim. Sci. 40:727−733.
Giacomini, M. E., S. Utsumi, S. Lodge-Ivey, A. Cibils, S. Soto-Navarro, R. L. Endecott, and M. K. Petersen. 2006. Assessing the nutritive value of one-seed juniper in sheep. New Mexico cattle grower’s short course proceedings. p. 58.
Gilbert, R. A., N. S. Hale, D. M. Kinsman, and W. A. Cowan. 1973. Sawdust vs. hay in complete lamb ration. J. Anim. Sci. 37:367.
Goto, M., T. Morio, E. Kojima, Y. Nagano, Y. Yamada, A. Horigane, and H. Yamada. 2000. Characteristics of digestion dynamics of rice and oat straw relating to microbial digestion in the rumen of sheep given high-concentrate diets. J. Anim. Sci. 13:1219−1227.
Häikiö, E., M. Makkonen, R. Julkunen-Tiitto, J. Sitte, V. Freiwald, T. Silfver, V. Pandey, E. Beuker, T. Holopainen, and E. Oksanen. 2009. Performance and secondary chemistry of two hybrid aspen (*Populous tremula* L. x *Populous tremuloides* Michx.) clones in long-term elevated ozone exposure. J. Chem. Ecol. 35:664–678.
Hale, W. H., C. Lambeth, B. Theurer, and D. E. Ray. 1969. Digestibility and utilization of cottonseed hulls by cattle. J. Anim. Sci. 29:773−776.
Hawkins. 1955. Consumption and digestion of lespedeza sericea hay and alfalfa hay plus gallotannin J. Dairy Sci. 38:237−243.
Heinemann, W. W. 1976. Bluegrass straw, cottonseed hulls and wheat screenings in steer finishing rations. College of Agriculture, Washington State Univ. Bull. 832. Pullman: Wash. State Univ. *in NRC 1983, p. 235*
Hemingway, R. W. and P. E. Laks. 1992. Plant phenolics: Synthesis, properties, and significance. Plenum Press, New York, NY. p. 1053.
Horton, G. M., and G. M. Steacy. 1979. Effect of anhydrous ammonia treatment on the intake and digestibility of cereal straws by steers. J. Anim. Sci. 48:1239−1249.

Humphrey, A. J., and M. H. Beale. 2006. Chapter 3: Terpenes. In: A. Crozier, M. N. Clifford, and H. Ashihara, editors, Plant secondary metabolites: Occurrence, structure, and role in the human diet. Blackwell Pub., Ames, Iowa.
Huston, J. E., B. S. Rector, L. B. Merrill, and B. S. Engdahl. 1981. Nutritional value of range plants in the Edwards Plateau region of Texas. Texas Agric. Exp. Sta. Bull. B–1357.
Huston, J. E., C. A. Taylor, Jr., and E. J. Straka. 1994. Effects of juniper on livestock production. In: Proc. 1994 juniper symposium. TX Agr. Res. Sta. Tech. Rep. 94-2. Sonora. p. 45−51.
Hyldgaard, M., T. Mygind, and R. L. Meyer. 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front. Microbiol. 3:1−24.
Ioannidis, J. P. A. 2005. Why most published research findings are false. PLOS Med. 2:696−701. DOI:10.1371/journal.pmed.0020124.
International Species Inventory System (ISIS). 1995. ISIS Physiological Data Reference Values. Apply Valley, MN. www.worldcat.org/title/isis-physiological-data-reference-values/oclc/56726687
Jimenez-Ramsey L. M., J. C. Rogler, T. L. Housley, L. G. Butler, and R. G. Elkin. 1994. Absorption and distribution of 14C-labeled condensed tannins and related sorghum phenolics in chickens. J Agri. Food Chem. 42:963−967.
Johnson, A. E., L. F. James, and J. Spillett. 1976. The abortifacient and toxic effects of big sagebrush (*Artemisia tridentate*) and juniper (*Juniperus osteosperma*) on domestic sheep. J. Range Manage. 29:278–280.
Kafilzadeh, F., N. Heidary, and S. Bahraminejad. 2012. Variety effect on composition, kinetics of fermentation and *in vitro* digestibility of oat (*Avena sativa* L.) straw and its neutral detergent fibre. S. African J. Anim. Sci. 42:406−415.
Keen, C. L., and T. W. Graham. 1989. Trace elements. In: J. J. Kaneko editor, Clinical biochemistry of domestic animals, 4th ed. Acad. Press, San Diego, CA. pp. 757−765.
Kingsbury, J. M. 1964. Poisonous plants of the United States and Canada. Prentice-Hall, Inc., Englewood Cliffs, NJ.
Kohl, K. D., and M. D. Dearing. 2011. Induced and constitutive responses of digestive enzymes to plant toxins in an herbivorous mammal. J. Exp. Biol. 214:4133−4140.
Kosonen, M., S. Keski-Saari, T. Ruuhola, C. P. Constabel, and R. Julkunen-Tiitto. 2012. Effects of overproduction of condensed tannins and elevated temperature on chemical and ecological traits of genetically modified hybrid aspens (*Populus tremula* × *P. tremuloides*). J. Chem. Ecol. 38:1235–1246.
Kramer, J. W. 1989. Clinical enzymology. In: J. J. Kaneko, editor, Clinical biochemistry of domestic animals. Academic Press, N.Y.
Kubitzki, K., and O. R. Gottlieb. 1984. Phytochemical aspects of angiosperm origin and evolution. Acta Botanica Neerlandica. 33:457–468.
Kutchan T, and R. A. Dixon. 2005. Physiology and metabolism. Secondary metabolism: nature's chemical reservoir under deconvolution. Curr. Opin. Plant Biol. 8:227−229.
Lans, C., N. Turner, T. Khan, G. Brauer, and W. W. Boepple. 2007. Ethanoveterinary medicines used for ruminants in British Columbia, Canada. J. Ethnobiol. Ethnomed. 3:1–22.
Larick, D. K., H. B. Hedrick, M. E. Bailey, J. E. Williams, D. L. Hancock, G. B. Garner, and R. E. Morrow. 1987. Flavor constituents of beef as influenced by forage- and grain-feeding. J. Food Sci. 52:245–251.
Launchbaugh, K. L., F. D. Provenza, and E. A. Burritt. 1993. How herbivores track variable environments: response to variability of phytotoxins. J. Chem. Ecol. 19:1047–1056.
Launchbaugh, K. L., C. A. Taylor, Jr., E. J. Straka, and R. Pritz. 1997. Juniper as forage: An unlikely candidate? Juniper Symposium TX Agric. Exp. Stat., San Angelo.
Lohmeyer, H. 2013. Grinding towards success. Delta County Independent Newspaper. Delta, CO. Feb. 27.
Makkar, H. P. S., P. Sidhuraju, and K. Becker. 2010. Plant secondary metabolites. Humana Press, New York, NY.

Malachek, J. C., and C. L. Leinweber. 1972. Forage selectivity by goats on lightly and heavily grazed ranges. J. Range Manage. 25:105−111.
Malecky, M., and L. P. Broudiscou. 2009. Disappearance of nine monoterpenes exposed *in vitro* to the rumen microflora of dairy goats: Effects of inoculum source, redox potential, and vancomycin. J. Anim. Sci. 87:1366−1373.
Malecky, M., L. P. Broudiscou, and P. Schmidely. 2009. Effects of two levels of monoterpene blend on rumen fermentation, terpene and nutrient flows in the duodenum and milk production in dairy goats. Anim. Feed Sci. Technol. 15424−35.
Manach, C., G. Williamson, C. Morand, A. Scalbert, and C. Ramsey. 2005. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am. J. Clin. Nutr. 81:230S–242S.
Mansfield, J. L., P. S. Curtis, D. R. Zak, and K. S. Pregitzer. 1999. Genotypic variation for condensed tannin production in trembling aspen (*Populus tremuloides*, Salicaceae) under elevated CO_2 and in high- and low-fertility soil. Am. J. Bot. 86:1154–1159.
Mariaca, R. G., T. F. H. Berger, R. Gauch, M. I. Imhof, B. Jeangros, and J. O. Bosset. 1997. Occurrence of volatile mono- and sesquiterpenoids in highland and lowland plant species as possible precursors for flavour compounds in milk and dairy products. J. Agric. Food Chem. 45:4423–4434.
Marion, P. F., C. E. Fisher, and E. D. Robinson. 1957. Ground mesquite wood as a roughage in rations for yearling steers. Tex. Ag. Exp. Sta. Progress Rep. 1972. TX Agricultural Experiment Station, College Station.
Marion, P. F., C. E. Fisher, and E. D. Robinson. 1959. Ground mesquite wood as a roughage for yearling steers. J. Anim. Sci. 18:1174.
Martin, R. J., L. L. Wilson, R. L. Cowan, and J. D. Sink. 1973. Effects of fasting and diet on enzyme profiles in ovine liver. J. Anim. Sci. 36:101−106.
Maruri, J. L., and D. K. Larick. 1992. Volatile concentration and flavor of beef as influenced by diet. J. Food Sci. 57:1275–1281.
Mateo, J. J., and M. Jiménez. 2000. Monoterpenes in grape juice and wines. J. Chromat. 881:557−567.
Mathison. G. W., and L. P. Milligan, and R. D. Weisenburger. 1986. Evaluation of aspen as a feedstuff for cattle. Univ. Alberta Agric. Forage Bull. (Suppl. 9):53−55.
Maynard, L. A. 1920. War-time sources of feeding-stuffs in Germany. J. Anim. Sci. 1920:97−102.
McGarvey, D. J., and R. Croteau. 1995. Terpenoids metabolism. The Plant Cell. 7:1015−1026.
McRae, J. M., and J. A. Kennedy. 2011. Wine and grape tannin interactions with salivary proteins and their impact on astringency: A review of current research. Molecules. 16:2348–2364.
McWilliam, E. L. 2004. The effect of poplar (*Populus* spp.) and willow (*Salix* spp.) supplementation on reproductive performance of ewes grazing low quality drought pasture during mating. Thesis. Institute of Veterinary Animal Biomedical Sci. Massey, Univ.
McWilliam, E. L., T. N. Barry, and N. L. Villalobos. 2005. Organic matter digestibility of popular (*Populus*) and willow (*Salix*) forage trees and its *in vitro* prediction. J. Sci. Food Agric. 85:1098−1104.
McWilliam, E. L., T. N. Barry, N. L. Villalobos, P. N. Cameron, and P. D. Kemp. 2004. The effect of different levels of poplar (*Populus*) supplementation on the reproductive performance of ewes grazing low quality drought pasture during mating. Anim. Feed Sci. Technol. 115:1–18.
Mellenberger, R. W., L. D. Satter, M. A. Millett, and A. J. Baker. 1971. Digestion of aspen, alkali-heated aspen and aspen bark by goats. J. Anim. Sci. 32:756−763.
Micko, M. M. 1987. Alberta aspen vs. black poplar. Wood quality differences. Aspen Bibliography. Paper 3495. http://digitalcommons.usu.edu/aspen_bib/3495. *in Peterson and Peterson, 1992*.
Millett, M. A., A. J. Baker, W. C. Feist, R. W. Mellenberger, and L. D. Satter. 1970. Modifying wood to increase its *in vitro* digestibility. J. Anim. Sci. 31:781−788.
Mittal, A., C. A. Elmets, and S. K. Katiyar. 2003. Dietary feeding of proanthocyanidins from grape seeds prevents phtocarcinogenesis in SKH-1 hairless mice: relationship to decreased fat and lipid peroxidation. Carcinogenesis. 24:1379–1388.

Moen, A. N. 1973. Wildlife ecology: An analytical approach. In: W. H. Freeman and Co., San Francisco, Calif. 458 p.
Moore, D. A., T. H. Terrill, B. Kouakou, S. A. Shaik, J. A. Mosjidis, J. E. Miller, M. Vanguru, G. Kannan, and J. M. Burke. 2008. The effects of feeding sericea lespedeza hay on growth rate of goats naturally infected with gastrointestinal nematodes. J. Anim. Sci. 86:2328−2337.
Morand-Fehr, P., V. Fedele, M. Decandia, Y. Le Frileux. 2007. Influence of farming and feeding systems on composition and quality of goat and sheep milk. Small Rumin. Res. 68:20–34.
Morrison, F. B., G. C. Humphrey, and R. S. Hulce. 1922. Hydrolyzed sawdust for dairy cows. Unpublished report, filed with the U.S. Dept. Ag., Forest Service, Forest Products Lab, Madison, WI. File No. L-13:563. In: J. G. Archibald, The composition, digestibility, and feeding value of hydrolyzed sawdust. J. Dairy Sci. 9:257−271.
Murdiati T.B., C. S. McSweeney, R. S. F. Campbell, and D. S. Stoltz. 1990. Prevention of hydrolysable tannin toxicity in goats fed *Clidemia hirta* by calcium hydroxide supplementation. J. Appl. Toxicol. 10:325–331.
Myung, K. H., and J. J. Kennelly. 1992. Effect of alkaline hydrogen peroxide and peracetic acid on *in sacco* ruminal digestibility of aspen sawdust. Asian-Australasian J. Anim. Sci. 5:635−641.
Nagy J. G., and R. P. Tengerdy. 1968. Antibacterial action of essential oils of *Artemisia* as an ecological factor. Appl. Microbiol. 16:441−444.
Nakyinsige, K. A. Q. Sazili, Z. A. Aghwan, I. Zulkifli, Y. M. Goh, and A. B. Fatimah. 2013. Changes in blood constituents of rabbits subjected to transportation under hot, humid tropical conditions. Asian Austr. J. Anim. Sci. 26:874−878.
Ndlovu, L. R., and J. G. Buchanan-Smith. 1985. Utilization of poor quality roughages by sheep: Effects of alfalfa supplementation on ruminal parameters, fiber digestion and rate of passage from the rumen. Can. J. Anim. Sci. 65:693−703.
NRC. 2000. Nutrient Requirements of Beef Cattle. 7th edition. Nat. Acad. Press, Washington, DC.
NRC. 1981. Nutrient Requirements of goats. Nat. Acad. Press, Washington, DC.
NRC. 2003. Nutrient Requirements of nonhuman primates. 3rd edition. Nat. Acad. Press, Washington, DC.
NRC. 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. Nat. Acad. Press, Washington, DC.
NRC. 1983. Underutilized resources as animal feedstuffs. Nat. Acad. Press, Washington. DC.
Oh, H. K., T. Sakai, M. B. Jones, and W. M. Longhurst. 1967. Effect of various essential oils isolated from Douglas fir needles upon sheep and deer rumen microbial activity. Appl. Micro. 15:777–784.
Otter, A. 2013. Diagnostic blood biochemistry and hematology in cattle. In Practice. 35:7−16.
Owens, C. J., C. B. Scott, C. A. Taylor, Jr., E. S. Campbell, and R. Brantley. 2010. Redberry juniper consumption does not adversely affect reproduction in meat goats. TX J. Agric. Nat. Res. 23:71−82.
Owens, M. K., E. J. Straka, C. J. Carroll, and C. A. Taylor, Jr. 1998. A comparison of techniques for extracting monoterpenoids from *Juniperus* (*Cupressaceae*) species. J. Range Manage. 51:540−544.
Parker, H. 1982. Mesquite utilization. Proceedings of the Symposium. Oct. 29 to 30. TX Tech Univ., Lubbock, TX.
Parr, A. J., and G. P. Bowell. 2000. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. J. Sci. Food Agricul. 80:985−1012.
Paumgartten, F. J., I. F. Delgado, E. N. Alves, A. C. Nogueira, R. C. de-Farias, and D. Neubert. 1990. Single dose toxicity study of beta-myrcene, a natural analgesic substance. Braz. J. Med. Biol. Res. 23:873−877.
Personius, T. L., C. L. Wambolt, J. R. Stephens, and R. G. Kelsey. 1987. Crude terpenoid influence on mule deer preference for sagebrush. J. Range Manage. 40:84−88.
Peterson, E. B., and N. M. Peterson. 1992. Ecology, management, and use of aspen and balsam poplar in the prairie provinces. NW Region. Special Report 1. Forestry Canada, Edmonton, Alberta.

Piluzza, G., L. Sulas, and S. Bullitta. 2013.Tannins in forage plants and their role in animal husbandry and environmental sustainability: a review. Grass and Forage Sci. 1−17.
Poulopoulou, I., E. Zoidis, T. Massouras, and I. Hadjigeorgiou. 2012. Terpenes transfer to milk and cheese after oral administration to sheep fed indoors. J. Anim. Physiol. Anim. Nutr. 96:172–181.
Prache, S., A. Cornu, J. L. Berdagué, and A. Priolo. 2005. Traceability of animal feeding diet in the meat and milk of small ruminants. Small Rumin. Res. 59:157−168.
Priolo, A., A. Cornu, S. Prache, M. Krogmann, N. Kondjoyan, D. Micol, and J. L. Berdagué. 2004. Fat volatiles tracers of grass feeding in sheep. Meat Sci. 66:475–481.
Pritz, R. K., K. L. Launchbaugh, and C. A. Taylor, Jr. 1997. Effects of breed and dietary experience on juniper consumption by goats. J. Range Manage. 50:600–606.
Reed, J. D. 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. J. Anim. Sci. 73:1516−1528.
Rexen, B. 1977. Enzyme solubility - A method for evaluating the digestibility of alkali treated straw. Anim. Feed Sci. Technol. 2:205−218.
Rice, P. J., and J. R. Coats. 1994. Structural requirements for monoterpenoid activity against insects. In: Proc. Symp. Bioregulators for Crop Protection and Pest Control. Amer. Chem. Soc. Series No. 557, Washington, DC. p. 92–108.
Riddle, R. R., C. A. Taylor, Jr., J. E. Huston, and M. M. Kothmann, Jr. 1999. Intake of Ashe juniper and live oak by Angora goats. J. Range Manage. 52:161–165.
Riddle, R. R., C. A. Taylor, Jr., M. M. Kothmann, and J. E. Huston. 1996. Volatile oil contents of ashe and redberry juniper and its relationship to preference by Angora and Spanish goats. J. Range Manage. 49:35−41.
Rios L. Y., R. N. Bennett, S. A. Lazarus, C. Remesy, A. Scalbert, and G. Williamson. 2002. Cocoa procyanidins are stable during gastric transit in humans. Am. J. Clin. Nutr. 76:1106 –10.
Ross, D. B. 1966. The diagnosis, prevention and treatment of chronic copper poisoning in housed lambs. Brit. Vet. J. 122:279−284.
RTECS. 2005. NIOSH Registry of toxic effects of chemical substances.
Rusoff, L. L., D. M. Seath, and G. D. Miller. 1946. Digestibility of Lespedeza hay. J. Dairy Sci. 29:613−615.
Salminen, J-P., and M. Karonen. 2011. Chemical ecology of tannins and other phenolics: We need a change in approach. Funct. Ecol. 25:325−338.
Sanchez-Moreno, C., G. Cao, B. Ou, and R. L. Prior. 2003. Anthocyanin and proanthocyanidin content in selected white and red wines. Oxygen radical absorbance capacity comparison with nontraditional wines obtained from highbush blueberry. J. Agric. Food Chem. 51:4889–4896.
Satter, L. D., A. J. Baker, and M. A. Millet. 1970. Aspen sawdust as a partial roughage substitute in a high-concentrate dairy ration. J. Dairy Sci. 53:1455−1460.
Satter, L. D., R. L. Lang, A. J. Baker, and M. A. Millet. 1973. Value of aspen sawdust as a roughage replacement in high-concentrate dairy rations. J. Dairy Sci. 56:1291−1297.
Schimel, J. P., K. Van Cleve, R. G. Cates, T. P. Clausen, and P. B., Reichardt. 1996. Effects of balsam popular (*Populus balsamifera*) tannins and low molecular weight phenolics on microbial activity in taiga floodplain soil: implications for changes in N cycling during succession. Canadian J. Botany. 74:84−90.
Schingoethe, D. J., D. S. Kipp, and L. D. Kamstra. 1981. Aspen pellets as partial roughage replacement for lactating dairy cows. J. Dairy Sci. 64:698−702.
Schweitzer , J. A., M. D. Madritch, J. K. Bailey, C. J. LeRoy, D. G. Fischer, B. J. Rehill, R. L. Lindroth, A. E. Hagerman, S. C. Wooley, S. C. Hart, and T. G. Whitham. 2008. From genes to ecosystems: the genetic basis of condensed tannins and their role in nutrient regulation in a *Populus* model system. Ecosystems. 11:1005−1020.
Serrano, E., A. Cornu, N. Kondjoyan, J. Agabriel, and D. Micol. 2011. Traceability of grass feeding in beef: terpenes, 2,3-octanedione and skatole accumulation in adipose tissue of young bulls. Animal. 5:641–649.

Serrano, E., A. Cornu, N. Kondjoyan, G. Figueredo, J. Agabriel, and D. Micol. 2007. Terpene accumulation in muscle and fatty tissues of calves supplemented with essential oils. J. Anim. Feed Sci. 16:168−179.

Serrano, J., R. Puupponen-Pimia, A. Dauer, A. M. Aura, and F. Saura-Calixto. 2009. Tannins: Current knowledge of food sources, intake bioavailability and biological effects. Mol. Nutr. Food Res. 53:S310−S329.

Shaw, M. D., C. Karunakaran, and L. G. Tabil. 2009. Physicochemical characteristics of densified untreated and steam exploded poplar wood and wheat straw grinds. Biosys. Eng. 103:198–207.

Sherrard, E. C., and G. W. Blanco. 1921. The preparation and analysis of a cattle food consisting of hydrolyzed sawdust. J. Indus. Eng. Chem. xiii. 61−65; also ibid., First progress report (unpublished). In: J. G. Archibald, The composition, digestibility, and feeding value of hydrolyzed sawdust. J. Dairy Sci. 9:257−271.

Singh, M. 1978. Utilization of whole aspen tree material as a ruminant feed component. Ph.D. Thesis. SD State Univ.

Singleton, V. L. 1981. Naturally occurring food toxicants: Phenolic substances of plant origin common in foods. Advan. Food Res. 27:149−242.

Slyter, A. L., and L. D. Kamstra. 1974. Utilization of pine sawdust as a roughage substitute in beef finishing rations. J. Anim. Sci. 28:692–696.

Spencer J. P., F. Chaudry, A. S. Pannala, S. K. Srai, E. Debnam, and C. Rice-Evans. 2000. Decomposition of cocoa procyanidins in the gastric milieu. Biochem. Biophysiol. Res. Commun. 272:236–41.

Stevens, M. T., and R. L. Lindroth. 2005. Induced resistance in the indeterminate growth of aspen (*Populus tremuloides*). Oecologia. 145:298−306.

Stevens, M. T., D. M. Waller, and R. L. Lindroth. 2007. Resistance and tolerance in *Populus tremuloides*: genetic variation, costs, and environmental dependency. Evol. Ecol. 21:829−847.

Stewart, W. C., T. R. Whitney, E. J. Scholljegerdes, R. P. Adams, H. D. Naumann, N. M. Cherry, K. D. Welch, and D. R. Gardner. 2014. Effects of juniper species and stage of plant maturity on nutritional, digestive, and plant secondary compound characteristics. Proceedings. West. Sec. Amer. Soc. Anim. Sci. 65:59–63.

Straka, E. J. 2000. The physiological effects of monoterpenes on Spanish and Angora goats. Ph.D. Dissertation. Texas A&M Univ., College Station.

Straka, E. J., 1993. Preferences for redberry and blueberry juniper exhibited by cattle, sheep, and goats. Thesis, Texas A&M University, College Station.

Straka E, C. B. Scott, C. A. Taylor, Jr., and E. M. Bailey. 2004. Chapter 65: Biological control of the toxic shrub juniper. In: T Acamovic, C. S. Stewart, and T. W. Pennycott, editors, Poisonous plants and related toxins. CABI Publishing, Wallingford, Oxon, UK. p. 436−442.

Suzuky, J., and M. E. Bailey. 1985. Direct sampling capillary GLC analysis of flavor volatiles from ovine fat. J. Agric. Food Chem. 33:343–347.

Swanson, E. W., and H. A. Herman. 1944. The digestibility of Korean Lespedeza hay and Ground Korean Lespedeza seed for dairy heifers. J. Dairy Sci. 27:263−268.

Terrill, T. H., J. A. Mosjidis, D. A. Moore, S. A. Shaik, J. E. Miller, J. M. Burke, J. P. Muir, and R. Wolfe. 2007. Effect of pelleting on efficacy of sericea lespedeza hay as a natural dewormer in goats. Vet. Parasitol. 146:117–122.

Terrill, T. H., G. C. Waghorn, D. J. Wooley, W. C. McNabb, and T. N. Barry. 1994. Assay and digestion of 14C-labelled condensed tannins in the gastrointestinal tract of sheep. Br. J. Nutr. 72:467−477.

Terrill, T. H., W. R. Windham, C. S. Hoveland, and H. E. Amos. 1989. Forage preservation method influences on tannin concentration, intake, and digestibility of Sericea lespedeza by sheep. Agron. J. 81:435−439.

Todd, J. R., and R. H. Thompson. 1963. Studies on chronic copper poisoning: II. Biochemical studies on the blood of sheep during the hemolytic crisis. Brit. Vet. J. 119:161−173.

Tornambé, G., A. Cornu, P. Pradel, N. Kondjoyan, A. P. Carnat, M. Petit, and B. Martin. 2006. Changes in terpene content in milk from pasture-fed cows. J. Dairy Sci. 89:2309–2319.
Torregrossa, A-M., A. V. Azzara, and M. D. Dearing. 2012. Testing the diet-breadth trade-off hypothesis: differential regulation of novel plant secondary compounds by a specialist and a generalist herbivore. Oecologia. 168:711−718.
Ullrey, D. E., W. G. Youatt, H. E. Johnson, A. B. Cowan, R. L. Covert, and W. T. Magee. 1972. Digestibility and estimated metabolizability of aspen browse for white-tailed deer. J. Wildl. Manage. 36:885−891.
Unknown. 1975. Cattle fatten up on old aspen trees. Monday, Dec. 1. The Billings Gazette.
USDA. 2004. USDA database for the proanthocyanidin content of selected foods. U.S. Dept. Agriculture, Beltsville, Maryland.
Utley, P. R., and W. C. McCormick. 1972. Level of peanut hulls as a roughage source in beef cattle finishing diets. J. Anim. Sci. 34:146.
Utsumi, S. A., A. F. Cibils, R. E. Estell, S. A. Soto-Navarro, and D. Van Leeuwen. 2009. Seasonal changes in one seed juniper intake by sheep and goats in relation to dietary protein and plant secondary metabolites. Small Rumin. Res. 81:152−162.
Utsumi, S. A., A. F. Cibils, R. E. Estell, and Y. F. Wang. 2006. Influence of plant material handling protocols on terpenoid profiles of one-seed juniper saplings. Rangel. Ecol. Manage. 59:668−673.
Van Soest, P. J. 1982. Nutritional Ecology of the Ruminant. Chpt. 11. O&B Books, Inc., Corvallis, OR. p. 178−194.
Vasta, V., A. G. D'Alessandro, A. Priolo, K. Petrotos, and G. Martemucci. 2012. Volatile compound profile of ewe's milk and meat of their suckling lambs in relation to pasture vs. indoor feeding system. Small Rumin. Res. 105:16–21.
Vasta, V., and A. Priolo. 2006. Ruminant fat volatiles as affected by diet. A review. Meat Sci. 73:218−228.
Vera-Badillo, F. E., R. Shapiro, A. Ocana, E. Amir, and I. F. Tannock. 2013. Bias in reporting of end points of efficacy and toxicity in randomized, clinical trials for women with breast cancer. Annals Oncol. 00:1−6. doi:10.1093/annonc/mds636.
Vetter, R. L. 1973. Evaluation of chemical and nutritional properties of crop residues. In: Crop residue symposium. Lincoln: Univ. of Nebraska. *in NRC, 1983, p. 234;*
Viallon, C., B. Martin, I. Verdier-Metz, P. Pradel, J.-P. Garel, J.-B. Coulon, and J.-L. Berdague. 2000. Transfer of monoterpenes and sesquiterpenes from forages into milk fat. Lait. 80:635−641.
Viallon, C., I. Verdier-Metz, C. Denoyzer, P. Pradel, J. B. Coulon, and J. L. Berdague´. 1999. Desorbed terpenes and sesquiterpenes from forages and cheeses. J. Dairy Res. 66:319–326.
Viuda-Martos, M., Y. Ruiz-Navajas, J. Fernandez-Lopez, and J. A. Perez-Alvarez. 2010. Effect of added citrus fibre and spice essential oils on quality characteristics and shelf-life of *mortadella*. Meat Sci. 85:568−576.
Waghorn G.C., and W. C. McNabb. 2003. Consequences of plant phenolic compounds for productivity and health of ruminants. Proc. Nutr. Soc. 62:383–392.
Wagner, P. 2006. A reassessment for camphor. Action memorandum submitted to US EPA. Jan. 27.
Welch, B. L., and J. C. Pederson. 1981. *In vitro* digestibility among accessions of big sagebrush by wild mule deer and its relationship to monoterpenoid content. J. Range. Manage. 34:497−500.
Welch, K. D., D. Cook, D. R. Gardner, C. Parsons, and J. A. Pfister. 2013. A comparison of the abortifacient risk of western juniper trees in Oregon. Rangelands. Feb. 40−44.
White, S. M., B. L. Welch, and J. T. Flinders. 1982. Monoterpenoid content of pygmy rabbit stomach contents. J. Range Manage. 35:107−109.
Whitney, T. R., A. E. Lee, D. R. Klein, C. B. Scott, and T. M. Craig. 2010. A modified *in vitro* larvae migration inhibition assay using rumen fluid to evaluate *Haemonchus contortus* viability. Vet. Parisitol. 176:217–225.

Whitney, T. R., A. E. Lee, M. G. Williamson, C. D. Swening, and R. L. Noland. 2011. Use of the Penn State forage particle separator to evaluate coarse-ground juniper as a supplemental feed limiter for lambs. Anim. Feed Sci. Technol. 168:21–29.

Whitney, T. R., C. J. Lupton, J. P. Muir, R. P. Adams, and W. C. Stewart. 2014. Effects of using ground redberry juniper and dried distillers grains with solubles in lamb feedlot diets: Growth, blood serum, fecal, and wool characteristics. J. Anim. Sci. 92:1119–1132.

Whitney, T. R., and J. P. Muir. 2010. Redberry juniper as a roughage source in lamb feedlot rations: performance and serum nonesterified fatty acids, urea nitrogen, and insulin-like growth factor-1 concentrations. J. Anim. Sci. 88:1492–1502.

Whitney, T. R., S. Wildeus, and A. M. Zajac. 2013. Effect of using redberry juniper (*Juniperus pinchotii*) to reduce *Haemonchus contortus* fecal eggs and increase ivermectin efficacy. Vet. Parasitol. 197:182−188.

Williams, R. L., and M. S. Elliot. 1997. Chapter 9: Antioxidants in grapes and wine: Chemistry and health effects. In: Natural antioxidants: Chemistry, health effects, and applications. Old Dominion Univ. Enological Res. Facility, Dept. of Chem. and Biochem. 150−173.

Wingren, A. 2005. Ethanol from softwood: Techno-economic evaluation for development of the enzymatic process, Doctoral Thesis. Depart. Chem. Engin., Lund University. *http://lup.lub.lu.se/record/545138*

Xu, L., and U. Tschriner. 2012. Peracetic acid pretreatment of alfalfa stem and aspen biomass. Bioresources. 7:203–206.

Yoshida, T., Hatano, T., Ito, H., and Okuda, T. 2000. Chemical and biological perspectives of ellagitannins oligomers from medicinal plants. Studies in Nat. Prod. Chem. 23:395−453.

Young, O. A., J. L. Berdague´, C. Viallon, S. Rousset-Akrim, and M. Theriez. 1997. Fat-borne volatiles and sheepmeat odour. Meat Sci. 45:183–200.

Yu, H., E. Bao, R. Zhao, and Q. Lv. 2007. Effect of transportation stress on heat shock protein 70 concentration and mRNA expression in heart and kidney tissues and serum enzyme activities and hormone concentrations of pigs. Amer. J. Vet. Res. 68:1145−1150.

Yu, C, H. Wu, T Chen, R. Yeh, and K. Chen. 2009. The valuation of blood and tissue enzymes for clinical diagnosis in New Zealand white and rex rabbits. Taiwan Vet J. 35:1−8.

APPENDIX A

Expert Opinion Letter

Request for a New AAFCO Ingredient Definition: Ground *Juniperus pinchotii* and *Juniperus ashei*

After a critical and thorough evaluation of available literature and after carefully considering the use of the proposed ingredient and similar woody products in mixed diets for ruminant animals, it has been concluded (at this time) with reasonable certainty that ground juniper is safe for use as a feed ingredient in ruminant animal diets, according to good feeding practices and the intended use as cited in the enclosed proposal. In addition, edible animal products from ruminant animals consuming ground juniper do not pose any known hazards and are safe for human consumption.

Therefore, it is anticipated that AAFCO and FDA will concur that the information presented fully supports the claim that ground juniper (*Juniperus pinchotii* and *J. ashei* species) is safe for use as a feed ingredient in ruminant animal diets; thus, published as an official AAFCO feed ingredient definition.

7-1-2014

Travis R. Whitney, Ph.D.   Date
Associate Professor
Texas A&M AgriLife Research
San Angelo, TX 76901
325-653-4576
trwhitney@ag.tamu.edu

APPENDIX B

Author's Vitae
Dr. Travis R. Whitney

Texas A&M AgriLife Research, San Angelo, TX 76901
325-653-4576 / ext. 235; trwhitney@ag.tamu.edu
http://sanangelo.tamu.edu/people/faculty/dr-travis-r-whitney-associate-professor-livestock-nutrition

Education

2004: University of Arizona, Tucson. Ph.D. Major: Ruminant Nutrition; Minor: Rangeland Ecology and Management; Major Professor: Dr. Glenn Duff (Cum Laude).
1999: Texas A&M University, College Station. M.S. Major: Agricultural Education; Major Professor: Dr. James Christiansen (Cum Laude).
1996: Southwest Texas State University, San Marcos. B.S. Major: Animal Science (Cum Laude).

Experience

2011 – current: Associate Professor, Texas AgriLife Research, San Angelo. Research leader for the AgriLife Livestock Nutrition Program.
2005 – 2011: Assistant Professor, Texas AgriLife Research, San Angelo.
2005 – current: Adjunct Faculty at Angelo State University (San Angelo, TX) and Tarleton State Univ. (Stephenville, TX). Research advisor for multiple graduate students.
2004 – 2005: Post-doctorate, Montana State University, Animal and Range Science Department, Bozeman. Evaluated livestock grazing behavior and microbial function. Supervised two undergraduate and two graduate students.
2001 – 2004: Graduate Research and Teaching Assistant, University of Arizona, Animal Science Department, Tucson. Performed ruminant nutrition research and guest lectured livestock nutrition classes.
2000: Lecturer, Palo Alto Junior College, San Antonio, Texas. Taught Animal Science classes.
1998 – 1999: Graduate Assistant, Texas A&M University, Center for Grazinglands and Ranch Management. Assisted with conference development, client consultation, and database management.
1998 – 1999: Graduate Assistant, Texas A&M University, Instructional Materials Service. Developed Animal Production curriculum for high school students.

Current Professional and Scientific Society Activities

1. Member, American Society of Animal Science (2003 to present)
2. Member, Southern Section (ASAS) Small Ruminant Production Program Committee (2013 to present)
3. Member of the International Goat Association (2007 to present)
4. Member, Texas Sheep and Goat Raisers' Association (2006 to present)
5. Member, American Registry of Professional Animal Scientists (2005 to present)
6. Member, Society for Range Management (2005 to present)
7. Editorial Review Board, Small Ruminant Research (2005 to 2011)
8. ad hoc Reviewer, J. Anim. Sci. and other various journals (2007 to present)
9. Member, past president, Western Extension, Research, and Academic Coordinating Committee (WERA 039. Coordination of Sheep and Goat Research and Education Programs for the Western States. (2007 to present)

10. Co-chairman, Texas Forage and Beef Workers' committee
11. Chair, Western Section of the American Society of Animal Science Extension Committee (2011 to present)
12. Member, AgriLife Research Council of Principal Investigators (2011 to present)
13. Member, AgriLife Agricultural Animal Care and Use Committee (2012 to present)

Selected Refereed Publications out of 27 (* denotes graduate student)

Whitney, T. R., G. C. Duff, S. P. Cuneo, D.A. Henderson, D. W. Schafer, D. M. Hallford, R. J. Collier, and P. C. Gentry. 2006. Effects of weaning programs on serum metabolites and hepatic IGF-1 mRNA of first-calf heifers and mature cows. J. Food Agric. Environ. 4:49–53.

Whitney, T. R., G. C. Duff, J. K. Collins, D. W. Schafer, and D. M. Hallford. 2006. Effects of diet for early-weaned crossbred beef steers on metabolic profiles and febrile response to an infectious bovine herpesvirus-1 challenge. Livest. Prod. Sci. 101:1–9.

*McEachern, J., **T. R. Whitney**, C. B. Scott, C. J. Lupton, and M. W. Salisbury. 2009. Substituting distillers dried grains for cottonseed meal in lamb-finishing diets: growth performance, wool characteristics, and serum NEFA, urea N, and IGF-1 concentrations. Sheep and Goat Res. J. 24:32–40.

Whitney, T. R., and J. P. Muir. 2010. Redberry juniper as a roughage source in lamb feedlot rations: performance and serum nonesterified fatty acids, urea nitrogen, and insulin-like growth factor-1 concentrations. J. Anim. Sci. 88:1492–1502.

Whitney, T. R., and C. J. Lupton. 2010. Evaluating percentage of roughage in lamb finishing diets containing 40% dried distillers grains: Growth, serum urea nitrogen, nonesterified fatty acids, and insulin growth factor-1 concentrations and wool, carcass, and fatty acid characteristics. J. Anim. Sci. 88:3030–3040.

Whitney, T. R., and K. W. Braden. 2010. Substituting cottonseed meal with dried distillers grains in lamb feedlot rations: carcass and meat characteristics. Sheep and Goat Res. J. 25:49–56.

Whitney, T. R., A. E. Lee, D. R. Klein, C. B. Scott, and T. M. Craig. 2010. A modified in vitro larvae migration inhibition assay using rumen fluid to evaluate Haemonchus contortus viability. Vet. Parisitol. 176:217–225.

Whitney, T. R., A. E. Lee, M. G. Williamson, C. D. Swening, and R. L. Noland. 2011. Use of the Penn State forage particle separator to evaluate coarse-ground juniper as a supplemental feed limiter for lambs. Anim. Feed Sci. Technol. 168:21–29.

Whitney, T. R., S. Smith, and C. J. Lupton. 2011. Redberry juniper as a roughage source in lamb feedlot rations: wool and carcass characteristics, meat fatty acid profiles, and sensory panel traits. Meat Sci. 89:160–165.

Whitney, T. R., C. J. Lupton, J. P. Muir, R. P. Adams, and W. C. Stewart. 2014. Effects of using ground redberry juniper and dried distillers grains with solubles in lamb feedlot diets: Growth, blood serum, fecal, and wool characteristics. J. Anim. Sci. 92:1119−1132.

External Grants

- over $520,000

Other Activities

- Supervised 2 Ph.D. candidates, 8 M.S. students, and more than 12 student workers.
- Delivered over 30 presentations at scientific meetings and livestock producer field days

APPENDIX C

Research Trials: Use of *Juniperus pinchotii* and *J. ashei* in mixed diets

Please Note:

a. At times, key information was taken directly from the research manuscript and either paraphrased or reported within quotes.
b. For clarity among all of the different journal styles and nomenclature, paraphrased or exact quotes from authors' were edited within this proposal. This proposal used the style and format of the Journal of Animal Science. Also, for an example, if authors used "ashe juniper" as a common name for *J. ashei*, this was changed to "blueberry juniper" within this proposal, even if it was a direct quote from those authors.
c. At times, direct references were made to the authors' figures or tables. To distinguish between authors' tables and figures, tables and figures referring to this proposal are in **bold** font.
d. Unpublished data is listed in the Literature cited section; however, instead of a publication year, the reference is noted by a, b, etc…

Whitney et al., 2014. Effects of using ground redberry juniper and dried distillers grains with solubles in lamb feedlot diets: Growth, blood serum, fecal, and wool characteristics. J. Anim. Sci. 92:1119–1132.

* *This study was completed by Dr. Whitney's TX AgriLife Research Nutrition Program, San Angelo.*

This trial evaluated effects of using ground redberry juniper (*J. pinchotii*: leaves and stems) in Rambouillet wether lamb (n = 45) feedlot diets on growth, blood serum, fecal, and wool characteristics. In a randomized design study with 2 feeding periods (Period 1 = 64% concentrate diet, 35 d; Period 2 = 85% concentrate diet, 56 d), lambs were individually pen-fed isonitrogenous corn DDGS-based diets where 0% (0JUN), 33% (33JUN), 66% (66JUN), or 100% (100JUN) of the oat hay was replaced by juniper (**Table 15**).

During the spring, redberry juniper branches < 3.6 cm diameter were cut from mature redberry juniper trees. Within 2 d, branches were mechanically chipped and dried for 4 h to approximately 93% DM in a drying trailer (26 to 31°C). Chipped material was fine-ground in a hammermill to pass a 4.76-mm sieve, bagged, and stored under cover. Oat hay was ground in a hammermill to pass a 6.35-mm sieve. During Period 1, lamb DMI, ADG, and G:F quadratically increased ($P < 0.01$; **Table 16**) as juniper increased in the diet. During Period 2, DMI was similar ($P > 0.19$), ADG increased linearly ($P = 0.03$) and G:F tended to decrease quadratically ($P = 0.06$) as juniper increased in the diet (**Table 16**). Serum urea N (SUN) increased quadratically ($P = 0.01$) and fecal N increased linearly ($P = 0.01$; **Table 17**), which was partially be attributed to greater dietary urea and CT intake. Most wool characteristics were not affected, but wool growth/kg of BW decreased quadratically ($P = 0.04$) as percentage of juniper increased in the diet. A treatment × day interaction was observed for BW ($P = 0.004$; **Fig. 6**) and within the DDGS-based diets, lamb BW increased quadratically ($P < 0.01$) as percentage of juniper incrementally increased in the diet. Results indicated that replacing all of the ground oat hay with ground juniper in lamb growing and finishing diets is not detrimental to animal performance or health.

Conclusions:

"… Results also indicate that ground juniper leaves and stems can effectively replace all of the oat hay in corn DDGS-based growing and finishing diets without negatively affecting animal health, performance, or wool characteristics. However, using a combination of juniper and oat hay during the growing period (Period 1; high roughage diet) increased growth performance and reduced total feedlot costs as compared to using juniper or oat hay as the sole roughage source. The economics of processing, storing, and mixing two roughage sources will need to be considered, but it appears that the most economical feeding regimen in this trial would have been to feed 66JUN during the growing period and then feed 100JUN during the finishing period."

Summary Notes:

- % total CT in ground juniper = 6.0%
- % total CT in ground sorghum grain = 0.55%
- No adverse effects on animal health were reported in either feeding period.

Period 1:

- Values reported below are on a DM basis.
- tIVDMD: ground oat hay = 57.4%; ground juniper = 55%
- Average total daily DMI for lambs fed 100JUN = 1,020 g/d
- Ground juniper daily intake for lambs fed 100JUN =
  367 g of juniper/d = **12.2 g** of juniper/kg BW
- % ground juniper in 100JUN diet = **36%**
- Estimated total CT intake for lambs fed CNTL, 0JUN, 33JUN, 66JUN, and 100JUN =
  3.5, 0.8, 11.0, 19.8, and 22.9 g CT/d, respectively, which =
  0.10, 0.02, 0.32, 0.58, and 0.76 g CT/kg BW, respectively."
- Estimated total volatile oil intake for lambs fed CNTL, 0JUN, 33JUN, 66JUN, or 100JUN =
  0.04, 0.42, 1.56, 0.94, and 1.12 g oil/d, respectively, which =
  0.001, 0.013, 0.046, 0.027, and 0.037 g oil/kg BW (BW at d 35), respectively."

Period 2:

- Total daily DMI for lambs fed 100JUN = 1,380 g/d
- Ground juniper daily intake for lambs fed 100JUN =
  207 g of juniper/d = **4.73 g** of juniper/kg BW
- % ground juniper in diet = **15%**
- Estimated CT intake for lambs fed CNTL, 0JUN, 33JUN, 66JUN, and 100JUN =
  5.58, 2.47, 6.6, 11.5, and 15.1 g CT/d, respectively, which =
  0.118, 0.057, 0.143, 0.247, and 0.345 g CT/kg BW, respectively.

Table 15. Ingredient, chemical composition (% DM basis), and digestibility of treatment diets

	Diet[1]							
	Period 1				Period 2			
Item[2]	0JUN	33JUN	66JUN	100JUN	0JUN	33JUN	66JUN	100JUN
Ground juniper	–	12.0	24.0	36.0	–	5.0	10.0	15.0
Oat hay	36.0	24.0	12.0	–	15.0	10.0	5.0	–
Dried distillers grains	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Cottonseed meal	–	–	–	–	–	–	–	–
Sorghum grain	14.15	14.55	14.93	15.32	35.05	35.19	35.31	35.43
Molasses	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Limestone	2.75	2.2	1.67	1.13	2.85	2.62	2.40	2.19
Ammonium chloride	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Salt	0.9	0.9	0.9	.9	0.9	0.9	0.9	0.9
Mineral premix	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Urea	–	0.15	0.30	0.45	–	0.09	0.19	0.28
Nutrient Composition, %								
DM	91.8	91.9	91.9	92.0	91.0	91.7	91.8	91.8
CP	22.8	22.9	22.5	22.0	23.3	24.1	23.2	23.9
ADICP	0.7	0.8	1.0	1.1	0.7	1.3	1.8	1.7
NDF	33.8	32.9	30.7	29.7	28.0	27.2	26.1	24.2
ADF	17.2	18.1	18.0	19.0	13.8	14.6	15.3	13.9
Crude fat	7.0	7.6	8.0	8.2	7.5	8.0	7.9	8.2
Lignin	3.8	5.3	5.6	7.7	2.5	3.5	5.2	5.2
Ca	1.2	1.1	1.1	1.0	1.4	1.4	1.5	1.3
P	0.6	0.6	0.6	0.6	0.62	0.67	0.66	0.68
Ca:P	2.5	1.8	1.8	1.7	2.3	2.1	2.3	1.9
Ash	10.1	8.7	8.4	7.7	10.2	9.7	9.7	7.5
Volatile oil, %	0.04	0.11	0.07	0.11	0.04	0.03	0.03	0.03
True IVDMD, %	74.5	73.8	71.9	72.2	81.1	81.5	80.0	79.6

[1]Treatment diets were isonitrogenous, non-agglomerated feedlot growing rations containing ground juniper that replaced 0% (0JUN), 33% (33JUN), 66% (66JUN), or 100% (100JUN) of the ground oat hay. During Period 1 (d 0 to 35), lambs were fed a 64% concentrate ration. Lambs were transitioned over 4 d into Period 2 (d 36 to 91) onto an 85% concentrate ration.

[2]ADICP = acid detergent insoluble CP; true IVDMD = true 48-h *in vitro* dry matter digestibility.

Table 16. Effects of replacing oat hay with ground juniper on lamb performance

Item/d[3]	Diet[1] 0JUN	33JUN	66JUN	100JUN	SEM[4]	*P*-value[2] Linear	Quadratic
Period 1							
DMI, kg; overall	0.96	1.38	1.30	1.02	0.07	0.73	<0.001
ADG, kg; overall	0.14	0.22	0.22	0.13	0.02	0.41	<0.001
G:F, kg/kg; overall	0.15	0.16	0.17	0.12	0.01	0.17	0.007
Period 2							
DMI, kg/d; d 42 to 91	1.28	1.34	1.45	1.38	0.06	0.19	0.31
ADG, kg; d 42 to 91	0.24	0.25	0.25	0.28	0.01	0.03	0.29
G:F, kg/kg; overall	0.19	0.18	0.18	0.20	0.01	0.41	0.06
Entire trial, d 0 to 91							
DMI	1.16	1.34	1.39	1.24	0.06	0.27	0.004
ADG	0.20	0.24	0.24	0.22	0.01	0.30	0.02
G:F	0.18	0.18	0.17	0.18	0.01	0.87	0.68
BW, kg; final shorn	43.5	46.1	46.6	43.8	1.1	0.74	0.01

[1]Treatment diets were isonitrogenous and non-agglomerated, and contained ground juniper that replaced 0% (0JUN), 33% (33JUN), 66% (66JUN), or 100% (100JUN) of the ground oat hay.

[2]Linear and quadratic orthogonal contrasts.

[3]During Period 1 (d 0 to 35), lambs were fed a 64% concentrate ration. Lambs were transitioned over 4 d into Period 2 (d 36 to 91) onto an 85% concentrate ration.

[4]SEM = greatest standard error of the mean.

Whitney et al., 2013. Effect of using redberry juniper (*Juniperus pinchotii*) to reduce *Haemonchus contortus* fecal eggs and increase ivermectin efficacy. Vet. Parasitol. 197:182−188.

* *This study was completed (in part) by Dr. Whitney's TX AgriLife Research Nutrition in collaboration with the Agricultural Res. Station (VA State Univ., Petersburg, VA) and the Dept. of Biomedical Sci. and Pathobiology (VA Tech, Blacksburg, VA).*

Paraphrased abstract:

Objective: determine if a redberry juniper-based diet can reduce fecal egg counts (**FEC**) and increase ivermectin (**IVM**) efficacy in IVM-resistant *Haemonchus contortus*. After natural infection was established, cross-bred lambs (n = 64; 6 mo old; BW = approximately 22.3 kg) were randomly assigned to pens and fed a pelleted treatment diet (4 pens/treatment and 8 lambs/pen) consisting of traditional feed ingredients mixed with either 30% hay (CNTL) or 30% ground juniper leaves and stems (JUN; **Table 17**). Redberry juniper branches < 3.6 cm diameter were cut from mature redberry juniper trees, chipped, and dried for 4 h to 93% DM in a drying trailer (26 to 31°C). Chipped material was fine-ground in a hammermill to pass a 4.76-mm sieve, bagged, and stored under cover. Sorghum sudangrass hay was ground in a hammermill to pass a 6.35-mm sieve.

Lambs were fed during two periods: Period 1 (d 0 to 28) and Period 2 (d 28 to 42). On d 28, half of the lambs from each treatment and pen were treated with IVM orally (0.2 mg/kg), creating four treatment groups: lambs fed CNTL or JUN and either not treated (CNTLn, JUNn) or treated (CNTLi, JUNi) with IVM. During Period 1, lambs fed CNTL had greater ($P < 0.001$) ADG than lambs fed JUN (0.09 vs. 0.04 kg/d), which was probably caused by the CNTL diet having greater protein and less ADF, lignin, and CT than the JUN diet. During Period 2, CNTLi lambs had greater ($P < 0.05$) ADG than JUNn and JUNi lambs.

Summary Notes:

- Values reported below are on a DM basis.
- Total average daily DMI for a 22.3-kg goat = 910 g/d
- % ground juniper in diet = **30%**
- Maximum daily juniper intake for lambs fed JUN =
  273 g juniper/d [910 × 0.3] = **12.24 g** juniper/kg BW [273g/22.3 kg BW]
- % total CT in ground sorghum sudangrass hay = 0.91%
- % total CT in juniper material = 5.66%
- % total CT in CNTL diet = 1.06%
- % total CT in JUN diet = 2.11%
- % total volatile oil in JUN diet = 0.06%
- Average CT intake for lambs fed JUN =
  19.2 g/d [910 g total intake × 0.0211] = **0.86 g**/kg BW [19.2g/22.3 kg BW]
- Maximum volatile oil intake for lambs fed JUN =
  0.546 g/d [910 g total intake × 0.0006] = **0.02 g**/kg BW [0.0546g/22.3 kg BW]
- Average daily gain was low, but positive, for lambs in all treatment groups.
- No adverse effects on animal health were reported.

Table 17. Ingredient and chemical composition (DM basis) of sorghum sudangrass hay, dried juniper material, sorghum grain, and treatment supplement

	Ingredient			Diet[1]	
Item, %[2]	Hay	Juniper	Sorghum grain	CNTL	JUN
Ingredient					
Sorghum sudan grass hay				30	0
Redberry juniper				0	30
DDGS				20	20
Cottonseed meal				2	2
Sorghum grain				40.76	41.38
Molasses				3	3
Limestone				1.95	1.06
Ammonia Cl				0.54	0.81
Salt				0.85	0.85
Mineral premix				0.6	0.6
Pellet binder				0.3	0.3
Chemical composition, %					
CP	8.7	7	11.4	19.1	16.8
Crude fat	1.2	7.2	6.4	5.9	6
ADICP	1.4	1.4	1.3	1.8	1.8
NDF	71.2	43.6	9.6	30.2	29.6
ADF	48.5	33.6	4.6	18.2	22.8
ADL	7.3	17	1	5.6	9.1
Ash	14.7	6.2	1.9	8.5	7.3
Ca	0.5	1.8	0.07	0.9	1
P	0.2	0.1	0.5	0.7	0.5
CT, total	0.91	5.66	0.53	1.06	2.11
CT, extractable	0	3.35	0	0	0.71
CT, fiber-bound	0.55	0.54	0.26	0.68	0.68
CT, protein-bound	0.36	1.77	0.27	0.38	0.72
Volatile oil, total		0.43		0.01	0.06

[1]Supplements were pelleted and contained either ground sorghum sudan grass hay (CNTL) or ground redberry juniper leaves and stems (JUN).

[2]Sorghum sudan grass hay = ground to pass a 6.35-mm sieve; redberry juniper = ground leaves and stems < 3.6 cm diameter, fine-ground to pass a 4.76-mm sieve; DDGS = corn dried distillers grains with solubles; ADICP = acid detergent insoluble crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CT = condensed tannins.

Stewart et al. Unpublished data. Effects of using ground redberry juniper in ewe supplements on growth, blood serum, wool characteristics, and on lamb birth and weaning weights.

* *This trial was recently completed by Dr. Whitney's TX AgriLife Research Nutrition Program, San Angelo in collaboration with NM State Univ., Las Cruces; thus,* ***data have not been statistically analyzed or submitted for publication****.*

Approximately 56 d after conception, mature, pregnant Rambouillet ewes (n = 28) were randomly allocated to individual pens, fed a basal Sorghum sudangrass ground hay diet, and assigned to 1 of 4 pelleted treatment supplements that contained either 0% (CNTL), 18%

(18JUN), 36% (36JUN), or 54% (54JUN) ground juniper (**Table 18**); supplements were fed until lambing. Mature redberry juniper trees were cut, chipped, and allowed to air-dry under cover for 7 to 12 d to approximately 78% DM. Chipped material was then fine-ground in a hammermill to pass a 4.76-mm sieve. Sorghum sudangrass hay that was used as the basal diet and in the supplements was ground in a hammermill to pass a 6.35-mm sieve.

Summary Notes:

- Values reported below are on a DM basis.
- Maximum juniper intake for ewes supplemented with 54JUN = **463.3 g** juniper/d = **5.5 g** juniper/kg BW
- % total CT in the ground juniper = 4.2%
- % total CT in 54JUN supplement (based solely upon CT in the juniper) = **2.27%**
- Maximum CT consumed/d = **19.5 g** CT/d = **0.23 g** CT/kg BW
- Maximum volatile oil consumed/d = **6.18 g** oil/d = **0.07 g** oil/kg BW
- % ground juniper consumed in relation to total daily DMI = **19.4%**
- No adverse effects on ewe health (daily visual evaluation, change in body condition), lambing, lamb birth weight, or lamb weaning weight were observed (**Table 19**).

Table 18. Ingredient and chemical composition (DM basis) pelleted treatment supplements

	Supplement			
Item[1]	CNTL	18JUN	36JUN	54JUN
Ground juniper	–	18	36	54
Ground hay	54	36	18	–
DDGS	29.75	29.75	29.75	29.75
Cottonseed meal	3	3	3	3
Sorghum grain	6	6	6	6
Molasses	4	4	4	4
Ammonium chloride	0.75	0.75	0.75	0.75
Salt	1	1	1	1
Mineral premix	1.5	1.5	1.5	1.5
Chemical composition, %				
CP	16	15.3	14.5	13.9
NDF	36	38	41	42
ADF	19	24	30	35
CT	0	0.76	1.51	2.27
Volatile oil	0	0.22	0.41	0.72
tIVDMD, %	75.5	72.5	64.7	50.1

[1]CT calculated based only on CT in the juniper portion in the diet. Ground hay = sorghum sudangrass hay; DDGS = corn dried distillers grains with solubles.

Table 19. Effects of using ground redberry juniper in ewe supplements on DMI, ewe milk quality lamb survival, birth weight, and growth performance[1]

	Supplement Group[2]			
Item[3]	CNTL	18JUN	36JUN	54JUN
BW of ewe at max juniper intake, kg	97.5	94.8	78.5	84.8
Maximum supplement intake, g/d	1,006	992	821	858
Hay intake, g/d	1,241	1,213	1,288	1,526
Total DMI, g/d (basal + supplement)	2,247	2,205	2,109	2,384
Maximum juniper intake, g/d	0	179	296	463
Maximum juniper intake, g/kg of BW	0	1.88	3.77	5.46
CT intake, g/d	0	7.5	12.4	19.5
CT intake, g/kg BW	0	0.08	0.16	0.23
Volatile oil intake, g/d	0	2.18	3.37	6.18
Volatile oil intake, g/kg BW	0	0.02	0.04	0.07
Milk fat, %	3.8	3.3	3.5	3.2
Milk protein, %	3.5	3.5	3.5	3.4
# lambs that died within 14 d of parturition	2	2	0	1
Lamb BW				
0 d after parturition	5.1	5.1	5.0	5.2
14 d after parturition	12	9.7	8.4	11.8
50 d after parturition (weaning)	22.8	21.1	22.0	21.2
Lamb ADG				
0 to 14 d after parturition	0.48	0.33	0.24	0.46
14 to 50 d after parturition	0.30	0.32	0.36	0.28

[1]**Note: Data has not been statistically analyzed or published.**

[2]Ewes fed a basal hay diet and supplemented daily with one of 4 pelleted feeds that contained either 0% (CNTL), 18% (18JUN), 36% (36JUN), or 54% (54JUN) ground juniper.

[3]Intake data represent the day of maximum consumption of supplement by a ewe within each supplement treatment group. Lamb BW and ADG represent only the lambs that were born as single lambs. After parturition, ewes and lambs were placed in a drylot for approximately 14 d and fed sorghum sudangrass hay. Ewes and lambs were then placed into a pasture for the duration of the study (d 14 to 56).

Whitney et al., 2011. Use of the Penn State forage particle separator to evaluate coarse-ground juniper as a supplemental feed limiter for lambs. Anim. Feed Sci. Technol. 168:21–29.

* *This study was completed in by Dr. Whitney's TX AgriLife Research Nutrition Program, San Angelo.*

Paraphrased Abstract:

This study determine if molasses can reduce sorting of course-ground juniper when juniper is used as a feed intake limiter for lambs. Rambouillet wether lambs (n = 21; approximate age = 6 mo; initial BW = 43.5 kg) were fed *ad libitum* treatments in the morning that consisted of juniper material, DDGS, and either no water or molasses (CNTL; 50:50:0), water (WAT; 45:45:10), or a 50:50 water:cane molasses solution (MOL; 45:45:10); lambs were fed an *ad libitum* basal pelleted diet in the afternoon. Redberry juniper branches < 3.6 cm diameter were cut from mature trees, chipped using a wood chipper, dried for 5 to 6 h to approximately 92% DM in a drying trailer, bagged, and stored under cover. Chipped material was not hammermilled. The water or water–molasses solution was sprayed onto the chipped juniper/DDGS mixture using a hand-pump sprayer and mixed by hand to ensure even distribution. Typical particle size distribution is shown in **Figure 7**. Ingredients of treatments and chemical composition (g/kg of DM) of basal diet, DDGS, juniper, and treatments are presented in **Table 20**.

Lambs were transitioned over 4 d onto an *ad libitum* basal pelleted diet and *ad libitum* treatment diet; *ad libitum* calculated for each lamb as previous day's intake plus 150 g. Lamb final BW, ADG, intake of treatment and basal diet, total DMI, and G:F are presented in **Table 21**. No differences ($P > 0.17$) were observed for final BW, ADG, basal diet and total DMI, and G:F. Treatment DMI of MOL was greater than CNTL but similar to WAT ($P < 0.09$); however, basal diet and total DMI remained similar among lambs ($P > 0.70$).

Summary Notes:

- Values reported below are on a DM basis.
- Maximum daily chipped juniper intake (approximate due to feed refusals containing various amounts of juniper) =
  89 g of juniper = **1.87 g** of juniper/kg BW (mid-study BW = 47.5 kg).
- Basal pelleted diet intake = 1,479 g
- % juniper consumed in relation to total daily DMI = **5.3%**
- Daily volatile intake (if assume 0.43% volatile oil in the dried juniper, DM basis) =
  0.383 g volatile oil = **0.008 g** volatile oil/kg BW
- Daily CT intake (if assume 5.5% CT in the juniper, DM basis) =
  4.88 g CT = **0.103 g** CT/kg BW
- Daily visual observation of each lamb revealed no adverse effects on animal health.

Fig. 7. Particle distribution of a supplement (control) separated by the Penn State Particle Separator; left to right: material remaining on 19.0, 8.0, and 1.18-mm sieves, and in bottom pan (< 1.18 mm).

Table 20. Ingredients of treatments and chemical composition (DM basis) of basal diet, DDGS, juniper forage, and treatments

				Treatment[1]		
Item, %[2]	Basal	DDGS	Juniper	CNTL	WAT	MOL
Ingredient						
Juniper				50.0	45.0	45.0
DDGS				50.0	45.0	45.0
Water				-	10.0	-
Molasses:water (50:50)				-	-	10.0
Chemical composition						
DM	92.1	94.8	94.0	92.4	89.0	90.9
CP	17.9	26.8	4.8	13.9	14.6	14.3
ADF	18.7	12.7	45.9	30.5	26.9	28.3
aNDF	30.3	37.3	54.0	47.8	44.9	43.9
Crude fat		8.6	3.4	7.2	7.3	6.6
Ash	6.8	4.3	4.8	4.7	4.3	5.0

[1]Treatments consisted of coarse-ground juniper leaves and stems and DDGS containing either no water or molasses (CNTL), water (WAT), or a 50:50 solution of molasses and water (MOL).

[2]Juniper forage consisted of ground juniper leaves and stems (pre-harvest stem diameter < 3.6 cm) dried to approximately 92% DM; aNDF = NDF assayed with a heat stable amylase and expressed inclusive of residual ash.

Table 21. Effects of adding water or molasses to ground juniper forage and DDGS on lamb performance[1]

	Treatment[2]				*P*-value[3]		
Item	CNTL	WAT	MOL	SEM[4]	t	d	t × d
Initial BW, kg	43.8	43.8	42.9	1.7	0.97	<0.001	0.09
Final BW, kg	50.6	50.8	52.0	1.8	0.97	<0.001	0.09
ADG, g	240	260	320	30	0.21	0.003	0.20
Treatment DMI, g	94[b]	107[ab]	197[a]	38	0.09	0.02	0.81
Basal diet DMI, g	1,470	1,500	1,480	79	0.95	<0.001	0.91
Total DMI, g	1,560	1,610	1,680	97	0.70	<0.001	0.86
G:F, kg gain/kg intake	0.16	0.16	0.20	0.02	0.18	<0.001	0.13

[1]Means within a row without a common superscript differ ($P < 0.10$).

[2]Treatments consisted of coarse-ground juniper leaves and stems and DDGS containing either no water or molasses (CNTL; 50:50), water (WAT; 45:45:10), or a 50:50 solution of cane molasses and water (MOL; 45:45:10).

[3]t = treatment; d = day

[4]Standard error of the means.

Whitney and Muir, 2010. Redberry juniper as a roughage source in lamb feedlot rations: performance and serum nonesterified fatty acids, urea nitrogen, and insulin-like growth factor-1 concentrations. J. Anim. Sci. 88:1492–1502.

* *This study was completed by Dr. Whitney's TX AgriLife Research Nutrition Program, San Angelo.*

Rambouillet ram lambs (n = 24, initial BW = 29 kg) were fed during 2 periods (Period 1 = 65% concentrate ration, 28 d; Period 2 = 85% concentrate ration, 49 d). Lambs were individually fed *ad libitum* diets containing cottonseed hulls (control; CSH), half of the cottonseed hulls replaced by air-dried juniper leaves (CSHJ), or all the cottonseed hulls replaced by air-dried juniper leaves (JUN; **Tables 22 and 23**). Leaves were removed from mature redberry juniper tree branches after the branches were cut and air-dried approximately 30 d.

Lamb BW was similar on d 0 and 14, but increasing juniper in the diet linearly reduced (P = 0.04) BW on d 28 (**Fig. 8**). Differences in BW during Period 1 are attributed to ADG and average daily DMI linearly decreasing (P < 0.001) with increasing concentrations of juniper, with lambs fed CSH, CSHJ, or JUN diets having ADG of 0.34, 0.30, and 0.14 kg, respectively (**Table 24**). Differences in DMI are partially attributed to secondary compounds in the cottonseed hulls and juniper (**Table 23**). Lambs fed CSHJ diets had the greatest (P = 0.04) G:F compared with lambs fed CSH and JUN during Period 1 (**Table 24**). Lambs fed JUN diets tended to have the greatest (P = 0.09) serum non-esterified fatty acid (**NEFA**) concentrations during Period 1, and increasing juniper in the diet linearly reduced (P = 0.006) serum urea N (**SUN**) and serum insulin-like growth factor-1 (**IGF-1**) on d 14 and 28, respectively.

During Period 2, DMI and ADG of lambs fed JUN diet rapidly increased, resulting in all lambs having similar ADG, DMI, G:F, and BW (**Table 24**). Results indicated that air-dried redberry juniper leaves can replace all of the cottonseed hulls in lamb feedlot rations. Feeding 30% juniper in the diet for a longer period of time during the initial feeding period probably would have further reduced growth performance.

Conclusions:

"Results indicate that air-dried redberry juniper leaves can effectively be used as a roughage source and can replace all of the cottonseed hulls in lamb feedlot rations, but may reduce intake and consecutively growth at greater inclusion levels. Results also indicate that secondary compounds and nutrient-toxin interactions should be considered when evaluating the nutritional quality of a feedstuff and nutrient requirements of the animal and its rumen microbial populations. Utilization of juniper as a roughage source could provide ranchers with a readily available on-site feed resource and possibly lessen the negative impact of this undesirable invasive brush species…"

Summary Notes:

- Values reported below are on a DM basis.
- tIVDMD: CSH = 20.8%; juniper leaves = 67%
- % total CT in juniper = 5.46%   % total CT in JUN diet = 4.4%
- % total volatile oil in juniper during Periods 1 and 2 = 0.52% and 2.22%, respectively.
- Daily visual observation of each lamb revealed no adverse effects on animal health.

Period 1:

- Average BW of lambs fed JUN diet = 30 kg
- Average total daily DMI = 1,190 g/d
- Maximum % juniper leaves in diet = **30%**
- Average daily juniper leaf intake for lambs fed JUN diet =
  357 g of juniper/d = **11.9 g** of juniper/kg of BW
- Average CT consumed/d (based upon CT of JUN diet during Period 1) =
  52.4 g CT/d = **1.75 g** CT/kg BW
- Average volatile oil consumed/d =
  1.86 g oil/d = **0.06 g** oil/kg BW

Period 2:

- Average BW of lambs fed JUN diet = 48 kg
- Total daily DMI = 1,620 g/d
- Maximum % ground juniper in diet = **10%**
- Average daily juniper leaf intake for lambs fed JUN =
  162 g juniper/d = **3.38 g** juniper/kg BW
- Average CT consumed/d (based solely on % CT in juniper portion of the diet; CT not analyzed in diets fed during Period 2) =
  8.85 g CT/d = **0.184 g** CT/kg BW
- Average volatile oil consumed/d =
  3.6 g oil/d = **0.075 g** oil/kg BW

Table 22. Ingredient and chemical composition (DM basis) of dry juniper leaves, cottonseed hulls, and treatment diets

			Diet[1]					
			Period 1			Period 2		
Item	Juniper Leaves	Cottonseed Hulls	CSH	CSHJ	JUN	CSH	CSHJ	JUN
Juniper leaves			0.0	15.0	30.0	0.0	5.0	10.0
Cottonseed hulls			30.0	15.0	0.0	10.0	5.0	0.0
Alfalfa meal, dehy			5.0	5.0	5.0	5.0	5.0	5.0
Crushed milo			46.0	46.0	46.0	66.0	66.0	66.0
Cottonseed meal			11.2	11.2	11.2	11.0	11.0	11.0
Molasses			5.0	5.0	5.0	5.0	5.0	5.0
Limestone			1.0	1.0	1.0	1.2	1.2	1.2
Ammonium Cl			0.8	0.8	0.8	0.8	0.8	0.8
Mineral premix			1.0	1.0	1.0	1.0	1.0	1.0
CP, %	7.1	6.6	15.1	14.7	15.1	16.3	16.2	14.6
Crude fat, %	8.7	0.9	2.9	4.9	6.6	4.2	-	4.2
NDF, %	37.8	79.9	38.3	34.1	30.8	22.8	24.1	19.5
ADF, %	31.2	69.5	29.0	24.7	22.8	14.3	16.0	12.6
Ash, %	5.3	2.8	7.0	6.7	7.8	7.1	-	5.2
TDN, %	63	33	68	76	77	74	79	80
Ca, %	1.46	0.18	0.87	1.06	1.45	0.80	1.03	0.82
P, %	0.07	0.10	0.43	0.40	0.37	0.45	0.50	0.36
Ca:P	20.9	1.80	2.02	2.65	3.92	1.78	2.06	2.28
Mg, %	0.15	0.20	0.31	0.29	0.29	0.29	0.33	0.23
Na, %	0.02	0.02	0.35	0.34	0.37	0.37	0.40	0.40
S, %	0.07	0.09	0.31	0.27	0.27	0.27	0.31	0.22
Fe, ppm	602	580	296	340	338	425	731	778
Zn, ppm	15	14	92	91	87	82	98	69
Cu, ppm	2	5	7	7	5	5	7	5
Mn, ppm	28	17	83	87	85	98	92	62
Mo, ppm	< 1	0.4	1.1	1.8	< 1	1.3	2.6	1.2

[1]Treatment diets contained either cottonseed hulls (control; CSH), and half (CSHJ) or all (JUN) of the cottonseed hulls replaced by dry juniper leaves.

Table 23. Solubility, digestibility, particle-associated bacteria, and condensed tannins (DM basis) of dry juniper leaves, cottonseed hulls, and treatment diets during Period 1[1]

Item[3]	juniper leaves	cottonseed hulls	Diet[2] CSH	CSHJ	JUN
Soluble DM, % of initial DM	36.9	11.7	25.9	29.6	34.4
Soluble CP, % of initial DM	2.02	1.39	4.91	3.96	4.41
48-h true IVDMD, %	67.0	20.8	67.2	75.0	79.9
Degradable CP, % of initial DM	4.44	3.35	12.7	12.3	12.4
CP digestion, % of initial CP	62.5	51.2	84.2	83.5	81.8
UIP, % of initial DM	2.7	3.2	2.4	2.4	2.8
PAB, mg CP	13.6	9.9	22.6	24.0	24.4
Condensed tannins (CT)					
extractable CT, %	4.12	3.26	0.43	2.19	2.31
protein-bound CT, %	0.96	2.19	0.80	0.50	0.43
fiber-bound CT, %	0.38	0.18	1.84	2.04	1.66
total CT, %	5.46	5.64	3.06	4.72	4.40

[1]During Period 1 (d 0 to 28), lambs were fed a 65% concentrate ration.

[2] Treatment diets contained either cottonseed hulls (control; CSH), and half (CSHJ) or all (JUN) of the cottonseed hulls replaced by dry juniper leaves.

[3]Juniper leaves collected from air-dried branches. PAB, particle-associated bacteria = (g N (× 6.25) after *in vitro* digestion but before NDF rinse) – (g N (× 6.25) after digestion and NDF rinse).

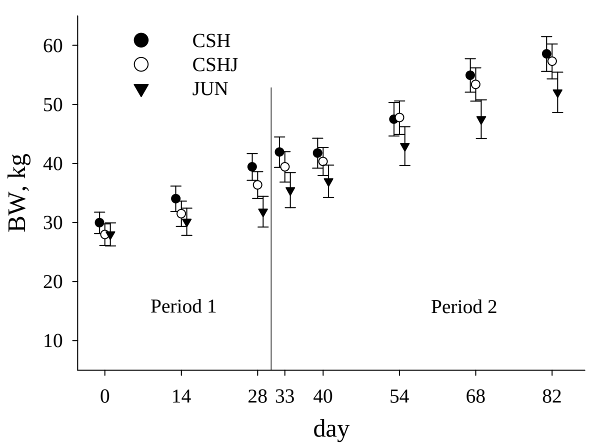
Table 24. Effects of replacing cottonseed hulls with juniper leaves on lamb performance

Item/d	Diet[1] CSH	CSHJ	JUN	SEM	*P*-value[2] Linear	Quadratic
Period 1						
ADG, kg	0.34	0.30	0.14	0.03	< 0.001	0.08
DMI, kg	2.10	1.62	1.19	0.13	< 0.001	0.86
G:F, kg/kg	0.16	0.19	0.12	0.02	0.07	0.04
Period 2						
ADG, kg	0.32	0.34	0.32	0.03	0.87	0.62
DMI, kg	1.85	1.88	1.62	0.12	0.16	0.28
G:F, kg/kg	0.17	0.18	0.20	0.02	0.14	0.85

[1] Treatment diets contained either cottonseed hulls (control; CSH), and half (CSHJ) or all (JUN) of the cottonseed hulls replaced by dry juniper leaves.

[2]Linear and quadratic orthogonal polynomial contrasts.

Fig. 8. Effects of replacing CSH with juniper leaves in rations on lamb BW. A treatment × d interaction was observed during Period 1 (*P* < 0.001) but not Period 2 (*P* > 0.18). Lamb BW was similar (*P* > 0.12) on d 0 and 14, but on d 28, lamb BW linearly (*P* < 0.04) decreased as percentage of juniper increased in the diet.



APPENDIX D

Research Trials: Use of *Juniperus* species other than *J. pinchotii* and *J. ashei* in mixed diets

Giacomini et al., 2006. Assessing the nutritive value of one-seed juniper in sheep. New Mexico cattle grower's short course proceedings.

Five ruminally fistulated wethers (BW = 55 kg) were used in 2 cross-over experiments composed of two 20-d periods to estimate nutritive value of one-seed juniper (JM; 73% DM, 71.7% NDF, 6.0% CP, DM basis) as a forage. Harvested juniper consisted of leaves stripped from the ends of branches of immature shrubs and stored at 4°C. Trial 1: sheep were fed either 100% buffalo grass straw (BS; 93% DM, 76.5% NDF, 4.5% CP, DM basis) or a mixed diet of 75% buffalo grass straw and 25% JM (BS+JM) at 2% of BW. Trial 2: either soybean meal (**SBM**) or fish meal (**FM**) was added to BS+JM. Protein sources of differing rumen degradabilities were fed to determine the potential for associative effects. Nutrient composition of the grass straw and one-seed juniper are reported in **Table 25.** Sheep were gradually adapted to a diet fed at 2% of BW. Rumen NDF and DM fill were similar ($P > 0.05$) among sheep and diets. The BS+JM diet showed greater ($P < 0.05$) DM and NDF digestibilities vs. 100% BS diet (BS+JM: 56.2, 65.9%; BS: 47.7, 54.4, for DM and NDF digestibility, respectively). Results indicated that consumption of juniper in a diet similar to dormant native range increased total diet digestibility. Trial 2: Addition of SBM or FM to the mixed diet had no influence ($P \geq 0.15$) on DM or NDF digestibility (SBM: 49.1, 50.9; FM: 57.4, 61.3% for DM and NDF digestibility, respectively). This indicates that there is a lack of associative effects associated with total diet digestibility when one-seed juniper is fed with protein supplements.

Conclusion:

"… juniper may indeed be a feasible feedstuff when browsed by small ruminants. Although laboratory measurements indicate that juniper is highly digestible, palatability may reduce its consumption in a practical setting. Thus, adaptation to diets containing juniper may be necessary, and may also reduce the antimicrobial effects of secondary metabolites found in juniper. Digestibility of the diet containing juniper was higher than that of the basal buffalo grass straw diet, implying that the addition of juniper to a range diet will not negatively impact overall diet digestibility over a short period of time. Testing of liver-specific enzymes [ALT transferase and alkaline phosphatase] also yielded no evidence of toxicosis due to the secondary metabolites found in juniper, and although juniper was not analyzed, we believe that juniper consumption did not compromise hepatic function in sheep used in this study."

Summary Notes:

- Feed intake was reported on an as-fed basis (Dr. Mark Petersen, *co-author*).
- Values below were calculated on a DM basis.
- Maximum total daily DMI = 969.1 g/d
- Maximum daily juniper leaf intake for lambs fed BS + JM diet =
  200.2 g of juniper/d = **3.64 g** of juniper/kg of BW
- Maximum % ground juniper as a percentage of the diet = **20.7%**
- If one-seed juniper leaves contained 2.18% volatile oil (DM basis; Utsumi et al., 2006; Utsumi et al., 2009), then maximum total daily volatile oil intake =

4.36 g [200.2 × 0.0218] = **0.079 g** volatile oil/kg BW [4.36 g/55 kg BW]

- If one-seed juniper leaves contained 5.92% CT (DM basis; Utsumi et al., 2009), then maximum total daily CT intake =
  11.85 g [200.2 × 0.0592] = **0.215 g CT**/kg BW [11.85 g/55 kg BW]
- No adverse effects on animal health were reported.

Table 25. Nutrient composition (DM basis) of buffalo grass straw and one-seed juniper

Item, %	Buffalo Grass Straw	One-seed Juniper
DM	93.2	72.8
NDF	76.5	71.7
CP	4.7	6.0

Animut et al., 2004. Effects of methods of exposure to Eastern red cedar foliage on cedar consumption by Boer crossbred wether goats. Small Rumin. Res. 54:197–212.

Twenty-four Boer crossbred yearling wethers (initial BW = 23.5 kg) were used to determine effects of stepwise increases in dietary level of fresh Eastern red cedar foliage (CF) compared with a constant relatively high level and subsequent availability of low-quality forage, on present and later consumption of CF. Animals were penned individually in Phases 1 (8 wk) and 3 (2 wk); wethers were kept in a pasture not containing cedar trees and were fed wheat hay during Phase 2 (6 wk). In Phase 1, a concentrate-based diet (CBD, 12.6% CP and 35.5% NDF) was offered at approximately 85% of the maintenance energy requirement alone (Control) or with weekly stepwise (Step) increases in level of substitution of CF for CBD (0, 1.25, 2.5, 5, 10, 15, 20, and 25% in wk 1 to 8, respectively; DM basis) or substitution of 25% CF in week 2 to 8 (Set). In Phase 3 (2 wk), all wethers were offered 75% CBD and 25% CF as previously, without or with separate free-choice access to low-quality grass hay.

Phase 1: intake of CF as a percentage of that offered was greater ($P < 0.05$) for Step vs. Set in wk 2 to 8 (wk 2: 84% and 68%; wk 3: 86% and 48%; wk 4: 89% and 56%; wk 5: 90% and 71%; wk 6: 96% and 81%; wk 7: 93% and 63%; wk 8: 96% and 84%), although CF intake as g/d was greater ($P < 0.05$) for Set vs. Step in all but wk 7 and 8.

Phase 3: CBD intake was similar among treatments, and hay intake when offered averaged 149, 134 and 124 g/d for Step, Set, and Control, respectively. For wethers not receiving hay, CF intake as g/d for Step was greatest among treatments ($P < 0.05$) but was not different from treatments with offered hay (67, 37, 30, 55, 53 and 56 g/d for Step, Set, and Control without and with hay, respectively). Similarly, CF intake as a percentage of that offered ranked ($P < 0.05$) Step > Set > Control without hay, but was not different between Step without hay and treatments with hay (78, 41, 34, 61, 57 and 60% for Step, Set and Control without and with hay, respectively). “Because there was not a decrease in CF intake with advancing wk of Phase 1 by Set wethers, feedback aversions may not have been present or appreciable.” Concentrations of various blood constituents [Na, K, Cl, carbon dioxide, urea N, P, glucose, Ca, total protein, albumin, globulin, bilirubin, lactate dehydrogenase, alkaline phosphatase, creatine kinase, γ-glutamyltransferase, aspartate aminotransferase] at the end of Phases 1 and 3 did not indicate adverse health effects of CF consumption. In conclusion, gradual increases in dietary level of CF deserve further research as a potential means to elevate present and future CF consumption, with attention also directed to effects of type and level of other feedstuffs offered.

Conclusion:

"CF consumed for 7 wk at up to 25% of the diet did not adversely affect growth rate or health of yearling goats. Slow, stepwise adaptation to CF resulted in slightly greater CF intake in the last 2 week of adaptation as a proportion of that offered than use of a constant dietary level, although the quantity consumed was similar. Addition of hay to CF-containing diets did not lessen CF intake, but rather elicited CF intake as great."

Summary Notes:

- Values reported below are on a DM basis.
- Maximum CF intake = **84 g** CF/d = **3.57 g** CF/kg BW
- Average total daily DMI at time (wk 8) of maximum CF intake = 372 g
- % juniper consumed as a % of daily DMI = **22.6%**
- Maximum volatile oil in CF = 3.18%
- Sabinene, limonene, safrole, terpin-4-ol and elemol were in greatest concentrations in CF.
- Maximum daily volatile oil consumed = **0.719 g** oil/d = **0.031 g** oil/kg BW
- "Replacement of up to 25% CBD by CF did not accentuate BW loss in either Phase 1 or 3."
- "Concentrations of various blood constituents at the end of Phases 1 and 3 did not indicate adverse health effects of CF consumption."
- No adverse effects on animal health were reported; based on blood constituents listed above.

APPENDIX E

Research Trials: Use of *Populus* species in mixed diets

Dinius et al., 1970. Intake and digestibility by sheep of rations containing various roughage substitutes. J. Anim. Sci. 30:309–312.

Note: Authors do not state if data is reported on an as-fed or DM basis; however, percentage of DM in all of the ingredients was probably fairly similar. Thus, data presented in the publication (and in the summary below) should closely represent what would be calculated on a DM-basis.

Wether sheep (n = 3) were fed a high concentrate (90%) diet consisting of 10% roughage (e.g. corn cobs, aspen, or oak sawdust). Diets were fed *ad libitum* for 3 wk. Concentrate portion of the diet was shelled, cracked corn (35.5%), rolled barley (34%), SBM (25%), dried molasses (5%), and a mineral premix (1.5%).

Conclusion:

"Several potential roughage substitutes were fed to sheep at a rate of 10% of the complete ration to determine the acceptability and digestibility of rations containing these products. Materials evaluated include: wood products (sawdust, shavings, flooring waste)… with ground corn cobs serving as the control. The DMI of the rations containing wood by-products… were not significantly different from that of the control ration with ground corn cobs… A variety of roughage substitutes can be incorporated into complete feeds without adversely affecting digestible energy intake…. The presence of the substitutes tended to increase the digestibility of the concentrate fraction of the rations."

Summary Notes:

- Values reported below are on a DM basis.
- Apparent DMD of diets containing aspen or oak sawdust was 77% and 75%, respectively. Apparent DMD of diets containing corn cobs (control) was 77.5%.
- Total intake of the diet containing aspen sawdust = **74.8 g**/kg $BW^{0.75}$, which was similar to the control diet with corn cobs (**71.5 g**/kg $BW^{0.75}$).
- Roughage substitutes tended to increase digestibility of concentrate-portion of the diet.
- If assume (not reported) that BW = 25 kg, then total DMI 836 g/d and aspen intake = **84 g**/d = **3.36 g** aspen/kg BW.
- One sheep fed a diet with oak sawdust developed a "mild case of [ruminal] laminitis" but it was not attributed to the feed.
- No "health problems attributed to the feeding of these roughage substitutes."

Satter et al., 1970. Aspen sawdust as a partial roughage substitute in a high-concentrate dairy ration. J. Dairy Sci. 53:1455−1460.

Note: authors do not state if diet composition is reported on an as-fed or DM basis; however, percentage of DM in all of the ingredients was probably similar. Thus, data presented in the publication (and in the summary below) should closely represent what would be calculated on a DM-basis.

Lactating cows (n = 12; 4/group) were fed either: (1) equal parts of hay and pelleted concentrate; (2) limited hay and pelleted concentrate containing 32% aspen sawdust; or (3) limited hay and pelleted concentrate. The air-dried, hammermilled aspen sawdust came from a sawmill that sawed bark-free logs of *Populus grandidentata*. The diet containing 32% aspen consisted of ground shelled corn (42.7%), SBM (19.6%), molasses (3.3%), and mineral (2%). Cows were fed *ad libitum* for 38 d.

Aspen sawdust was effective as a partial roughage substitute in a high-grain dairy ration. Cows receiving 2.3 kg of hay and about 17 kg of pelleted grain, one-third of which was aspen sawdust, maintained a normal milk fat level. Cows receiving a similar ration without sawdust had a milk fat content half as great. The ratio of ruminal acetate to propionate was much higher in the cows fed aspen and was closer to the ratio in the group of cows fed equal amounts of hay and concentrate. Inclusion of aspen in a high-concentrate ration nearly doubled ruminating time. If less dietary aspen would be equally as effective in complete pelleted dairy rations, aspen sawdust could become an attractive roughage substitute in areas where hay is expensive and difficult to obtain.

Summary Notes:

- Values reported below are on a DM basis.
- In the beginning, cows were reluctant to consume pellets containing aspen for 3 to 4 d. "There appeared to be a repulsive component in the aspen because the cows would readily detect a difference between treatments… Once consumption started however, there was no problem with feed intake."
- Cows fed pellets containing aspen had the greatest intake.
- Total daily DMI of diet with aspen sawdust = 2.0 kg hay + 14.9 kg treatment
- Total aspen sawdust consumed/d = **4.83 kg**
- Assume that mature cows BW (not stated by authors) = 635 kg, then daily aspen consumed = **7.6 g**/kg BW [4,830 g/635 kg BW]
- No adverse effects on animal health were reported.

Satter et al., 1973. Value of aspen sawdust as a roughage replacement in high-concentrate dairy rations. J. Dairy Sci. 56:1291−1297.

Note: authors do not state if diet composition is reported on an as-fed or DM basis; however, percentage of DM in all of the ingredients was probably similar. Thus, diet composition data should closely represent what would be calculated on a DM-basis. In addition, intake is reported on an air-dried basis in the publication, but reported on a DM-basis below.

Paraphrased Abstract:

This trial was used to determine if aspen sawdust (*P. grandidentata* Michx.) could be a roughage replacement in high-concentrate dairy rations. The sawdust was "from a mill that sawed bark-free logs and after air-drying, the sawdust was hammermilled through a 0.16-cm screen." Lactating Holstein cows (n = 20; 161 d post-partum) were separated into four groups and fed *ad libitum* one of the following pelleted rations for 6 wk: (1) grain mix with 10% sawdust; (2) grain mix with 10% sawdust, 5% Na bentonite, and 2% Na bicarbonate; (3) grain mix with 20% sawdust; or (4) grain mix with 30% sawdust. Feed intake was similar and cows fed the diet with 30% sawdust consumed approximately 5.32 kg of aspen/d (DM basis). No differences in milk production, percent milk protein, percent milk solids-not-fat, feed intake, or BW were observed. In addition, "a pelleted ration of 30% aspen sawdust is as effective as 50% long hay to maintain normal luminal acetate-to-propionate ratios."

In summary, aspen sawdust can be a partial roughage substitute in lactating dairy cow rations and it is helpful in maintaining near-normal milk fat content in high-concentrate rations. The sawdust cannot, however, serve as the only source of roughage for lactating cows because of the irregular feed intake that results if no other forage is fed.

Summary Notes:

- Values reported below are on a DM basis.
- Total aspen sawdust consumed/d = **5.32 kg**
- Assume that mature cows BW (not stated by authors) = 635 kg, then daily aspen consumed = **8.38 g**/kg BW [5,320 g/635 kg BW]
- No adverse effects on animal health reported.

Schingoethe et al., 1981. Aspen pellets as partial roughage replacement for lactating dairy cows. J. Dairy Sci. 64:698−702.

Paraphrased Abstract:

Ten mid-lactation dairy cows were in a complete switchback design with three periods of 5 wk each to determine if aspen could serve as part of their roughage. Aspen pellets made from whole aspen trees contained: 1.9% CP, 80.3% NDF, 64.5% ADF, and 16.9% lignin (DM basis). The aspen was chipped, dried, coarsely ground, and pelleted. ASPEN diet contained 30% aspen pellets, 30% corn silage, and 40% concentrate mix; CNTL diet contained 60% corn silage and 40% concentrate mix (DM basis). Cows consumed 19.4 kg/d (DM basis) of ASPEN diet and 19.3 kg/d (DM basis) of CNTL diet.

Summary Notes:

- Values reported below are on a DM basis.
- Cows consumed approximately **5.82 kg** aspen/d, which = **9.77 g**/kg BW
- Ruminal pH was greater in cows fed ASPEN vs. CNTL
- Ruminal contents from cows fed CNTL contained a greater concentration of total VFA, but similar concentrations of the various individual VFA and ammonia.
- Milk production, composition, and flavor were similar from cows fed both diets.
- No adverse effects on animal health were reported.
- Aspen can serve as part of the roughage fed to lactating cows past peak production.
- Note: Authors state that "the amount of fiber in the aspen ration may limit feed intake due to gut fill during peak of lactation. For this reason feeding more than 30% of the total ration DM as aspen would not be recommended, and even 30% … might be too high for cows in early lactation."
  - Dr. Whitney's Comment: Considering that total DMI and milk production were similar for cows fed ASPEN or CNTL, the proceeding statement does not seem warranted.

Mathison et al, 1986. Ruminant feed evaluation unit: evaluation of aspen as a feedstuff for cattle. Univ. Alberta Agric. Forage Bull. (Suppl. 9):53–55.

Paraphrased Abstract:

The digestibility and intake of diets containing ground whole aspen trees were measured for sheep and cattle. The diet containing 58% hay and **42% aspen** (DM basis) was 53% digestible in cattle and 49% sheep; digestibility of the aspen was calculated to be 37% and 31%, respectively.

Summary Notes:

- Values reported below are on a DM basis.
- 72-h *in situ* digestibility for ground aspen was 25%.
- Cattle BW = 400 kg [6 kg total intake/0.015 intake as % of diet]
- Cattle consumed a total of 6 kg of the mixture/d, which was "77% of the intake … when only hay was fed.
- Total aspen consumed/d = **2.52 kg** = **6.3 g**/kg BW [2,520 g/400 kg BW]
- Sheep ate "61% as much of the aspen-hay mixture" vs. hay alone.
- No adverse effects on animal health were reported.
- Conclusion: "… the feeding value of unprocessed aspen in ruminant diets is less than 75% of the feeding value of straw."

APPENDIX F

Research Trials: Use of woody plants other than *Juniperus* and *Populus* species in mixed diets

Marion et al., 1957. Ground mesquite wood as a roughage in rations for yearling steers. Tex. Ag. Exp. Sta. Progress Rep. 1972.
Marion et al., 1959. Ground mesquite wood as a roughage for yearling steers. J. Anim. Sci. 18:1174. Abstract.

Green mesquite stems (2.54 to 7.62 cm in diameter) were cut weekly, allowed to air-dry for 5 to 7 d, chipped, hammermilled (0.476-cm screen), and mixed with other ingredients. Chemical composition of the mesquite and CSH are reported in **Table 26**.

Note: authors do not state if data is reported on an as-fed or DM basis, but data are believed to be reported on an as-fed basis. Thus, the following assumptions were used to calculate data on a DM basis: DM of mesquite = 94.4%; CSH = 90.6%; molasses = 75%; grain = 92%; and CSM = 92%.

Preliminary Trial (1954 to 1955):
Calves (initial BW = 204 kg; n = 4) were fed 1 of 2 treatment diets (2 calves/treatment): (**1**) CNTL diet = CSM (0.835 kg [0.907 kg as-fed basis × 0.92 DM]), sorghum grain (0.834 kg), molasses (0.68 kg), and CSH (2.47 kg); or (**2**) **MESQ** diet = CSM (0.835 kg), sorghum grain (0.834 kg), molasses (0.68 kg), and ground mesquite (2.57 kg).

Mesquite intake summary for Preliminary Trial (data reported below are on a DM basis):
- Total DMI = 4.92 kg
- Mesquite intake = **2.57 kg** = **12.6 g**/kg BW [2,570 g/204 kg BW]
- Assuming that all of the diet was consumed, % mesquite in the diet (DM basis) = **52.2%** [2.57/4.92) × 100]
- ADG for calves fed the CNTL or MESQ diet were 0.70 and 0.61 kg/d, respectively.
- Calves fed CNTL experienced night-blindness (related to Vitamin A deficiency), but calves fed MESQ had normal night vision.
- No negative health effects were observed.

Trial 1 (1955 to 1956):
Steers (initial BW = 293 kg; n = 8) were fed 1 of 2 treatment diets (4 calves/treatment) for 140 d, consisting of either CSH or mesquite as the main roughage source. During the 1st 54 d, percentage of ground mesquite in the diet was **34.7%** (DM basis); from d 55 to 140, percentage of ground mesquite in the diet was approximately **46%** (DM basis). ADG of steers fed a diet containing mesquite was 1 kg/d vs. 1.04 kg/d for steers fed a diet without mesquite.

Mesquite intake summary for Trial 1 (data reported below are on a DM basis):
First 54 d:

- Average total DMI = 8.89 kg
- Maximum mesquite intake = **3.084 kg** = **10.53 g**/kg BW [3,084 g/293 kg BW]
- % mesquite in the diet = **34.7%** [3.084/8.888) × 100]

Days 55 to 140:
- Average total DMI = 9.31 kg
- Maximum mesquite intake = **4.28 kg** = **14.61 g**/kg BW [4,280 g/293 kg BW]
- % mesquite in the diet = **46%** [4.28/9.31) × 100]
- ADG for calves fed the CNTL or MESQ diet were 1.04 kg/d and 1.0 kg/d, respectively.
- No negative health effects were observed.

Trial 2 (1956 to 1957):
Steers (initial BW = 396 kg; n = 8) were fed 1 of 2 treatment diets (4 calves/treatment) for 112 d, containing either no ground mesquite (CNTL) or ground mesquite at approximately 34.9% (DM basis) of the diet. The starting ration for the mesquite-fed steers contained 2.61 kg (DM basis) of ground mesquite/head per day. *Paraphrased from Marion et al (1957)* "This amount was increased each wk, and the amount of hulls and bundles were decreased until the entire roughage consisted of ground mesquite. At the end of 70 d, steers were eating **5.23 kg**/d (DM basis) of ground mesquite and 6.53 kg/d of concentrates (DM basis assuming that the mesquite and concentrates contained 72% and 90% DM, respectively). ADG for steers fed diets with no mesquite vs. mesquite was10.23 kg/d vs. 1.15 kg/d, respectively." Authors also report (Marion et al., 1959) that cows were maintained 140 d on a mesquite wood ration, calved normally, and were in better condition vs. cows fed CSH.

In addition, Marion et al. (1957) also reported that a rancher fed cows a diet containing ground mesquite (454 kg), molasses (227 kg), grain (90.7 kg), and CSM (90.7 kg) with no reported negative effect on animal health.

Mesquite intake summary for Trial 2 (data reported below are on a DM basis)**:**
- Total DMI = 12.8 kg
- Average mesquite intake = **5.24 kg** = **13.2 g**/kg BW [5,240 g/396 kg BW]
- Average % mesquite in the diet =
  40.9% [5.24/12.8) × 100]
- Maximum mesquite intake reported by the authors (assume the concentrates contained 92% DM) = **6.85 kg** = **17.3 g**/kg BW [6,850 g/396 kg BW]
- Maximum % mesquite in the diet =
  50.6 % [6.85/(6.85 juniper + 6.68 kg concentrate) × 100]
- No negative health effects were observed.

In conclusion, the authors stated "No ill effects resulted from feeding the ground wood."

Table 26. Chemical composition (DM basis) of ground mesquite wood and cottonseed hulls

Feed	CP, %	Fat, %	NFE[1], %	Fiber, %	Ash, %	P, %	Carotene, ppm
Ground mesquite wood	6.29	0.83	37.5	51.5	3.8	0.06	26
Cottonseed hulls	4.53	0.99	52.5	52.5	3	0.03	0

[1]NFE = nitrogen-free extract = 100 – (CP, fat, water, ash, and fiber).

Dinius and Baumgardt, 1968. Ration dilution and feed intake in the sheep. J. Anim. Sci. 27:1767. Abstract.

Paraphrased Abstract

Objectives of this study were to determine voluntary feed and energy intake of rations varying in digestibility and density, and to determine parameters for rations on which intake is limited by digestive tract capacity. A basic concentrate mixture was diluted from 5 to 50%, at 5% increments, with each of 4 diluents: (A) sawdust, (B) sawdust with constant 3% clay, (C) verxite expanded hydrobiotite, (D) same as "A" except percent nitrogen was kept constant. Thus, 40 rations were evaluated. Each pelleted ration sequence (A to D) was fed to four sheep maintained in metabolism stalls. DM intake per kg $BW^{0.75}$ generally increased for all ration sequences through the 35% dilution and thereafter decreased. The DE intake (kcal/kg $BW^{0.75}$) remained nearly constant through the 35% dilution (average 203 kcal) and was 170, 151 and 126 kcal for the 40, 45 and 50% dilutions, respectively. The DE intake at 45% dilution was lower ($P = 0.05$) than that at 35% and lesser dilutions; at 50% it was lower than that at 40% and lesser dilutions. Reduction in intake began at a DE of 2.3 kcal/g or a DE of 3.4 kcal/ml using density measured by water displacement.

Summary:

- DMI decreased with increasing percentage of sawdust, but this was attributed to energy dilution of the diet and not a reduction in animal health.

Ellis, 1969. Wintering cows on ground mesquite. M.S. Thesis. TX Tech. Univ., Lubbock.

Mesquite (6-yr regrowth) was chopped, dried, and ground (0.95-cm screen). The ground mesquite contained the following (assuming that the authors reported on an as-fed basis; assumption is based upon CP concentration of the ground mesquite): CP (8.2 to 11.4%), ash (5.6 %), Ca (0.12 to 0.13 %), and P (0.13 to 0.16%). This study was a preliminary trial and did not have a control group. Crossbred cows (n = 5) were fed a diet that initially consisted of 4.08 kg of concentrate (sorghum grain and a vitamin-mineral premix), 0.45 kg of molasses, and 0.45 kg of mesquite (as-fed basis). Mesquite was increased 0.45 kg/d until cows were consuming 0.68 kg of concentrate (1/3 sorghum grain, 1/3 CSM, 1/3 premix), 0.45 kg of molasses + 10 parts water, and *ad libitum* mesquite. Mesquite consumption/cow was between 6.35 and 7.26 kg/d.

Results suggested that the cows “were not on a high enough nutritional plane to support milk production.” Authors report negative health in some of the cows, but state that they do not know if these effects were due to the diet or state that “the death did not appear to be related to the ration” or “such weight loss is recognized as normal for cows being wintered on the range.” Authors also report that “data indicate that the ration containing mesquite was reasonably adequate for maintenance. The sharp weight loss post parturition suggests that the wood was inadequate as a major component of the ration for suckling cows.”

Summary Notes:

- Authors ground the entire mesquite tree, which included leaves; thus, results of this trial need to take into account that mesquite leaves (> 5% of the diet) have been shown to reduce intake and BW gain in sheep (Baptista and Launchbaugh, 2001).

- Authors do not state if % of ingredient in the diet or consumption is on an as-fed or DM basis; however, it appears that results are on an as-fed basis. Thus, assuming that DM of the concentrate mixture, dried mesquite, and molasses was 90%, 94%, and 75%, respectively, cows initially were fed diets containing approximately **10%** mesquite. Quantity of mesquite was gradually increased until cows were consuming approximately **88%** mesquite.
- No adverse effects on animal health were reported. However, because of the small numbers of cows "in these two trials, no definite conclusion can be drawn as to the feasibility of maintaining a large herd on mesquite."

El-Sabban et al., 1971. Utilization of oak sawdust as a roughage substitute in beef cattle finishing rations. J. Anim. Sci. 32:749−755.

Note: authors do not state if data is reported on an as-fed or DM basis; however, % of DM in all of the ingredients was probably fairly similar. Thus, data presented in the publication (and in the summary below) should closely represent what would be calculated on a DM-basis.

Trial 1:

Herford steers (n = 30; initial BW = 352 kg) were group-fed *ad libitum* during a 103-d finishing trial. There were 5 high concentrate treatment diets with 6 steers/treatment, but only 1 pen/treatment. The control diet contained 5% ground timothy hay (CNTL), but no sawdust (SD) and the maximum concentration of course SD in a treatment diet was 15% (SD15). Steers fed CNTL or SD15 had similar growth performance (DMI, ADG, final BW, G:F) and carcass characteristics.

Trial 2:

Herford steers (n = 48; initial BW = 349 kg) were fed *ad libitum* during a 103-d finishing trial. There were 8 treatment diets and only 1 pen/treatment. The control diet contained 15% ground timothy hay (CNTL), but no sawdust (SD) and the maximum concentration of course SD in a treatment diet was 15% (SD15). Steers fed CNTL or SD15 had similar growth performance (DMI, ADG, final BW, G:F) and carcass characteristics.

Summary Notes:
- "In this study, feedlot performance data of steers suggest that raw oak sawdust, particularly larger particles, can be successfully used as a roughage substitute in high energy rations at levels up to 15% of the ration."
- "Performance attained by the incorporation of coarser sawdust particles in rations, particularly at the 15% level, may be attributed to a greater associative effect…"
- "…the incidence of liver abscesses tended to be reduced as the level and particle size of sawdust increase."
- "Rumens of steers fed the rations containing sawdust were parakeratotic" (however, steers fed CNTL were also parakeratotic). "However, improvement in rumen condition was observed when sawdust was included in rations at 15% level, and when coarser sawdust particles were used…" "No apparent problems resulted from feeding sawdust at levels up to 15% to beef cattle."

Cody et al., 1972. Effect of dietary screened sawdust on health, feed intake, and performance of the bovine. J. Anim. Sci. 35:460−465.

Note: Authors do not state if data is reported on an as-fed or DM basis; however, % of DM in all of the ingredients was probably similar. Thus, data presented in the publication (and in the summary below) should closely represent what would be calculated on a DM basis.

Abbreviated Abstract:

This study evaluated the effect of dietary sawdust on animal health (especially on the gastrointestinal tract; **GI**) and examined the merit of sawdust (**SD**) as a roughage substitute and/or intake regulator. Kiln-dried, screened sawdust from shortleaf southern pine was combined at various levels with concentrates and fed to calves 2 wk old and older. Feeds containing screened SD at levels of 10, 15, 25, 35 and **45%** were fed for periods of up to 20 mo. Certain experimental groups were observed for performance; other groups were slaughtered after specific feeding periods. Gross and microscopic pathologic examinations of GI sections and major visceral organs were conducted. Results indicated that rations containing screened SD did not physically injure the GI lining nor was "any toxic effect apparent." Twenty-five percent SD appeared to be the most desirable level; higher levels occasionally induced impaction of digesta.

Summary Notes:

Trial 1:

- There was only 1 animal/treatment, thus data could not be analyzed statistically. Calves were fed individually from 6 wk to 10 mo of age. As a percentage of total consumption, average SD consumption was approximately **26%** (DM basis). "Forty-five % SD depressed intake… Therefore, the percentage of SD fed to animal D was reduced to 35%." No gross lesions were attributed to the SD, but a calf fed a control diet (no sawdust) had a liver abscess.
- The calf fed 35% SD "displayed moderate abdominal distention, although clinical signs of impaction were not apparent. At slaughter, this animal's rumen contents were "firmer and appeared less moist than those from calves not fed" SD and this animal had an enlarged omasum, "containing an accumulation of SD."

Trial 2:

- "Consumption of concentrate mixtures containing 15% SD was comparable with that of rations containing no SD. No adverse effects of SD on health were noted.

Additional Investigations:

Used 10 calves 6 wk to 8 mo old and 1 mature rumen-fistulated cow. "With rations containing 25% SD, rumen and reticular mucosa appeared to remain normal; that percentage of SD did not induce rumino-reticular or omasal compaction, nor did it obstruct muscle sphincters. At 35% SD, tureen distention was noted; however, anorexia was observed in only one animal receiving this SD level. Postmortem examination revealed ruminal and omasal impaction in each of four calves receiving 35% SD as the only roughage. Anorexia was attributed to impaction of the pyloric sphincter. When 2.3 kg of baled bromegrass hay was fed daily to each of six heifers receiving a pelleted concentrate containing 35% SD, rumen distention was not apparent nor was health or appetite noticeably impaired. This ration was fed up to and during gestation. No apparent adverse effect on parturition was noted."

Summary Notes:

- No adverse effects on animal health were reported. "Histological examination revealed no tissue destruction or penetration of the GI mucosa, at any SD level."

Gilbert et al., 1973. Sawdust vs. hay in complete lamb ration. J. Anim. Sci. 37:367. Abstract.

Ram and ewe lambs (n = 19) purebred Dorset ram and ewe lambs (n = 19) were used to compare 15% ground hay and **15**% sawdust rations. Trial 1: Lambs were randomly assigned to the treatments and placed in acclimation pens until reaching 27.2 kg. They were then placed in their previously determined treatment groups, divided according to sex, weighed weekly and carried to 40.8 kg at which time they were weighed off the trial and G:F, ADG, and feed consumption were calculated. Results indicated a significant difference ($P < 0.05$) in ADG and G:F in favor of the 15% hay ration. Trial 2: 16 crossbred and four Hampshire ram lambs were used to evaluate three levels of sawdust and one level of hay. There was a significant ($P < 0.05$) linear relationship between the 3 levels of sawdust 15%, 20%, and 25%, when compared to hay for G:F indicating that as the level of sawdust increases, G:F also increases and comes closer to G:F of the hay ration.

Slyter and Kamstra, 1974. Utilization of pine sawdust as a roughage substitute in beef finishing rations. J. Anim. Sci. 28:692−696.

Paraphrased:

Yearling Hereford heifers (n = 36) were randomly allotted to four treatment groups. The rations were (1) all concentrate, (2) concentrate + 15% alfalfa, (3) concentrate + 15% pine sawdust, and (4) concentrate + 5% alfalfa and 10% sawdust. Note: Authors do not state if these values are on an as-fed or DM basis. Animals were fed *ad libitum* in South Dakota during Oct. to Feb. for 126 d. Coarse ponderosa pine sawdust was obtained from a local sawmill. Average DMI of heifers fed the diet with 15% sawdust diet was 6.59 kg vs. 6.96 kg for heifers fed the diet with 15% alfalfa. Heifers fed the diet with 15% sawdust had less final shrunk BW and ADG vs. heifers fed a diet with 15% alfalfa; however, carcass characteristics were similar. In addition, 11% of the heifers fed the diet with 15% alfalfa had abscessed livers vs. 22% of the heifers fed the diet with 15% sawdust.

Summary Notes:

- Values reported below are on a DM basis.
- Heifers fed 15% sawdust had BW = 270.2 kg
- Sawdust intake **= 988.5 g**/d [6,590 g × 0.15] = **3.66 g**/kg BW [988.5 g/270.2 kg BW]
- No adverse effects on animal health were reported.

Dinius and Williams, 1975. Sawdust as a diluent for adapting cattle to concentrate diets. J. Anim. Sci. 41:1170−1179.

Paraphrased Abstract:

Four experiments were conducted with sawdust as a dietary diluent as cattle were adapted to an all-concentrate diet. Trial 1: individually fed ruminally fistulated cattle (n = 24; BW = 370 kg) were abruptly switched from a forage diet to concentrate diets containing 20, 35 or 50% oak sawdust for 5 or 10 d, and then the concentrate diet with no sawdust was fed for another 30 d. The oak sawdust was from a circular saw and was air-dried before being used. "Oak sawdust is essentially non-digestible (Dinius and Baumgardt, 1970)." There were no differences in grain intake related to level of sawdust or to interval of sawdust feeding. Ruminal fluid pH dropped markedly during the first 2 d for all treatments. In general, cattle fed the 20% sawdust diet had lower ruminal fluid pH than those fed the 50% sawdust diet during the sawdust feeding interval.

Trials 2, 3 and 4: total of 160 steers were group fed and abruptly switched from forage to the concentrate diet diluted with varying percentages of sawdust, fed for 5 or 10 d, and then fed only concentrate for another 10 to 30 d. Steers tended to go "off feed" when abruptly switched from forage to 20 or 35% sawdust diets, or from the 50% sawdust diet to the all-concentrate diet. There was less fluctuation in daily grain intake when dietary sawdust was reduced from 50% to 0% in three steps than when the sawdust was abruptly withdrawn. In general, control steers that were switched by decreasing the dietary percentage of forage while increasing the percentage of concentrate during a 10-d interval had fewer off-feed problems and tended to have higher weight gains for the total feeding period than sawdust-fed cattle.

Summary Notes:

- Values reported below are on a DM basis.
- Trial 1: Average total DMI (assuming that values reported were on an as-fed basis and assuming that the diet was 90% DM) = 9 kg/d
- Trial 1: Maximum amount of oak sawdust consumed/d =
  4.5 kg sawdust/d = **11.9 g** sawdust/kg BW (assuming BW = 378 kg)
- Trial 2: Average total DMI (assuming that values reported were on an as-fed basis and assuming that the diet was 90% DM) = 2.45 kg/d
- Trial 2: Average amount of sawdust consumed/d =
  1.23 kg sawdust/d = **4.9 g** sawdust/kg BW (assuming BW = 252 kg)
- The only negative health problems resulted when steers (from all treatment groups) were abruptly transitioned from a high-roughage to high-concentrate diet. Authors state, "…the more conventional system of gradually reducing dietary forage while increasing concentrate resulted in fewer off-feed problems" in all the diets fed.

APPENDIX G

Research Trials: Known and Potential Safety Issues

Riddle et al., 1996. Volatile oil contents of ashe and redberry juniper and its relationship to preference by Angora and Spanish goats. J. Range Manage. 49:35−41.

Paraphrased Abstract:

Angora and Spanish goats were exposed (10 min, 3 times/d for 10 d) to blueberry and redberry branches in cafeteria style feeding trials. Preferences were consistent across seasons except winter. Spanish goats generally consumed more juniper than Angoras. Both breeds preferred blueberry over redberry juniper. Concentrations of volatile oils varied significantly between species of juniper and among seasons, but not between seasons. Significant correlation of oil concentration with juniper consumption indicated that specific oils were influencing preference for juniper. Correlations were similar for Angora and Spanish goats, indicating no differences between goat breeds in sensitivity to oils.

Summary:

- Values reported below are reported on a DM basis; however, the authors do not state DM contents of the feed or juniper leaves, intake of basal diet (needed to calculate % juniper consumption), if juniper leaf intake values are reported on an as-fed or DM basis, or goat BW. Thus, DMI reported below assumes that the fresh juniper leaves contained 50% DM and goat BW was 50 kg. For clarity, the author's as-fed values are converted to a DM basis in **Table 27**. In addition, volatile oil values reported in the publication (in the authors' Table 4) should have been "mg/g" and not "µg/g."
- Maximum daily blueberry juniper leaf intake = **35.4** g/d = **0.71** g/kg BW
- Maximum daily blueberry juniper volatile oil intake = **0.53** g/d = **0.01** g/kg BW
- Maximum daily redberry juniper leaf intake = **12.8** g/d = **0.26** g/kg BW
- Maximum daily redberry juniper volatile oil intake = **0.25** g/d = **0.005** g/kg BW
- No negative effects on animal health were reported.

Table 27. Converting volatile oil analysis reported by Riddle et al. (1996) from an as-fed basis to a DM basis

		DM of Juniper Leaves (assumed)		
	blueberry	**0.5**		
	as-fed	DM-basis, mg/g	% of Leaf DM	% of Total Oil
a-pinene	0.37	0.74	0.074	4.92
camphene	0.69	1.38	0.138	9.18
sabinene + B pinene	0.18	0.36	0.036	2.39
myrcene	0.34	0.68	0.068	4.52
cymene	0.22	0.44	0.044	2.93
limonene	1.28	2.56	0.256	17.02
camphor	3.1	6.20	0.620	41.22
terpineol	0.04	0.08	0.008	0.53
carvone	0.09	0.18	0.018	1.20
bornyl acetate	1.21	2.42	0.242	16.09
blueberry	7.52	**15.04**	**1.50**	100.00

		DM of Juniper Leaves (assumed)		
	redberry	**0.5**		
	as-fed	DM-basis, mg/g	% of Leaf DM	% of Total Oil
a-pinene	0.52	1.04	0.104	5.30
camphene	0.24	0.48	0.048	2.44
sabinene + B pinene	4.02	8.04	0.804	40.94
myrcene	0.89	1.78	0.178	9.06
cymene	0.21	0.42	0.042	2.14
limonene	1.2	2.40	0.240	12.22
camphor	1.61	3.22	0.322	16.40
terpineol	0.56	1.12	0.112	5.70
carvone	0.01	0.02	0.002	0.10
bornyl acetate	0.56	1.12	0.112	5.70
redberry	9.82	**19.64**	**1.96**	100.00

Pritz et al., 1997. Effects of breed and dietary experience on juniper consumption by goats. J. Range Manage. 50:600–606.

Paraphrased Abstract:

This study examined: (1) if redberry juniper consumption could be increased by exposing goats to essential oils early in life; (2) if goat breeds differed in juniper consumption; (3) if differences in juniper consumption were related to detoxification abilities of goats; and (4) if differences in digestibility and nitrogen or energy balance could explain juniper consumption patterns. "Conditioning Period": Spanish and Angora goats (6 to 7 wk old) were bolused every other day for 1 mo with essential oils distilled from fresh redberry juniper leaves, while control animals received empty capsules. Trial 2 ("Acceptance Testing": goats offered fresh redberry juniper branches and Spanish goats consumed more ($P < 0.01$) juniper than Angoras. Goats previously dosed with essential oils ingested less ($P < 0.09$) juniper than goats not dosed with essential oils. Liver-specific enzymes in blood serum were compared before and after the acceptance trial to examine potential liver damage and Spanish goats apparently experienced less tissue damage in response to juniper consumption than Angora goats. Trial 3 (digestion and metabolism of juniper): Spanish goats consumed more ($P < 0.01$) juniper (as a percentage of BW) than Angora goats though Angoras digested juniper more completely; probably a result of their lower consumption. The metabolic fate of dietary nitrogen and energy was similar for both breeds and unaffected by exposure to essential oils early in life.

Summary:

- This study is discussed in detail because it has been cited incorrectly (numerous publications) in regards to juniper leaf consumption causing hepatic insult.
- This article has been referenced numerous times, as having reported that maximum intake of fresh juniper leaves by goats is approximately 33% of the diet. However, it is unclear how 33% was derived from the data presented in this publication. Because feed ingredients have different moisture contents (e.g., 92% in hay vs. 40% in silage), it is critical that intake of an individual feed ingredient, as a % of total intake, is cited on a DM basis or at least present data needed for the reader to convert to a DM basis; numerous authors have incorrectly cited percentages on an as-fed basis.

Calculations below **assume** the following (*not presented by the authors*):

- Kid goat BW = 13.6 kg
- Juniper leaves and basal diet contained 50% and 90% DM, respectively.
- Basal diet DMI (maintenance; 2.5% BW) was 340 g.
- Juniper leaves contained between 2% to 3% volatile oil (DM basis).

All data reported on a DM basis

- Maximum daily DMI of juniper leaves =
  48.3 g of juniper = **3.55** g/kg of BW (authors' Fig. 4)
- Total daily DMI = [340 g basal + 48.3 g juniper] = 388 g
- Percentage of juniper in the diet = [48.3/388] = **12.4%**
- Maximum juniper volatile oil dose (note: 1 ml of oil = 0.825 g) =
  2.468 g oil dose = **3** ml oil dose [0.22 ml/kg BW dose/(0.825 g/ml oil × 13.6 kg BW)]
  0.181 g oil/kg BW [2.468 g oil/13.6 kg BW]

- Assume 2% oil: Maximum volatile oil intake from maximum leaf consumption (authors' Fig. 5) = [3.55 g/kg BW × 13.6 kg BW × **0.02** oil] = **0.966 g** oil intake = **1.17** mL oil
  = **0.07 g** oil/kg BW
- Assume 3% oil: Maximum volatile oil intake from maximum leaf consumption (authors' Fig. 5) = [3.55 g/kg BW × 13.6 kg BW × **0.03** oil] = **1.448 g** oil intake = **1.76** mL oil
  = **0.106 g** oil/kg BW
- Authors concluded that juniper consumption resulted in hepatic insult; however, the data and their corresponding discussion do not support this conclusion. Authors stated, "Spanish goats apparently experienced less tissue damage in response to juniper consumption than Angora goats" (in authors' Abstract).
  - This was stated even though the maximum difference in juniper leaf consumption between Angora and Spanish goats was only 25.2 g (approximately 1.85 g juniper/kg BW, DM basis; authors' Fig. 1). In addition, even though "there was no difference in serum enzyme (AST and GGT) concentrations due to exposure to essential oils during conditioning, they also stated, "Essential oils may have caused damage to detoxification organs (liver and kidneys)… making the group with prior exposure to essential oils less able to detoxify juniper ingested during the acceptance trial." They also stated that "lack of treatment differences (during conditioning)… does not indicate that treatments had no detrimental effects. It however confirms that exposure to essential oils did not affect specific metabolic activities involving AST and GGT." To explain the lack of effect of juniper consumption on AST and GGT, they then stated that it could have been due to "differences in terpene composition or amount of essential oils in the fresh juniper."
- The following facts should have been considered: (1) within a any given day, the juniper oil dose delivered during the conditioning period was approximately 70% greater than the maximum amount of volatile oil the goats consumed (through consuming juniper) during the acceptance trial (DM basis); (2) evaluating only 2 liver enzymes, neither of which are liver specific, would not have given conclusive evidence for hepatic insult, even if differences would have been observed; (3) all goats were fed juniper during the Acceptance Period, thus it is unknown if juniper intake was responsible for any change in serum enzymes; and (4) during the Acceptance Period, juniper leaves were the sole feed and DMI/goat was = 8.2 g and 45.6 g/d; thus, starvation during this period was a confounding factor, especially in regards to the 2 blood enzymes they evaluated (see below for more detail). Furthermore, reporting how many goats that did not consume any juniper (thus, any feed at all) during the Acceptance Period would have been relevant.
- The conclusion that hepatic insult resulted from consumption of juniper leaves was based on 1 blood enzyme (AST; aspartate aminotransferase) being greater at the end of the "Acceptance Trial," when the goats were starving. The other blood enzyme (GGT; gamma glutamyltransferase), actually declined in the Angora goats and was less in Angora vs. Spanish goats at the end of the Acceptance Trial; both AST and GGT were predicted by the authors to increase due to juniper consumption causing "hepatic necrosis." Authors stated that "…an increase in GGT would be a good indicator of liver damage… However, a decrease in GGT of this small magnitude has little diagnostic value;" the same should have been stated about the increase in AST (Spanish goats).
- Furthermore, AST remained within the normal physiological range for caprines (Carlson, 1996), starvation caused hepatic insult and greater AST (Martin et al., 1973; Cal et al., 2009, for as little as 4 d), and neither AST or GGT are liver-specific (Carlson, 1996; Yu et al., 2009; Otter, 2013). Other factors that can increase AST are heat stress (Cerutti et al., 2003; Nakyinsige et al., 2013), physical stress (Yu et al., 2007), copper toxicity (Todd and

Thompson, 1963; Ross, 1966; Buckley and Tait, 1981; Keen and Graham, 1989), and hemolysis due to factors such as amount of time before serum is separated from the blood (Carlson, 1996).

- It should also be noted, that even though livestock and deer consume juniper leaves while grazing, no reports by ranchers or veterinarians are known to exist that relate juniper leaf consumption to hepatic insult; especially at consumption levels reported in this trial. To Dr. Whitney's knowledge, when a veterinarian or other specialist evaluates a reported problem related to grazing livestock becoming ill, juniper consumption is not even considered during the diagnosis.
- In the second experiment, "Juniper Digestibility and Metabolism," authors stated "The higher juniper intake again indicates that Spanish goats have a greater ability to tolerate or avoid (through detoxification) negative consequences of jumper consumption." They also stated, "Apparently, high juniper consumption during the acceptance experiment limited the ability of goats to cope with essential oils during the digestion experiment."
    - As discussed below, these conclusions are not warranted.
- In regards to the discussion related to N retention, authors stated that juniper consumption resulted in negative N balance and that the "metabolism of juniper required more nitrogen than goats were provided by the juniper." However, basal diet DMI was not reported and the fact that redberry juniper contains CT (Whitney and Muir, 2010; Whitney et al., 2014) was not considered, even though CT can affect N retention.
- In the "Introduction", authors incorrectly stated that palatability of juniper is usually attributed to… essential oils through their "negative effect on liver metabolism (Huston et al., 1994).
    - Huston et al. (1994) was a review paper and did not state that low juniper palatability is attributed to liver metabolism. In contrast, Huston et al. (1994) stated that "this process [multifunctional oxidase enzyme systems; MFO] allows monoterpenes to be harmlessly excreted by the animal through its urine."

Riddle et al., 1999. Intake of Ashe juniper and live oak by Angora goats. J. Range Manage. 52:161–165.

Paraphrased Abstract:

Angora mutton goats (BW = 40 kg) were fed diets of either live oak, alfalfa hay, Coastal bermudagrass hay or female blueberry juniper plus Coastal bermudagrass hay (**CBH**) during the spring and fall of 1991 in a digestion and metabolism study. Nitrogen concentration of CBH was nearly equal to that of alfalfa hay; N concentration of the juniper and live oak were much lower than those of the hays and higher in fall than spring. Average DMI and dietary N intake were highest for alfalfa hay, intermediate for CBH, and lower for blueberry juniper and live oak. Goats retained more N when consuming alfalfa and CBH than juniper or live oak during fall, but differences were smaller ($P > 0.10$) during spring. Nitrogen balance was… positive for juniper for spring and fall. During fall, DMI of juniper … were significantly lower than alfalfa and CBH. We conclude that both blueberry juniper… foliage can provide nutrients for goats but only as portions of diets.

Summary (all reported on a DM basis):

- Authors stated (Results/Discussion section; authors' Table 1) average juniper intake/goat = **555 g**/d, which was part of a total intake of 975 g/d per goat. Thus, juniper intake was approximately **56.9%** of total DMI.
- Maximum daily juniper consumption (authors' Table 1) = **564 g** juniper DM/d = [564 g juniper/40 kg BW] = **14.1** juniper intake/kg BW
- Assuming: 1 mL of oil = 0.825 g; 3% volatile oil in the juniper leaves
  - Average daily volatile oil consumption = [555 × 0.03] = **16.65 g/d** per goat = **0.42** g/kg BW
  - Maximum volatile oil consumption = [564 × 0.03] = **16.92g** per goat = **0.42** g/kg BW
- Summary/Management Implications section, authors stated: "… Pritz et al. (1997) reported that N balance of goats may be negatively affected when substantial amounts of juniper are consumed."
  - As previously discussed in this proposal, "substantial amounts of juniper were not consumed in the cited study and the negative N balance reported Pritz et al. (1997) could have been due to CT and/or basal diet consumption, neither of which were reported.
- Authors also stated: "However, ashe juniper is of sufficiently high quality (i.e., 50% DMD) to significantly contribute to the diets of grazing animals that have access to other forages and/or supplemental feeds."

Straka, 2000. The physiological effects of monoterpenes on Spanish and Angora goats. Ph.D. Dissertation. Texas A&M Univ., College Station.

Paraphrased Abstract:

Goats used for brush control tend to self-limit juniper at 30% of the diet or less. Previous studies of free consumption by Angora and Spanish goats of *Juniperus* spp. have reported maximum intake values as 33.5% (6.7 g/kg BW) of diet composition (Pritz et al., 1997). Part I: addressed whether adding essential oil distilled from redberry juniper inhibited *in vitro* fermentation. There was no effect ($P > 0.05$) on *in vitro* fermentation of an alfalfa substrate. Secondly, this study addressed whether dosing goats with juniper caused a pre-conditioning effect. VFA levels increased following dosing goats with redberry juniper at 30% of their diet indicating a slight preconditioning effect. When goats were dosed with 40%, 50%, and 60% redberry juniper, VFA levels declined. There was no consistent difference in DMD and acetate:propionate ratios indicate an alteration in microbial species.

Part II: Investigated what physiological or toxic effect the redberry oil would have on Spanish and Angora goats. Ruminal dosing with juniper oil for nine d with 0.18 g oil/kg BW resulted in cachexia and mild hepatic lipid vacuolization. At higher dose levels, some hepatocellular necrosis and lobular encapsulation were evident. BW declined in Angora ($P < 0.002$) and Spanish ($P < 0.001$) goats during the dosing period as a result of a decline in consumption of the basal feed ration. Serum AST levels rose ($P = 0.03$) in Spanish goats after 9 d of dosing at the higher dose level and serum glucose levels decreased in all goats ($P < 0.05$), providing evidence of a catabolic state. In summary, post-ingestive effects of juniper oil consumption by goats at levels above 30% of the diet were alteration of normal microbial function, and inducement of a cachectic and catabolic state.

Summary (data reported on a DM basis):

- This study is discussed in detail because it has been cited incorrectly (numerous publications) in regards to juniper leaf consumption causing hepatic insult.
- After close examination, the data presented in this dissertation do not support the conclusions made by the author, that dosing goats with juniper volatile oil and/or juniper consumption caused negative effects on animal health. Even though the author stated that juniper consumption (and/or volatile oil dose) resulted in hepatic (and other GI) insult: (1) there were no control animals to compare necropsy results and numerous factors can result in GI insult (2) all of the animals necropsied had hepatic insult and "hepatic injury overall … was not severe and was within a range where compensation could occur"; (3) some of the animal health problems were discussed by the author to be related to not supplying a mineral block during the trial. With no control animals, it is unknown if goats had previous GI injury and/or developed GI injury due to an uncontrolled factor during the study (excess copper intake, starvation, etc.).
- Other issues: 1) The author also cited numerous times, that results are confounded by various issues; 2) author stated, "the dosing of oils led to a decrease in feed intake of the pelleted alfalfa ration…" but then states that feed consumption was not measured; 3) author stated [p. 57], "the most likely etiology for the hepatic lesions is fatty acid accumulation…" as a result of "depression of intake and a fasted state." Also, "the decrease in feed consumption … probably initiated a catabolic state." As discussed above in this proposal, a catabolic (not meeting maintenance requirements) state directly affects GI insult.
- Additional items:
    - (p. 3) Author cited Malachek and Leinweber (1972) as stating that in late fall and winter periods… juniper averaged roughly 11% of the browse consumed and at times, represented up to 31% of browse." However, this percentage is believed to be (authors do not state) reported in the cited reference on an as-fed basis." Therefore, this percentage would be different when reported on a DM basis considering that grasses, forbs, and browse have different DM concentrations.
    - Table 1(author's table) is reported on a DM basis, not "on a % total weight basis." Also, the concentration of carvone was incorrectly calculated.
- p. 11: Author incorrectly stated, "Results from metabolic studies of blueberry juniper consumption (Riddle et al., 1996) report a significant difference in digestible energy and metabolizable energy values of juniper fed goats. This indicates a significant loss of energy, presumably the oils, in the urine."
    - Riddle et al., 1996 did not report metabolism data. If the author was referring to Pritz et al. (1997), then the author's conclusion is still not supported by the data presented, especially when the discussion (in this proposal) related to that publication is reviewed.
- p. 12: Author stated, "Necrotizing rumenitis, cessation of rumen function and an associated reproductive toxicologies are reported following juniper administration to sheep at 1 lb/d (Johnson et al. 1976)."
    - In Experiment 1 of Johnson et al. (1976), all 6 sheep were orally dosed with 1 lb. of juniper leaf material; there were no control animals and thus, no way to determine if the juniper dose resulted in any animal health issues. During Experiment 2 (Johnson et al. 1976), out of the 4 sheep dose with 3/4 lb. of juniper, 3 had "normal twins" and "1 had a normal single lamb." They stated, "Attempts to confirm these findings by feeding juniper to other sheep during gestation days 60 to 90 were unsuccessful." They also stated that: (1) these 4 sheep had normal clinical chemical tests; (2) "Because of the acute nature of the toxicity, the clinical chemical tests were ineffective in predicting the onset of symptoms, but the normal values obtained throughout the experiment in those animals which tolerated the full 30-d plant feeding trial indicated that there was probably no

chronic tissue damage. This further supports the proposal that systemic shock probably caused mortality. Additionally, serious systemic disturbances would have increased the likelihood of interrupting pregnancy, which did not occur."

- p. 17: Author incorrectly cited Pritz et al. (1997) as Pritz et al. (1996) that maximum juniper leaf intake = 33.5%.
- p. 17: Calculations are incorrect. If 0.5 g of alfalfa was added to each 30-mL jar, then the quantity of juniper that makes up 30% of the diet should have also been included; thus, calculation should have been:
  - Total diet (theoretical) for a 30-mL jar = [0.5 g alfalfa/0.7] = **0.7143 g**
  - Thus, 30% juniper in the diet = 0.214 g (not 0.15 g as calculated by the author)
  - Also, Owens et al. (1998) reports redberry juniper oil concentration as approximately 1.83% of leaf DM (**Table 2**) and not 3% as reported by the author.
  - The author calculated "respective oil weights as: 30% of diet = 4.5 mg oil/30 mL solution.
    - This equals 0.0045 g oil/30 mL of solution = 0.00545 mL oil/30 mL solution.
      Note: 1 mL of oil = 0.825 g of oil
  - Conclusion: The oil concentrations/jar were much less than what would be represented if an animal consumed juniper at 30% of its diet.
  - Assuming that: (1) mature goat BW = 55 kg and (2) size of rumen = 8,000 mL, then total daily DMI at 2% BW = 1,100 g
    - 30% juniper in diet = [0.3 × 1,100 g total DMI] = **330 g**
    - Total daily oil consumption (assume 3% oil as stated by author) = [0.03 × 330] = **9.9 g** oil/d
    - Total mg oil to include in 30 mL of rumen fluid/buffer solution = [9.9/8,000 mL rumen] × 30 × 1000] = **37.13** mg
    - 4.5 mg/30mL reported by the author would relate to only 1.2 g of oil in the 55-kg goat previously discussed: [(0.0045 g/30 ml) × 8,000 mL rumen]
    - 4.5 mg/30mL reported by the author would relate to only 40 g of juniper leaf consumption (DM basis) in the 55-kg goat previously discussed. [(0.15 g juniper material/30mL) × 8,000 mL rumen]. In this scenario, 40 g of juniper intake would only represent 3.64% of total DMI and livestock consume more juniper than this while grazing with no reported negative effects on animal health or reproduction.
- p. 33: "Oh et al. (1967) reported that juniper oil can negatively affect rumen microbial activity."
  - Author correctly stated that the Oh et al. (1967) *in vitro* study used volatile oil doses that are much greater than what an animal would consume while grazing. Oh et al. (1967) used an *in vitro* volatile oil dose that would represent an 55-kg animal (8 L rumen) consuming 2,640 g of juniper within a given day; [(0.3 mL/25 mL solution) × (8,000 mL rumen × 0.825 g)/0.03 oil content] = **2,640 g** of juniper/d, DM basis; which would unrealistically represent 4.8% of BW.
- Results do not support the conclusion that juniper leaves and/or a volatile oil dose from juniper leaves, resulted in negative effects on rumen function and/or animal health.

Table 28. Converting volatile oil analysis reported by Owens et al. (1998) from an as-fed basis to a DM basis

		DM of Juniper Leaves		
	blueberry	**0.53**		
	as-fed	DM-basis, mg/g	% of Leaf DM	% of Total Oil
a-pinene	0.3	0.57	0.057	2.63
borneal	0.071	0.13	0.013	0.62
bornyl acetate	0.776	1.46	0.146	6.80
b-pinene	0	0.00	0.000	0.00
camphene	0.188	0.35	0.035	1.65
camphor	3.116	5.88	0.588	27.31
carvone	0.152	0.29	0.029	1.33
citronellol	0.356	0.67	0.067	3.12
cymene	0.21	0.40	0.040	1.84
fenchyl alcohol	0.023	0.04	0.004	0.20
y-terpinene	0.459	0.87	0.087	4.02
limonene	1.293	2.44	0.244	11.33
linalool	0.079	0.15	0.015	0.69
myrcene	0.447	0.84	0.084	3.92
sabinene	2.884	5.44	0.544	25.27
terpineol	0.125	0.24	0.024	1.10
terpin-4-ol	0.787	1.48	0.148	6.90
tricyclene	0.145	0.27	0.027	1.27
blueberry	11.411	**21.53**	**2.15**	100.00

		DM of Juniper Leaves		
	redberry	**0.55**		
	as-fed	DM-basis, mg/g	% of Leaf DM	% of Total Oil
a-pinene	0.27	0.49	0.049	2.69
borneal	0.104	0.19	0.019	1.04
bornyl acetate	0.818	1.49	0.149	8.14
b-pinene	0.003	0.01	0.001	0.03
camphene	0.188	0.34	0.034	1.87
camphor	2.713	4.93	0.493	27.00
carvone	0.014	0.03	0.003	0.14
citronellol	0.255	0.46	0.046	2.54
cymene	0.189	0.34	0.034	1.88
fenchyl alcohol	0.235	0.43	0.043	2.34
y-terpinene	0.432	0.79	0.079	4.30
limonene	1.202	2.19	0.219	11.96
linalool	0.097	0.18	0.018	0.97
myrcene	0.496	0.90	0.090	4.94
sabinene	1.863	3.39	0.339	18.54
terpineol	0.112	0.20	0.020	1.11
terpin-4-ol	0.84	1.53	0.153	8.36
tricyclene	0.217	0.39	0.039	2.16
redberry	10.048	**18.27**	**1.83**	100.00

Bisson et al., 2001. Activated charcoal and experience affect intake of juniper by goats. J. Range Manage. 54:274–278.

Paraphrased Abstract:

Our objective was to determine if dosing goats with the adsorptive compound activated charcoal (AC) would increase juniper consumption. Twenty Boer-cross goats (BW = 15 to 29 kg) were placed in individual pens; at 0800 h, 10 were dosed daily for 10 d with 1 g/kg BW of AC and 10 were not dosed. Goats were offered redberry juniper (Trial 1), blueberry juniper (Trial 2), or a choice between redberry and blueberry juniper (Trial 3). For each trial, juniper was offered to all goats for 2 h after dosing with AC. In Trial 1, goats dosed with AC consumed more ($P < 0.05$) redberry juniper during the first 5 d of exposure. In Trial 2, AC did not affect blueberry juniper intake. In Trial 3, dosing with AC did not affect juniper intake. Juniper intake increased across days of exposure for Trials 1 and 2, apparently because goats adapted to terpenoids in juniper through repeated exposure.

Summary:

- After discussing with one of the co-authors, it was determined that data were reported on an as-fed basis. Therefore, data below have been converted to a DM basis with the assumption that fresh juniper leaves and alfalfa pellets contained 50% and 90% DM, respectively.
- Abstract: authors stated, “Juniper intake increased … apparently because goats adapted to the terpenoids in juniper through repeated exposure.”
    - The data do not lead to this conclusion as being “apparent.”

- Introduction: authors stated, "If alternative forage is limited, herbivores may continue to consume the toxic plant but limit intake below toxic levels (Launchbaugh et al., 1993).
  - The cited literature did not study toxic plants.
- Introduction: authors stated, "Terpenoids in redberry juniper cause more digestive distress than those in ashe juniper, and goats prefer ashe to redberry juniper (Straka, 1993)."
  - This cited reference did not evaluate "digestive distress."
- Trial 1: all goats were offered juniper leaves and maximum **redberry juniper** DMI = approximately **3 g/kg** BW (DM basis) = 45 g and 87 g for a 15 and 29-kg goat, respectively. If average alfalfa pellet DMI = 180 [200 g × 0.9 DM], then percentage of juniper in the diet = **20 to 32.6%**, DM basis [e.g. 45 g juniper/(45 g + 180 g) × 100].
- Trial 1: Assume redberry juniper volatile oil concentration = 1.83% (Owens et al., 1998): then maximum juniper volatile oil consumption within a given day =
  - 15-kg goat = **0.82 g** oil [45 g juniper × 0.0183] = **0.055 g** oil/kg BW.
  - 29-kg goat = **1.59 g** oil [87 g juniper × 0.0183] = **0.055 g** oil/kg BW.
- Trials 2 and 3: maximum **blueberry juniper** consumption (within a given day) of **11.5 g/kg** of BW (DM Basis) =
  - **173 g** and **333.5 g**/d for a 15 and 29-kg goat.
  - If average alfalfa pellet daily DMI = 175 g/d [194 g × 0.9 DM], then % juniper in the diet = **49.7% and 65.6%**, DM basis [e.g. 173 g juniper/(173 g + 175 g) × 100].
- Trials 2 and 3: assume blueberry juniper volatile oil concentration = 2.15 (Owens et al., 1998): then maximum daily juniper volatile oil consumption within a given day =
  - 15-kg goat = **3.72 g/d** oil [173 g juniper × 0.0215] = **0.248 g** oil/kg BW
  - 29-kg goat = **7.17 g/d** oil [333.5 g juniper × 0.0215] = **0.248 g** oil/kg BW
- Discussion section: authors stated, "Daily increases in juniper consumption were probably caused by physiological changes that increased the liver's ability to metabolize the terpenoids in juniper (Launchbaugh et al., 1997)."
  - Two problems exist with this conclusion: 1) AST remained within the normal physiological range for caprines (Carlson, 1996) as previously discussed in this proposal (see discussion on Pritz et al., 1997); and 2) Launchbaugh et al. (1997) was a review paper and thus, did not study metabolism of terpenes and liver function.
- Discussion section: authors stated, "We contend that goats increased intake of juniper until a toxic threshold was reached. The toxic threshold represents the point at which toxin intake surpasses the liver's ability to oxidize and excrete the terpenoids. When the threshold was reached, goats probably experienced aversive postingestive feedback and decreased intake accordingly."
  - Data presented do not support this conclusion. Maximum juniper DMI, as a percentage of total DMI was 65.6% and no negative health effects were reported.
- Discussion section: authors stated, "Exposure to the terpenoids in juniper early in life causes liver damage which reduces subsequent juniper consumption and animal productivity (Pritz et al., 1997)."
  - See discussion above related to the Pritz et al. (1997) publication; the data reported by Pritz et al. (1997) do not support this conclusion. In fact, Pritz et al. (1997) stated, "Goats that received essential oils early in life tended to ingest less ($P < 0.09$) juniper than control goats during the 5-d trial. Thus, early exposure to essential oils did not increase the goats abilities to ingest juniper later in life as had been anticipated."

- Discussion section: authors stated, "… aversive postingestive feedback from terpenoids limit intake below maintenance levels (Riddle et al., 1999).
    - The cited reference states, "…juniper (even in a mixture with Coastal bermudagrass hay) though probably adequate for maintenance, were inadequate for goats that are either pregnant or lactating. Ashe juniper, … can provide nutrients at important periods in a goat's annual production cycle but probably only as partial diets. The intake of a pure diet of juniper forage would be too low for maintenance." Other "traditional" feedstuffs are also not adequate to meet maintenance requirements when fed as the sole diet.
- No negative effects on animal health were reported.

Ellis et al., 2005. Sire influence on juniper consumption by goats. Range. Ecol. Manage. 58:324–328.

Paraphrased Abstract:

Goats avoid eating redberry juniper when other palatable forages are available but will increase intake of juniper when exposed to the plant for several days. Intake of redberry juniper also differs among breeds and individual goats. The purpose of this study was to determine the influence of sires on fresh juniper leaf consumption. Heritability of redberry juniper intake was assessed for 3 yr placing male Boer-Spanish cross goats (BW = 30 kg) from 4–8 sires in individual pens and feeding juniper (200 g) daily for 2 h over 5 to 10 d. Goats increased juniper intake while in individual pens. These results suggest that an acceptance of juniper by goats can be conditioned through exposure to the plant after weaning…

Summary:

- Abstract: authors incorrectly stated, "Goats avoid eating redberry juniper when other palatable forages are available…"
    - Goats are known to consume redberry juniper even when other palatable forages are available.
- Abstract: 200 g of juniper offered, is on an as-fed basis; thus represents ~ **100** g of DM (assuming that the juniper leaves = 50% DM).
- Abstract: authors incorrectly stated, "Goats may consume juniper, but intake is limited because of terpenoid levels that kill rumen bacteria when intake exceeds 30% of the diet (Straka et al., 2003 [should be 2004])."
    - This references should be "Straka et al. (2004); these authors did not evaluate effects of terpenoid concentration on rumen microbial concentration and the statement "intake exceeds 30% of the diet" is incorrect.
- On average, fresh redberry juniper leaf consumption was 1.9 and 3.3 g/kg of BW (DM Basis) during 1997 and 1998, respectively. After discussing with Dr. Scott (co-author), it is believed that this data is reported on an as-fed basis. Thus, on a DM basis (assuming juniper DM = 50%), juniper consumption = **0.95 and 1.65 g**/kg BW during 1997 and 1998, respectively.
- Juniper in the diet (all data are reported on a DM basis):
    - Average daily alfalfa intake = 405 g alfalfa/d [30 kg BW × 1.5% BW × 1000 × 0.9 DM]
    - Total daily DMI = 505 [405 + 100]
    - Average % juniper in diet = **6.57 to 10.9%**
        - e.g. [(1.9 g/kg BW × 0.5 DM × 30 kg BW)/(28.5 g juniper + 405 g alfalfa) × 100]
    - Maximum daily juniper consumption (Table 2) =
      3.35 g/kg BW = **100 g**/goat [3.35 × 30 kg BW].

- **Maximum** % juniper in diet = **19.8%** [(100/505) × 100]
- Assume redberry juniper volatile oil concentration = 1.83 (Owens et al., 1998):
  - maximum juniper volatile oil consumption within a given day = **1.83 g** oil/d [100 g juniper × 0.0183] = **0.061 g** oil/kg BW

- Discussion: authors stated, "As intake increases, goats experience aversive postingestive feedback from overconsumption of terpenoids and decrease intake accordingly (Pritz et al., 1997). Apparently intake levels on d 5 were sufficient to induce aversive postingestive feedback and a subsequent decrease in intake."
  - Pritz et al. (1997) does not support this conclusion and thus, it is not absolutely "apparent" that aversive *postingestive* feedback decreased DMI.
- Discussion: authors stated, "This study and others (Bisson et al., 2001; …) have illustrated that goats will increase intake of juniper when it is fed at weaning.
  - Bisson et al. (2001) study does not support this conclusion; they did not evaluate the effects of juniper consumption at weaning on increasing juniper later in life.
- Discussion: authors stated, "Exposure to juniper before weaning may occur before rumen development, and cause liver and kidney damage (Pritz et al., 1997)."
  - As discussed in this proposal, data presented by Pritz et al. (1997) does not support this conclusion.
- No negative effects on animal health were reported.

Dunson et al., 2007. Chapter 64: Rumen function and the ability of goats to consume redberry juniper. In: K. E. Panter, T. L. Wierenga, and J. A. Pfister, editors, poisonous plants: global research and solutions. Wallingford, Oxon, United Kingdom: CABI Publ. p. 377–385.

Paraphrased Abstract/Methods:

Objective: determine if changes in the rumen microbial population are responsible for the detoxification and reduction of redberry juniper terpenoids in rumen fluid. If the mechanism of detoxification can be identified, it may be possible to select for or manipulate this process in an effort to increase redberry juniper utilization. For example, if rumen microbial populations are responsible for degradation of the terpenes found in redberry juniper, improving juniper consumption may simply involve feeding juniper in pens to improve acceptance.

Goats were placed in individual pens for 10 to 14 d and feeding redberry juniper. Juniper was stripped from stems and stored at 0°C. Intake, serum metabolite levels, terpene disappearance rates, volatile fatty acid profiles, and ammonia levels were monitored and compared between treatments (fed juniper or fed alfalfa). Fresh water and mineral were provided *ad libitum* to all goats during testing in individual pens. All goats also received alfalfa pellets (2% BW) daily to meet maintenance requirements.

Trial 1:

For the first experiment, 20 Boer-Spanish cross goats (BW = 20 kg; 10/treatment) and 6 ruminally cannulated Boer×Spanish goats were placed in individual pens. After a 14-d adjustment pretrial, where goats were fed only alfalfa (2% BW), 13 randomly selected goats were fed juniper for 2 h/d for 14 d. Three goats were randomly selected from the cannulated group and 10 from the 20 goats without cannulas. Serum metabolite levels were monitored to assess physiological status. In previous studies, dosing goats with juniper also affected serum constituent levels (AST, BUN, GGT, creatinine, and bilirubin; Pritz et al., 1997; Bisson et al., 2001). Pritz et al. (1997) noted increases in AST above the physiologically normal range. Bisson et al. (2001) noted a statistically significant increase in AST that remained within biologically normal range (ISIS, 1995). Changes in serum levels are indicative of toxicosis (Cornelius, 1989; Cheeke, 1998). It appears that a minor hepatic insult occurs when goats are exposed to juniper monoterpenes but the insult does not approach pathological levels.

Rumen fluid was collected from the cannulated goats on day 10 to assess changes in rumen microbial populations and toxin degradation. Four terpenes (myrcene, limonene, terpineol, α-pinene) were used in this experiment because of commercial availability and because three of them (myrcene, terpineol, α-pinene) have been negatively correlated with juniper intake (Riddle et al., 1996). Rumen fluid was collected from each goat and mixed with rumen fluid from goats fed the same diet. Concentrations used in this study were based on terpene levels from juniper found growing on the same collection site (Owens et al., 1998).

Trial 2:

Immediately following Experiment 1, rumen fluid was collected from each cannulated goat, mixed with rumen fluid from goats fed the same diet. The 10 goats not fed juniper in Experiment 1 were inoculated with rumen fluid from either cannulated goats fed juniper or cannulated goats fed only alfalfa. All 10 naive goats were fed redberry juniper for 2 h/d for 10 d thereafter and intake was monitored determine if inoculation from goats fed juniper would increase rate of adaptation to the plants toxins faster than inoculation from naive goats.

Summary:

- Abstract: "Most species of livestock do not utilize redberry juniper because the terpenoids found in the plant cause aversive postingestive feedback (Riddle et al., 1996; Pritz et al., 1997)."
  - This conclusion is not supported by the literature.
- Abstract: "Even when forced to consume juniper in pen-feeding situations, intake rarely exceeds 30% of the diet (Straka et al., 2004).
  - Others have shown that maximum intake can exceed 56% of DM (Riddle et al., 1999).
- Serum creatinine, AST, GGT, and BUN concentrations are presented in **Table 29** (below).
- Methods: "In previous studies, dosing goats with juniper also affected serum constituent levels (serum aspartate transaminase, blood urea nitrogen, gamma glutamyltransferase, creatinine, and bilirubin; Pritz et al., 1997; Bisson et al., 2001). Pritz et al. (1997) noted increases in AST above the physiologically normal range. Bisson et al. (2001) noted a significant increase in AST that remained within biologically normal range (ISIS, 1995).
  - Dr. Whitney's Comment related to Pritz et al. (1997): As discussed above in this proposal, AST remained within the physiological normal range for goats and more importantly, starvation was probably the main factor affecting the 2 serum enzymes that were evaluated; both of which are not liver-specific. Numerous other issues such as AST remaining within the normal physiological range for caprines (Carlson, 1996) are discussed above, which do not support the conclusion by Dunson et al. (2007).
  - Dr. Whitney's Comment related to Bisson et al. (2001): Those authors state, "… changes in serum metabolite levels do not provide conclusive evidence that toxicosis has occurred. Serum metabolite levels vary among healthy individuals, and levels can be affected by disease or tissue damage unrelated to toxicosis (Cornelius, 1989, Kramer, 1989). Other facts to consider: (1) all goats were offered juniper, thus there is no way to determine if juniper consumption was the

primary factor that increased AST; (2) AST decreased in goats when they were dosed with charcoal, but only when they consumed redberry juniper, not blueberry juniper; (3) GGT was not affected by charcoal dosing; (4) AST remained within the normal physiological range for caprines (Carlson, 1996)

- Methods: "It appears that a minor hepatic insult occurs when goats are exposed to juniper monoterpenes but the insult does not approach pathological levels."
  - The literature does not support this conclusion related to juniper causing hepatic insult.
- Discussion: "Juniper intake increased daily until d 11. At this point, goats were probably experiencing aversive postingestive feedback from the monoterpenes in juniper."
  - Results do not support this conclusion.
- Discussion: "Analysis of serum metabolite levels suggests that goats may have experienced some effects from toxicosis from over-ingestion of juniper. Levels of certain blood serum metabolites can be an indicator of toxicosis when elevated beyond normal ranges (Cheeke, 1998)."
  - Discussion related to toxicosis is not warranted, especially considering that the authors correctly state that, "Although serum metabolite levels were elevated in goats consuming juniper, none were outside the normal range for healthy goats (ISIS, 1995). Creatinine can be used as an indicator of kidney damage, but is such a sensitive indicator of muscle damage that, generally, only large increases are of clinical significance (Cornelius, 1989). AST and GGT are both used for diagnosis of liver damage (Cornelius, 1989), but elevated levels are irrelevant when still within the accepted range."
- When juniper intake exceeds 30% of the diet, microbial death occurs, which alters VFA levels (Straka, 2000).
  - Results of Straka (2000) do not support this statement.
- Given the lack of evidence supporting rumen degradation of monoterpenoids in this study, it appears the liver may be the primary site of detoxification in goats.
  - Even though disappearance of 3 (of the 4) terpenes were similar among goats fed juniper vs. not fed juniper, authors do present evidence that monoterpenoids were not degraded in the rumen. Thus, in combination with the other data reported, the statement related to the liver being the primary detoxification site in goats is not warranted.
- Average and maximum juniper intake (all data are reported on a DM basis):
  - Average daily alfalfa intake (authors' Fig. 1) = 324 g alfalfa [18 g/kg BW × 0.9 DM × 20 kg BW]
  - Average daily juniper intake (authors' Fig. 2) = 60 g [6 g/kg BW × 0.5 DM × 20 kg BW]
  - Average total daily DMI = 384 g [324 g + 60 g]
  - Average % juniper in diet = **15.6%** [60/384 × 100]
  - Maximum juniper intake within a given day (authors' Fig. 2) =
    94.7 g [9.47 g/kg BW × 0.5 DM × 20 kg BW] = **4.74 g** juniper/kg BW [94.7/20 kg BW]
  - Maximum alfalfa intake on the day of maximum juniper intake (authors' Fig. 1) =
    306 alfalfa [17 g/kg BW × 0.9 DM × 20 kg BW]
  - Maximum total daily DMI = 400.7 g [306 + 94.7]
  - Maximum % juniper in diet = **23.6%** [94.7/400.7 × 100]
  - Assume redberry juniper volatile oil concentration = 1.83% (Owens et al., 1998), then maximum juniper volatile oil consumption within a given day =
    1.73 g [94.7 g juniper × 0.0183] = **0.087 g**/kg BW
- No negative effects (visual) on animal health were reported.

Table 29 Serum metabolite levels for goats fed redberry juniper for 14 days and goats naive to juniper. Samples were collected on days 0, 5, 10, and 15 of the study. Serum levels on day 0 were used as a covariate to account for variation among individual goats.

	Treatment		
Serum metabolite	Fed Juniper	Fed Alfalfa	SEM
Creatinine (mg/dL)	0.60^{a}	0.54^{b}	0.01
AST (U/L)	62.57^{a}	61.26^{b}	1.93
GGT (U/L)	47.78^{a}	44.52^{b}	0.97
BUN (mg/dL)	22.54	23.62	0.51

$^{a\text{-}b}$Means within rows with different superscripts differ ($\underline{P}$<0.05)

Campbell et al., 2007. Effects of supplementation on juniper intake by goats. Rangeland Ecol. Manage. 60:588–595.

Paraphrased Abstract:

The first experiment evaluated the effect on juniper intake of either no supplementation (negative control) or supplementation with corn, alfalfa, or CSM fed at an isonitrogenous CP level of 1.5 g/kg BW for 12 d. Redberry juniper consumption by individually penned goats was measured on d 11 and 12. Each goat received each supplement in a complete 4 × 4 Latin square design. Juniper intake increased for goats supplemented with alfalfa and CSM (*P* = 0.001), but not for those supplemented with corn (*P* = 0.94). A second study investigated the effect of either no supplementation or SBM supplementation on juniper consumption by free grazing goats. Forty goats were assigned to four pasture groups by breed and previous juniper intake, and randomly allocated to either the treatment (supplementation) or control (no supplementation) in a complete block design. Juniper intake was highest for goats supplemented with SBM (*P* = 0.03).

Experiment 1 (Pen Trial):

Feed treatments included a negative control (NC, no supplemental feed), corn (C), alfalfa (A), and CSM. At the target rate, all animals were fed CP to 100% of maintenance protein requirements (NRC 1981). Two breeds and two crossbreeds of goats received 4 feed treatments in a complete 4 × 4 Latin square design with four replications/treatment. Goat breeds (4 animals/breed) were Angora (28.6 kg), Spanish (33.3 kg), Angora × Spanish (29.7 kg), and Spanish × Boer (37.0 kg) for a total of 16 mature (> 2 yr old) nannies. Each trial was 12 d long, with the first 10 d representing a preconditioning period. Each d, supplemental feed was offered from 0800 to 1200 h. Fresh juniper foliage was harvested daily and goats were offered redberry juniper *ad libitum* by attaching branches in each pen.

Experiment 2 (Grazing Trial)

Effects of a SBM supplement, goat breed, and propensity to graze juniper, on juniper intake by free-grazing goats was investigated during a 16-d period in midwinter. Soybean meal was used instead of CSM to prevent gossypol from possibly interacting with other allelochemicals and affecting consumption of juniper. Percentage juniper in the diet of goats for calculating genetic merit was estimated using near-infrared spectroscopy (NIRS) predictions of fecal samples collected when they were free-grazing on juniper-infested pastures. Goats were preconditioned to juniper by grazing on a 16-ha, juniper-infested pasture for a period exceeding 10 d before separating them into 4 pastures. Ten goats were assigned to each pasture by breed. Animals were allocated to either the treatment (supplementation) or control (no supplementation) for 4 d. Goats within a pasture grazed freely together but received supplementation individually. For supplementation, goats were placed in individual stalls at 1000 h for a 3-h period and released back to the pasture. Soybean meal was fed to half the animals at 0.33% BW/d. Supplemental feeding rate was calculated to provide 0.24 g N/kg BW. After 4 d, fecal samples for NIRS estimation of percentage juniper in the diet were collected manually at 1600 h.

Summary:

- Even after discussing with co-authors, it is still not apparent if intake data is presented on a DM or as-fed basis (not stated by the authors in the paper). Compared to other trials related to fresh juniper leaf intake, juniper intake/kg of BW data reported by the authors seems to suggest that it is on an as-fed basis; supported by the fact that these authors have published other literature in which intake is reported on an as-fed basis. However, assuming that authors reported juniper intake on a DM basis results in the greater juniper consumption vs. if they the intake is actually reported on an as-fed basis and is then converted to a DM basis. Thus, the following assumes that the intake data is reported on a DM basis.
- Introduction section: authors state, “Researchers using pen studies measuring juniper intake by … goats have reported maximum intake values of 33.5% (6.7 g/kg BW) of diet composition (Pritz et al., 1997).”
    - It is unclear how 33.5% of the diet was calculated, even if it was incorrectly calculated on an as-fed basis.
- Introduction section: authors state, “Even though juniper species can represent an important part of goat’s diets, the overall intake of juniper tends to be self-limited when juniper consumption is higher than 30% of the diet (Pritz et al., 1997; Bisson et al., 2001; Straka et al., 2004).”
    - The literature does not support this statement.
- Introduction section: authors state, “The restriction in juniper intake appears to be an attempt to regulate consumption of monoterpenes and avoid negative postingestive consequences of monoterpene exposure at higher levels.”
    - The literature does not support this statement; e.g. restricting juniper intake could be due to initial sensory characteristics such as smell, taste, and texture and could be due to CT.
- Introduction section: authors state, “Toxic monoterpenes in juniper deter goat browsing of juniper plants by reducing nutrient assimilation (Riddle et al., 1999)…”
    - The literature related to juniper leaf consumption does not seem to warrant the statement that the monoterpenes in the juniper are “toxic.” Furthermore, the cited literature does not support this statement; Riddle et al. (1999) report that N balance was positive when blueberry juniper was consumed and at times, similar to the N balance of goats that consumed coastal bermudagrass hay and no juniper. In addition: (1) the cited study did not evaluate the correlation of DMI with nutrient assimilation and (2) authors stated that blueberry juniper “is of sufficiently high quality to significantly contribute to the diets of grazing… animals that have access to other forages and/or supplemental feeds.”
- No negative effects on animal health were reported.

Trial 1 Results:

- CP of juniper leaves assumed to be = 6.5% (Table 3)
  - e.g. [(0.777 g/kg BW)/(11.953 g/kg BW)*100]
- Goats consumed (on average; authors' Table 3) **11.95** ± 10.2, **10.4** ± 4.7, **6.52** ± 5.5, and **6.39** ± 3.8 g/kg BW of juniper, respectively (assumed to be on a DM basis; not reported by the authors). Thus, **maximum** juniper intake (DM basis) =
  - **22.15 g**/kg BW [11.95 g/kg BW + 10.2] = **819.6 g** of juniper [22.15 g/kg BW × 37 kg BW]
  - Average supplement intake/d = 3.507 g of supplement /kg of BW = [1.473 g/0.42 CP] = 129.8 g of supplement [3.507 g/kg BW × 37 kg average BW]
  - Total DMI/d = [819.6 + 129.8] = 949 g
  - Juniper intake as % of total diet = **86.4%** [819.6 g/949) × 100]
  - Assume redberry juniper volatile oil concentration = 1.83% (Owens et al., 1998): then maximum daily juniper volatile oil consumption =
    15 g/d [819.6 g juniper × 0.0183] = **0.405 g**/kg BW
- Average juniper consumption (DM basis; Table 3) of the CSM treatment =
  - Juniper intake/d = **11.953 g** juniper/kg of BW = **382.5 g** [11.953 g/kg BW × 32 kg BW]
  - Supplement intake/d = 3.507 g supplement/kg BW = [1.473 g/0.42 CP] = 112.23 g [3.507 g/kg BW × 32 kg average BW]
  - Total DMI/d = [382.5 + 112.23] = 497.73
  - Juniper intake as percentage of total diet = **77.3%** [382.5/497.73) × 100]
  - Assume redberry juniper volatile oil concentration = 1.83% (Owens et al., 1998): then maximum daily juniper volatile oil consumption =
    7 **g/d** [382.5 g juniper × 0.0183] = **0.22 g**/kg BW
- Discussion: authors state, "This study showed that protein supplements increased juniper consumption by goats."
  - This conclusion is speculative considering that (1) goats in the negative control group were fed only juniper and (2) goats in the corn group were fed only corn when juniper was offered. In the Methods section, the authors report that "protein sources selected for this study reflected three winter supplements commonly used to correct seasonal forage nutrient deficiencies." In addition, authors conclude the paper with, "The current study was designed to utilize and evaluate typical winter supplementation practices …" However, treatments in Trial 1 do not represent traditional practices and do not represent a supplementation program because they were fed as the sole diet.
- Discussion: Authors discuss high-protein diets affecting detoxification, etc.
  - The study was not designed to evaluate detoxification mechanisms; the main effects of the treatment diets, as discussed by the authors, were probably mainly due to rumen physiology and not toxicology.
- Discussion: authors state, "Monoterpenes have the potential to exacerbate the negative effects of a high starch diet. Oxygenated monoterpenes in sagebrush inhibited cellulolytic bacteria populations in deer (Nagy and Tengerdy, 1968). In goats, VFA profiles of microbial populations before and after dosing with juniper oil shifted, implying a decrease in cellulolytic in favor of saccharolytic species (Straka et al., 2004)."
  - A reduction in cellulolytic and an increase in saccharolytic bacteria would actually be beneficial when consuming a high starch diet. In addition, in a previous publication (Straka et al., 2004), the author states, "Juniper consumption within 30% of diet may also result in a favorable shift of VFA production towards lower acetate:propionate ratios, thus improving feed efficiency" (p. 437).
  - Straka et al. (2004) was a review paper that makes a general inference related to effects of terpenes on microbial species composition; they did not evaluate microbial species composition.

- Even though Nagy and Tengerdy (1968) make references related to a decrease in certain types of bacteria, they state that "Identification of the microorganisms appearing in the rumen of deer was not the objective of this investigation." In addition, Nagy and Tengerdy (1968) evaluated the effects of volatile oils and not specific oxygenated monoterpenes.

- Discussion: authors state, "When feed refusals were compared in order to compare gross intake levels, the corn treatment group had the highest level of feed refusal. The most plausible explanation for the decline in feed intake **in this study** was due to attempts by goats to ''correct'' imbalances in the ruminal environment through their feeding behavior (Cooper et al., 1996)."
  - This seems to contradict the paragraph preceding this statement.

Trial 2 Results:

- Greatest level of juniper intake was by supplemented high-consumer goats = **31.4%** ± 2.7% of diet

Frost et al., 2008. Age and body condition of goats influences consumption of juniper and monoterpene treated feed. Rangeland Ecol. Manage. 61:48–54.

Objective: determine how age and BC of goats influence consumption of juniper and an artificial feed containing 4 monoterpenes.

Experiment 1: Intake of fresh redberry juniper foliage by 39 young (2 yr) or mature (> 6 yr) goats in high (HBC) or low condition (LBC) was examined. Redberry juniper branches were harvested daily and leaves were stripped and offered in excess to each goat from 0900 h until 1300 h each day for 5 d. Goats in LBC ate more ($P < 0.01$, 8.6 g/kg BW) juniper vs. HBC (2.3 g/kg BW) and young consumed more ($P < 0.05$, 7.2 g/kg BW) juniper vs. mature goats (3.9 g/kg BW).

Experiment 2: 36 young (2 yr) or mature (> 6 yr) goats either in HBC or LBC, were offered a feed treated with a mixture of 4 of the most prominent monoterpenes found in redberry juniper: α-pinene (13%), limonene (58%), myrcene (24%), and α-terpineol (5%). The terpenes were applied to a bluegrass pelleted feed at a concentration of 20.8 g of monoterpenes/kg feed (as-fed basis). On d 1 to 6, goats were offered 1/3 of their basal ration (0.5% BW) at 0900 h followed by a 4-h exposure to a feed treated with monoterpenes. Beginning on d 7, goats were offered access to the treated feed from 1100 to 1500 h; afterwards, goats were returned to a larger holding pen and allowed to drink for 30 min. Animals were then returned to individual pens and offered 1% BW of alfalfa pellets as the remainder of their basal ration. Orts were collected and intake recorded daily for 23 d. Goats in LBC ate more ($P < 0.01$, 25.3 g/kg BW) of the terpene-treated feed than those in HBC (17.5 g/kg BW), and young ate more ($P < 0.05$, 22.5 g/kg BW) vs. mature (20.3 g/kg BW) goats. Age and BC are important factors that influence intake of chemically defended plants.

Summary:

- Introduction section: authors state, "Secondly, monoterpenes limit intake presumably by producing conditioned flavor aversions from digestive malaise or illness following consumption (Estell et al., 1998, 2002;…)."
    - Estell et al. (1998, 2002) reported that only a few individual terpenes (e.g. α-pinene and camphor), not monoterpenes as a group, were negatively correlated to intake and do not state it as a fact." Estell et al. (2002) also state, "Monoterpenes are typically toxic to insects but safe for consumption by mammals (Rice and Coats, 1994). Because many monoterpenes are classified as "Generally Recognized as Safe" and are natural plant products that are abundant and easily synthesized (Rice and Coats, 1994), they are potential candidates for use in manipulating feeding patterns of browsing herbivores."
- Authors state that terpenes were applied to the pelleted feed at a concentration of 2.08% of the feed (as-fed basis); assuming pellets = 90% DM, this represents terpenes being applied at 2.31 % on a DM basis.

Experiment 1 Results:

- Authors' presented data on an as-fed basis. Data presented below is converted to a DM basis assuming that DM of fresh juniper foliage = 50%.
- All goats were offered alfalfa pellets (1.8% of BW; [2% × 0.9 DM]) and fresh juniper (for 4 hr/d) for 5 d.
- Average alfalfa pellet intake (DM basis) of goats in YLBC = 455.5 g [0.018 × 25.3 kg BW]
- Maximum daily DMI of fresh juniper leaves by goats in YLBC group (authors' Fig. 1) = **9.5 g/kg** BW [19 g/kg BW × 0.5 DM] = **240.4 g** juniper [9.5 g/kg BW × 25.3 kg BW]
- Maximum % of juniper in the diet (DM basis) =
  34.6% [240.4/(240.4 g juniper + 455.5 g alfalfa)]
- Assume redberry juniper volatile oil concentration = 1.83% (Owens et al., 1998): then maximum daily juniper volatile oil consumption =
  4.4 g/d [240.4 g × 0.0183] = **0.23** g/kg BW
- Notable quotes in Results/Discussion Sections:
    - "All goats consumed 100% of their basal ration of alfalfa pellets and were readily accepting the juniper by d 3 of the pretrial period."
    - "At the end of the trial period, all animals were consuming nearly 4 times the amount of juniper they had initially (authors' Fig. 1)."

Experiment 2 Results:

- Results/Discussion: Authors state, "Goats increased intake during the 6-d pretrial period and were readily accepting the feed by the first day of the trial."
    - This supports the fact that the volatile oils were not "toxic."
- Results/Discussion: Authors state (*paraphrased*), "Goats in LBC ate more ($P < 0.01$) of the terpene-treated pellets vs. goats in HBC (25.3 g/kg BW vs. 17.5 g/kg BW; authors' Fig. 2) and young animals ate more ($P < 0.05$) than mature goats across BC treatments (22.5 g/kg BW compared with 20.3 g/kg BW; authors' Fig. 3). Consequently,…, total daily intake of monoterpenes by the LBC group (0.49 g/kg BW) during this trial was roughly double the dose shown to create aversions (0.22 g/kg BW) by Pritz et al. (1997)."
    - Dr. Whitney's Comment: Pritz et al. (1997) dosed goats with 0.22 ml/kg BW, which does not equate to 0.22 g/kg BW considering that 1 mL oil = 0.825 g. In addition, in the discussion (within this proposal) related to the Pritz et al. (1997) study, considering that maximum juniper

consumption was 3.55 g/kg BW and assuming that juniper leaves contained 3% oil (DM basis) and goat BW = 13.6 kg, then volatile oil consumption would be approximately **0.106 g** oil/kg BW. Thus, in a "real world scenario," the dose used in Frost et al. (2008) is actually 4.6 times greater than Pritz et al. (1997) and still, no signs of toxicity were observed.

- Results/Discussion: Authors state (paraphrased), "… goats in low BC (Experiment 2) were not only capable of maintaining a high level of intake of a toxic food, but they were also capable of gaining weight (2.09 kg/animal; 95 g/d) over the course of the 22-d trial. This indicates that metabolism of the 4 monoterpenes did not result in an energy deficit, despite the high concentration of monoterpenes (twice the amount found on redberry juniper foliage), the relatively low quality of the bluegrass pellets, and the limited amount of alfalfa pellets (1.5% of BW). Dziba et al. (2006) found that dosing sheep in the rumen with 1,8-cineole (a monoterpene found in sagebrush) did not affect the acid–base balance of sheep. This suggests that terpene metabolism does not significantly alter acid:base homeostasis so that additional energy is not required for metabolism processes."
  - Dr. Whitney's Comment: This study, particularly the discussion above, supports the fact that juniper terpenes should not be generally defined as "toxic." Even though juniper volatile oil technically falls under the definition of "toxic," so does all other substance (e.g. water) when in "excess." As discussed in this literature, other literature suggests that consumption of juniper leaves is not toxic to herbivores.

Dietz et al., 2010. Feeding redberry juniper (*Juniperus pinchotii*) at weaning increases juniper consumption by goats on pasture. Rangel. Ecol. Manage. 63:366−372.

Paraphrased Abstract and Methods:

Objective was to improve efficacy of goats as a biological control mechanism for juniper through behavioral training. To test whether conditioning creates a longer-lasting increase in juniper preference, we determined if goats would continue to consume juniper on pasture for 1 yr after being fed juniper in pens for 14 d. Female Boer-cross goats (n = 40; average BW = 29.2 kg; 12 mo old) were randomly divided into 2 treatments: conditioned or naive to juniper. At approximately 12 mo of age, conditioned goats were placed in individual pens and fed redberry juniper 1 h/d for 14 d and alfalfa pellets (2% BW); naive goats received only alfalfa pellets (2% BW). Next, goats were placed in 1 of 4 pastures (10 goats/pasture) for 12 mo; 2 pastures housed conditioned goats and 2 pastures housed naive goats at a moderate stocking rate. Conditioned goats consistently ate more ($P < 0.05$) juniper than naive goats except for April and March. Seasonal changes of monoterpene levels in juniper had no apparent effect on juniper preference.

Summary:

- After discussing with Dr. Scott (co-author), it was determined that feed and juniper intake was reported on an as-fed basis. Therefore, data below have been converted to a DM basis with the assumption that fresh juniper leaves and alfalfa pellets contained 50% and 90% DM, respectively.

- Introduction section: authors state, "Most livestock species avoid juniper because of monoterpenoids found in the plant that cause aversive postingestive feedback and the formation of conditioned food aversions (Riddle et al. 1996; Pritz et al. 1997)."
  - Dr. Whitney's Comment: Although Riddle et al. (1996) reported that some terpenes were negatively correlated to juniper consumption, many others were not. In addition, goats only had access to fresh juniper branches for 10 min over a 10-d period. Furthermore, volatile oil concentration in juniper was greatest during the spring and summer (authors' Table 4), but juniper consumption was at times, was similar or greater during spring and summer vs. fall or winter; especially redberry juniper consumption (authors' Table 3).
  - Dr. Whitney's Comment: The discussion above (within this proposal) related to the study by Pritz et al. (1997) concludes that this citation does not adequately support this statement.
- Introduction section: authors state, "It appears that goats can adapt to the monoterpenoids in juniper if they are exposed to the plant slowly over several days (Bisson et al., 2001). Two studies confirmed this observation by feeding juniper to goats in individual pens for 10 to 14 d (Ellis et al., 2005; Dunson et al., 2007). Goats increased intake daily until an apparent toxic threshold was reached."
  - Dr. Whitney's Comment: as discussed above (in this proposal) the data presented by Bisson et al. (2001) does not support this conclusion.
  - Dr. Whitney's Comment: as discussed above (in this proposal) the data presented by Ellis et. al. (2005) does not support this conclusion. Even though intake did increase over time (except for a decrease on d 5 during 2 of the 3 yr) the authors did not design the experiment to evaluate effects of conditioning on juniper intake; all goats were offered juniper and thus, there was no control and no way to determine if intake of a browse plant that did not contain volatile oil would have also increased over time. In addition, since all goats were offered juniper during the first year of the study (1997), the reason for greater juniper consumption during the second year (1998) is purely speculative. Pritz et al. (1997); previously discussed in this proposal) stated, "Goats that received essential oils early in life tended to ingest less ($P < 0.09$) juniper than control goats during the 5-d trial. Thus, early exposure to essential oils did not increase the goats abilities to ingest juniper later in life as had been anticipated."
  - Dr. Whitney's Comment: Results reported by Dunson et al. (2007) do not support this statement. Even though juniper intake generally increased over the 14-d trial (authors' Fig. 2), it is unclear if previously conditioning goats to juniper was the cause. In addition, the authors reported that: (1) serum metabolite levels of GGT, AST, and creatinine increased by d, but remained within the normal range for healthy goats and (2) rumen microbial adaption does not appear to be the physiological processes allowing goats to consume juniper." They also state, "In Experiment 2, 10 goats were inoculated with rumen fluid from either goats familiar with juniper or goats naive to juniper and juniper intake was monitored daily for 10 d. Juniper intake should have been higher when inoculated with fluid from familiar goats, especially if the rumen was adapting to the toxin. However, both goats dosed with rumen fluid from goats consuming juniper and from those consuming alfalfa alone consumed juniper at similar levels."
  - Dr. Whitney's Comment: In regards to goats increasing intake until an apparent toxic threshold was reached: this statement is speculative because none of the cited studies evaluated toxic thresholds.

Pen Study Results:

- Alfalfa intake for 29.2-kg goat, DM basis =
  - 526 g alfalfa/d [29.2 kg BW × 1,000 g × 2% of BW × 0.9 DM]
  - 18 g alfalfa/kg BW•d^{-1}
- Maximum juniper intake (authors' Fig. 1), DM basis =

- **18.98 g** juniper [1.3 g juniper/kg BW × 29.2 kg × 0.5 DM]
- **0.65 g** juniper/kg BW•d^{-1}

- Total DMI = 545 g [526 g alfalfa + 18.98 g juniper]
- Maximum % juniper in diet, DM basis =
  - **3.48%** [18.98 g juniper/545 g total DMI]
- Assume redberry juniper volatile oil concentration = 1.83% (Owens et al., 1998), then maximum daily juniper volatile oil consumption = **0.35 g**/d [18.98 g juniper × 0.0183] = **0.012 g**/kg BW

Grazing Trial Results/Discussion:

- Maximum total bites (% of total bites) of juniper foliage (authors' Fig. 2) = **37%**
- Maximum volatile oil concentration (DM basis; authors' Fig. 3) in:
  - redberry juniper foliage = **24 mg volatile oil**/g DM = **2.4%**
  - blueberry juniper foliage = **17 mg volatile oil**/g DM = **1.7%**
- Notable quotes:
  - "Monoterpene levels appeared to have little impact on variations in juniper preference. We hypothesized that juniper preference would decline as monoterpene levels increased. This hypothesis was rejected; juniper preference was not related ($P > 0.05$) to individual or total monoterpene levels in either blueberry or redberry juniper…"
- Authors state, "Prior to the initiation of this study, it was clear that goats would increase consumption of juniper when fed the plant at weaning in individual pens (Bisson et al., 2001; Ellis et al., 2005; Dunson et al., 2007)." "Goats were reluctant to consume juniper initially, but by the end of the feeding trials, juniper would account for up to 32% of the diet."
  - Dr. Whitney's Comment: As discussed above, these 3 citations do not support this statement.
  - Dr. Whitney's Comment: "32% of the diet" was incorrectly calculated on an as-fed basis.
- Authors state, "A previous study (Dunson et al., 2007) illustrated that rumen function in goats does not change to the point of detoxifying the terpenes in juniper. Hepatic involvement is more likely."
  - Dr. Whitney's Comment: A few ruminal parameters were effected by juniper, but no differences were observed in terpene degradation. Hepatic involvement being "more likely" is not supported by the literature.
- Authors state, "Moderate doses of juniper oil at levels nearing exposure levels seen at maximal intake levels of free-ranging goats (0.18 g oil/kg BW) resulted in mild hepatic injury in the form of lipid vacuolization. At higher dose levels (0.36 g oil/kg BW), cellular necrosis and lobular encapsulation were evident (Straka et al., 2004)."
  - Dr. Whitney's Comment: This cited reference is a review paper and not a research publication. This data comes from Straka (2000). In addition, authors failed to continue the discussion by Straka et al. (2004), which states, "Hepatic injury overall due to vacuolization was not severe and was most likely attributable to fasting."
- Authors state, "Goats typically avoid juniper during the spring and summer and when alternative herbaceous forage is available…
  - Dr. Whitney's Comment: Goats do not "typically" avoid juniper during the spring and summer.

Owens et al., 2010. Redberry juniper consumption does not adversely affect reproduction of meat goats. TX J. Agricul. Nat. Res. 23:71−82.

Paraphrased Abstract and Methods:

Goat browsing can slow the encroachment of juniper onto rangelands, but potential detrimental effects of monoterpenoids on reproduction are unknown. We determined whether redberry juniper consumption by pregnant goats caused abortions or reduced offspring neonatal viability. Pregnant Boer-cross nannies (n = 19; BW = 54 kg) were randomly divided into 4 treatments, 3 treatments fed redberry juniper 1 h/d for 22 d during 1 of the 3 trimesters and a control group fed alfalfa pellets throughout gestation at 2% BW; fed in individual pens. In a pasture trial, pregnant nannies (n = 20) were placed on juniper-dominated rangeland throughout gestation; juniper preference was monitored once monthly via bite count surveys and fecal NIR analysis. In both trials, birth date, BW, offspring number, sex, and vigor scores were recorded at parturition. No abortions occurred as a result of redberry juniper consumption and no differences ($P > 0.05$) were observed in offspring number, vigor scores, or weight. Producers can use goats as a management tool for slowing juniper encroachment onto rangelands without causing abortions or reducing neonatal viability.

Summary:

- Introduction: authors state, "Monoterpenoids, a class of terpenes containing two isoprene units, found in juniper are known to cause aversive postingestive feedback (Riddle et al., 1996; Pritz et al., 1997) thereby limiting intake."
  - Dr. Whitney's Comment: The literature does not support this statement; e.g. restricting juniper intake could be due to initial sensory characteristics such as smell, taste, and texture and could be due to CT.
  - Dr. Whitney's Comment: Although Riddle et al. (1996) reported that some terpenes were negatively correlated to juniper consumption, many others were not. In addition, goats only had access to fresh juniper branches for 10 min. over a 10-d period. Furthermore, volatile oil concentration in juniper was greatest during the spring and summer (authors' Table 4), but juniper consumption was at times, was similar or greater during spring and summer vs. fall or winter; especially redberry juniper consumption (authors' Table 3).
  - Dr. Whitney's Comment: The discussion above (within this proposal) related to the study by Pritz et al. (1997) concludes that this citation does not adequately support this statement.
- Introduction: authors state, "During winter months when alternative forage availability and nutrient quality is limited, juniper consumption can range from 22-29% of the diet (Campbell et al., 2007)."
  - Dr. Whitney's Comment: Campbell et al. (2007) did not study juniper consumption.
- Introduction: authors state, "Although extensive research has gone into conditioning goats to consume juniper (Bisson et al., 2001; Ellis et al., 2005; Dunson et al., 2007)…"
  - Dr. Whitney's Comment: As discussed above (within this proposal) data presented by Bisson et al. (2001) do not support this conclusion.
  - Dr. Whitney's Comment: As discussed above (within in this proposal) data presented by Ellis et. al. (2005) do not support this conclusion. Even though intake did increase over time (except for a decrease on d 5 during 2 of the 3 yr) the authors did not design the experiment to evaluate effects of conditioning on juniper intake; all goats were offered juniper and thus, there was no control and no way to determine if intake of a browse plant that did not contain volatile oil would have also increased over time. In addition, since all goats were offered juniper during the first year of the study (1997), the reason for greater juniper consumption during the second year (1998) is

purely speculative. Pritz et al. (1997); previously discussed in this proposal) stated, "Goats that received essential oils early in life tended to ingest less ($P < 0.09$) juniper than control goats during the 5-d trial. Thus, early exposure to essential oils did not increase the goats abilities to ingest juniper later in life as had been anticipated."

- Dr. Whitney's Comment: Results reported by Dunson et al. (2007; previously discussed in this proposal) do not support this statement. Even though juniper intake generally increased over the 14-d trial (authors' Fig. 2), it is unclear if previously conditioning goats to juniper was the cause. In addition, the authors reported that: (1) serum metabolite levels of GGT, AST, and creatinine increased by d, but remained within the normal range for healthy goats and (2) rumen microbial adaption does not appear to be the physiological processes allowing goats to consume juniper." They also state, "In Experiment 2, 10 goats were inoculated with rumen fluid from either goats familiar with juniper or goats naive to juniper and juniper intake was monitored daily for 10 d. Juniper intake should have been higher when inoculated with fluid from familiar goats, especially if the rumen was adapting to the toxin. However, both goats dosed with rumen fluid from goats consuming juniper and from those consuming alfalfa alone consumed juniper at similar levels."

- Introduction: authors state, "Ingestion of ponderosa pine causes abortions in cattle due to acetyl isocupressic acid (**ICA**) which is converted to ICA in the rumen (Gardner et al., 1998)."
    - Dr. Whitney's Comment: The study by Gardner et al. (1998) did not use any control animals and thus, should be interpreted with caution.
    - Dr. Whitney's Comment: Redberry juniper does not contain any significant quantities of ICA (< 0.05) or labdane acids (< 0.14% of plant DM, Stewart et al., 2014; Kevin Welch and Dale Gardner, Ph.D., Personal Communication, May, 3, 2013).
- Introduction: authors state, "Johnson et al. (1976) discovered that feeding one-seeded juniper to sheep in the second and early third trimester caused abortions."
    - Dr. Whitney's Comment: In Experiment 1 of Johnson et al. (1976), all 6 sheep were orally dosed with 1 lb. of juniper leaf material; there were no control animals and thus, no way to determine if the juniper dose resulted in any animal health issues. During Experiment 2, out of the 4 sheep dose with 3/4 lb. of juniper, 3 had "normal twins" and "1 had a normal single lamb." They stated, "Attempts to confirm these findings by feeding juniper to other sheep during gestation days 60 to 90 were unsuccessful." They also stated that: (1) these 4 sheep had normal clinical chemical tests; (2) "Because of the acute nature of the toxicity, the clinical chemical tests were ineffective in predicting the onset of symptoms, but the normal values obtained throughout the experiment in those animals which tolerated the full 30-d plant feeding trial indicated that there was probably no chronic tissue damage. This further supports the proposal that systemic shock probably caused mortality. Additionally, serious systemic disturbances would have increased the likelihood of interrupting pregnancy, which did not occur."

Results/Discussion: Pen feeding trial (all data reported below are on a DM basis:

- Authors state, "Avoidance occurs because of monoterpenoids contained in juniper associated with aversive postingestive feedback (Riddle et al., 1996; Pritz et al., 1997)."
    - Dr. Whitney's Comment: As discussed above, these 2 citations do not support this conclusion.
- Authors state, "Gas chromatograph analysis of redberry juniper fed to goats revealed higher concentrations of monoterpenes inversely correlated to intake than that of a study conducted by Dietz et al. (2009 [should have been cited as 2010])."
    - Dr. Whitney's Comment: Dietz et al. (2010) concluded that "Seasonal changes of monoterpene levels in juniper had no apparent effect on juniper preference."
- Alfalfa intake for a 54.1-kg nannie =
  973.8 g alfalfa/d [54.1 kg BW × 1,000 g × 2% BW × 0.9 DM]

18 g alfalfa/kg BW•d^{-1}

- Maximum juniper intake (authors' Fig. 1) =
  54.1 g juniper [2 g juniper/kg BW × 54.1 kg × 0.5 DM] = **1 g** juniper/kg BW•d^{-1}
- Total DMI = 1,028 g [973.8 g alfalfa + 54.1 g juniper]
- Maximum % juniper in diet =
  5.26% [54.1 g juniper/1,028 g total]
- Assume from authors' Table 6, that the fresh redberry leaves contained 0.45% volatile oils [(((1.063% + 0.883% + 0.733%)/3) 0.5 DM)], then maximum volatile oil intake =
  0.243 g volatile oil [54.1 g juniper × 0.0045] = **0.0045 g** volatile oil/kg BW

Results/Discussion: Pasture trial:

- Table 30 (authors' Table 4) reports that nannies selected juniper up to 66% of their diet.

Table 30. Total herbaceous production and preferences of herbaceous vegetation, juniper or other shrubs

Table 4. Total herbaceous production (kg ha^{-1}) and preference of herbaceous vegetation, juniper, or other shrubs (percent of total bites) for pregnant nannies during 3 months of observation on juniper dominated rangeland.

	August	September	October	SEM
Herbaceous Production	1066.5	659.46	1459.2	309.04
Preference				
Herbaceous	6.8	53.7	33.9	1.2
Juniper	17.3	43.7	66.2	1.4
Other Shrubs	76.0	0.0	0.0	0.6

George et al., 2010. Supplements containing escape protein improve redberry juniper intake by goats. Range. Ecol. Manage. 63:655−661.

Paraphrased Abstract and Methods:

This study determined if protein supplements that differed in percentage of ruminal bypass protein, affected intake of fresh redberry juniper leaves by individually-penned wether Boer-cross goats (n = 47; BW = 23.6 kg). Treatments 1, 2, 3, and 4 received a protein supplement (from 0800 to 0900 h) and juniper leaves for 1 h/d (from 0900 to 1000 h) for 14 d, along with a basal diet of alfalfa pellets (2% BW). Treatment 1 contained CSM; Treatment 2 contained CSM and DDG; Treatment 3 contained SBM; Treatment 4 contained SBM and DDG; and Treatment 5 received only a basal diet of alfalfa pellets and juniper. Fresh juniper leaves were stripped from stems, composited, and stored at 4°C until fed. Supplementation with 1) CSM, 2) SBM, or 3) SBM and DDG did not influence ($P > 0.05$) fresh redberry juniper leaf intake. Conversely, goats supplemented with CSM and DDG ate more ($P < 0.05$) juniper than goats receiving only alfalfa.

Summary:

- Introduction section: Authors state, "Unfortunately, intake of juniper is at times limited because monoterpenoids found in the plant cause aversive postingestive feedback (Riddle et al., 1996; Pritz et al., 1997).
    - Dr. Whitney's Comment: Riddle et al. (1996) reported that some terpenes were negatively correlated to juniper consumption, but many other individual terpenes were not. In addition, animals only had access to fresh juniper branches for 10 min. over a 10-d period. Furthermore, volatile oil concentration in juniper was greatest during the spring and summer (authors' Table 4), but juniper consumption was at times, was similar or greater during spring and summer vs. fall or winter; especially redberry juniper consumption (authors' Table 3).
    - Dr. Whitney's Comment: The discussion above (within this proposal) related to the study by Pritz et al. (1997), suggests that this citation does not adequately support this statement.
- Authors present data on an as-fed basis. Due to authors not presenting percentage of DM of ingredients/supplements, the following assumptions were made based on other literature to present intake on a DM basis: DM of juniper leaves = 50%; DM of supplement = 90%.
- Average daily DMI of alfalfa and supplement (authors' Table 3) =
  505.5 g [21.42 g/kg BW × 23.6 kg BW]
- Maximum daily juniper DMI intake for goats fed CSMD (authors' Fig. 3) =
  2.25 g juniper/kg BW [4.5 g juniper/kg BW × 0.5 DM]
  53.1 g juniper/d [2.25 g juniper/kg BW × 23.6 kg BW]
- Maximum percentage of juniper in the diet =
  9.5% [53.1/(53.1 + 505.5 g of alfalfa and supplement) × 100]
- Assume redberry juniper volatile oil concentration = 1.83% (Owens et al., 1998), then maximum daily juniper volatile oil consumption =
  0.97 g/d [53.1 g juniper × 0.0183] = **0.04 g**/kg BW
- Authors stated, "… they [monoterpenoid levels] tend to be higher in winter and spring (Riddle et al., 1996)."
    - Dr. Whitney's Comment: Riddle et al. (1996) reported that "Concentrations of total oils were greater in the spring and summer than in the fall and winter."
- Authors stated, "Goats in all treatments increased juniper intake daily until d 12, and this pattern of intake has been clearly illustrated in other studies (Bisson et al., 2001; Ellis et al., 2005; Dunson et al., 2007). In addition, feeding juniper at weaning can increase acceptance of that plant that continues once goats are released on pasture (Dietz et al., 2010).
    - Comment: Juniper intake declined on d 10 (Bisson et al., 2001). In Ellis et al. (2005), juniper intake declined on d 6 and remained relatively constant thereafter during the first year of the study and declined on d 6 during the second year of the study.
- No adverse effects on animal health were reported.

Anderson et al., 2013. Using experience and supplementation to increase juniper consumption by three different breeds of sheep. Rangel. Ecol. Manage. 66:204−208.

Paraphrased Abstract and Methods:

Goats will consume up to 30% of their diet in juniper, but it is unknown if sheep will accept juniper to the same extent. Objectives were to determine if sheep can be conditioned to consume juniper and to compare intake among different breeds. Rambouillet (n = 10), Suffolk (n =10), and Dorper-cross (n = 10) lambs were randomly placed in individual pens for 31 d. A basal diet of alfalfa pellets (2.5% BW) and fresh redberry juniper leaves were fed to all lambs. Juniper was fed each morning from 0800 to 0830 h. Following the first 17 d, basal diet was reduced to 2% BW for 7 d and then reduced to 1.5% BW for the final 7 d. Serum metabolites and BW were measured to assess any adverse physiological effects from juniper consumption. In a second trial, lambs were again fed alfalfa (2.5% BW) and juniper; half of the lambs were also fed a 36% protein supplement. Lambs received alfalfa, juniper, and protein supplement for 22 d and juniper intake was similar ($P > 0.05$) among breeds of sheep. Lambs readily consumed juniper and increased ($P < 0.05$) intake of juniper as the amount of alfalfa fed was reduced. Protein supplementation did not improve juniper consumption. Concluded: sheep will consume a diet consisting of 24% juniper without experiencing any adverse effects.

Summary:

- Abstract: The amount of feed that was offered is on an as-fed basis; thus, on a DM basis, these percentages would be approximately 10% less (if assume alfalfa contained 90% DM).
- Abstract: Authors state, "We contend that sheep will consume a diet consisting of 24% juniper without experiencing any adverse effects."
  - Comment: This percentage is on an as-fed basis and should have be reported on a DM basis.
- Introduction: Authors state, "Livestock and wildlife typically avoid consuming both species of juniper because of monoterpenoids that cause aversive postingestive feedback and the formation of conditioned food aversions (Riddle et al., 1996; Pritz et al., 1997).
  - Comment: In regards to the first part of this statement: livestock do consume both species of juniper, albeit at different percentages of total DMI.
  - Comment: The statement related to redberry juniper consumption causing aversive **postingestive** feedback forming aversions is not supported by these 2 references. Although Riddle et al. (1996) reported that some terpenes were negatively correlated to juniper consumption, many others were not. Also, they did not determine if the effect was pre- (smell, taste, etc.) or postingestive. In addition, goats only had access to fresh juniper branches for 10 min over a 10-d period. Furthermore, volatile oil concentration in juniper was greatest during the spring and summer (authors' Table 4), but juniper consumption was at times, was similar or greater during spring and summer vs. fall or winter; especially redberry juniper consumption (authors' Table 3).
  - Comment: The discussion above (within this proposal) related to the study by Pritz et al. (1997) concludes that this citation does not adequately support this statement.
  - Comment: In addition, Dietz et al. (2010) state, "Seasonal changes of monoterpene levels in juniper had no apparent effect on juniper preference."
- Introduction section: Authors state, "Goats will consume juniper on pasture after conditioning a preference for the plant in individual pens (Dietz et al., 2010).
  - Comment: This statement contradicts the authors' statement related to livestock typically not consuming juniper.

- Comment: This statement seems to suggest that only conditioned goats will consume juniper on pasture. Authors should have stated that Dietz et al. (2010) reported that conditioning goats can increase juniper consumption on pasture.

- Introduction section: Authors state that juniper typically accounts for 30% of their diet.
  - Comment: "30%" is incorrect: Bisson et al. (2001): percentage of redberry and blueberry juniper in the diet = 30 to 32.6% and 49.7 to 65.6% (DM basis), respectively. Ellis et al. (2005): average redberry juniper in the diet = 6.57 to 10.9% and maximum redberry juniper intake = 19.8% of the diet (DM basis). Dunson et al. (2007): average redberry juniper in the diet = 15.6% and maximum redberry juniper intake = 23.6% of the diet (DM basis).
- Introduction section: Authors state, "Feeding more juniper initially or increasing the amount of juniper offered too quickly results in the formation of conditioned food aversions and avoidance of juniper (Dietz et al., 2010)."
  - Comment: Dietz et al. (2010) report that goats were reluctant to consume juniper from d 2 to 9, but this reference does not support the statement related to juniper being "offered too quickly results in the formation of conditioned food aversions and avoidance of juniper."
- Methods section: Authors state, "At this time, there is little available evidence regarding juniper acceptance by sheep."
  - Comment: As stated above, there are numerous references related to juniper acceptance by sheep.
- Methods section: Authors state, "Amount of juniper offered was initially limited to 50 g because over-ingestion can lead to the formation of condition food aversions."
  - Comment: The literature does not support this conclusion
- Methods section: Authors state, "A second trial was conducted the following summer because all lambs, regardless of breed, lost weight."
  - Comment: It is believed that the amount of feed, as a percentage of BW, was calculated on an as-fed basis. Thus, e.g., if 2.5% of BW was fed, they actually fed ~2.25% of BW [2.5% × 0.9 DM].
- Results: Authors state, "During the first 17 d, juniper made up 4.6% of diet, 15.3% during d 18 to 24, and 24.1% during d 25 to 31 (authors' Table 1)."
  - Comment: These percentages are on an as-fed basis and are less when reported on a DM basis.
- Results: Authors state, "Serum metabolite levels … differed ($P < 0.05$) by d of collection (authors' Table 4). Blood urea nitrogen levels were lower at the end of the study. Bilirubin levels were higher initially and declined as the study progressed. Creatinine levels increased as the study progressed. Serum AST levels were similar across all 4 collection periods. GGT levels also decreased as the study progressed. All serum metabolite levels remained within normal range for healthy individuals. In addition, most serum metabolite levels declined the longer sheep were fed juniper.
  - Comment: This discussion supports the fact that juniper leaves (and its oils) are not toxic.
- Discussion: Authors state, "Goats at 6 wk of age were fed blackbrush daily. Blackbrush contains CT that are toxic to ruminants. Goats introduced to blackbrush early in life consumed 95% more blackbrush than naive goats, were more efficient at digesting blackbrush, and excreted more uronic acid, apparently because of an increased ability to detoxify the tannins in blackbrush." (Distel and Provenza, 1991)
  - Comment: Results of Distel and Provenza (1991) do not support the statement that CT are toxic to ruminants and only "suggest" and assume that differences in consumption were due to detoxification mechanisms (see authors' conclusion).
- Discussion: Authors state, "Dunson et al. (2007) illustrated that goats did not adapt to juniper through changes to the rumen environment; rather, hepatic involvement seems more likely. Monoterpenoids are converted from lipophilic compounds to hydrophilic conjugated

compounds by phase 1 and phase 2 detoxification enzymes in the liver before urinary excretion (Foley et al., 1995).
  - Comment: As previously discussed in this proposal for the Dunson et al. (2007) citation, disappearance of 3 (of the 4) terpenes were similar among goats fed juniper vs. not fed juniper. Thus, in combination with the other data reported in Dunson et al. (2007), the statement related to the liver being the primary detoxification site is speculative.
- Discussion: Authors state, "Moderate doses of juniper oil at levels nearing exposure levels seen at maximal intake levels of free ranging goats (0.18 g oil/kg) resulted in mild hepatic injury in the form of lipid vacuolization. At higher dose levels (0.36 g oil/kg), cellular necrosis and lobular encapsulation were evident (Straka et al., 2004).
  - Comment: As previously discussed in this proposal for the Dietz et al. (2010) publication, Straka et al. (2004) is a review paper and not a research study. This statement comes directly from Straka (2000) and should be within quotation marks. In addition, authors failed to continue the discussion by Straka et al. (2004), which states, "Hepatic injury overall due to vacuolization was not severe and was most likely attributable to fasting."
- Discussion: Authors state, "Serum metabolite levels collected differed across 4 periods; however, all metabolite levels remained within normal range for sheep …
  - Comment: The amount of feed, as a % of BW, was calculated on an as-fed basis. Thus, for example, if 2.5% of BW was fed, they actually fed approximately 2.25% of BW [2.5% × 0.9].
- Levels seem to vary somewhat randomly. When toxicosis occurs, creatinine, AST, and GGT levels typically all increase."
  - Comment: As previously discussed in this proposal, AST and GGT are not liver specific.
- Discussion: Authors state, "During the first trial, juniper intake data from Period 1 illustrates that sheep will consume juniper in a pen situation even though their basal diet (2.5% BW) meets or exceeds nutritional requirements (NRC, 2007)."
  - Comment: This statement seems to contradict the authors' previous statement related to why lambs were losing BW in the first trial: "Lambs probably lost weight in the first trial because they were unable to meet their nutritional requirements, especially because the amount of alfalfa was reduced below the recommended level to meet maintenance requirements."
- Discussion: Authors state, "Indeed, goats will increase intake of juniper on pasture situations as forage availability declines (Dietz et al., 2010). Goats that were conditioned to consume juniper in the same manner increased intake, whereas naive goats increased intake of other shrubs in the pasture."
  - Comment: Dietz et al. (2010) did not study effects of forage availability on juniper consumption or forage quality.

Juniper intake data (calculations are on a DM basis):

- Alfalfa DMI on the d of maximum juniper intake =
  429.3 g [31.8 kg BW × 0.015 × 1000 g × 0.9 DM] = 13.5 g/kg BW [429.3/31.8 kg BW]
- Maximum juniper DMI intake (authors' Fig. 1; did not consider the last d as maximum) =
  87.5 g juniper/d [(5.5 g juniper/kg BW) × 0.5 DM × 31.8 kg BW]
  2.75 g/kg BW [87.5 g juniper/31.8 kg BW]
- Maximum percentage of juniper in the diet =
  16.93% [87.5/(87.5 g juniper + 429.3 g alfalfa) × 100]
- Assume redberry juniper volatile oil concentration = 1.83% (Owens et al., 1998): then maximum daily juniper volatile oil consumption =
  1.60 g/d [87.5 g juniper × 0.0183] = **0.05 g**/kg BW [1.6 g oil/31.8 kg BW].
- No adverse effects on animal health were reported.

Campbell et al., 2010. Pharmacokinetic differences in exposure to camphor after intraruminal dosing in selectively bred lines of goats. J. Anim. Sci. 2620−2626.

Paraphrased Abstract and Methods:

A pharmacokinetic dosing study with camphor was used to determine whether selection lines of high-juniper-consuming goats (HJC, n = 12) and low-juniper-consuming goats (LJC, n = 12) differed in disposition kinetics. Post-dosing plasma camphor concentrations were used to examine whether a timed single blood sample collected after intraruminal administration of camphor would be a useful screening test to aid in the identification of HJC. Yearling female goats (n = 24) received a single intraruminal dose of monoterpene cocktail (**0.270 g/kg BW**) containing 4 different monoterpenes that represented their composition previously reported for blueberry juniper. Camphor, the predominant monoterpene in blueberry juniper, was 49.6% of the mix and was the monoterpene analyzed for this study. Blood samples were taken at 15 time points after dosing and camphor was measured in plasma. Maximal plasma concentration of camphor was greater for LJC than HJC ($P = 0.01$). Total systemic exposure (area under the curve) to camphor was 5 times less in HJC goats. Concluded: 1) HJC goats possess internal mechanisms to reduce bioavailability of camphor, and 2) a blood sample taken at 45 min or at 60 min after intraruminal administration of camphor may be useful for identifying HJC individual animals from within large populations of goats.

- Introduction: Authors state, "One limitation is that goats self-regulate their consumption of juniper due to the presence of aversive monoterpenes in its foliage (Riddle et al., 1996).
    - Comment: As previously discussed in this proposal, this citation does not support this statement.
- Methods: Authors state, "The dose was chosen to represent the concentration and composition of monoterpenes present in blueberry juniper leaves (Riddle et al., 1996)…. The dose was chosen to be biologically relevant to the monoterpene concentration present in a diet of 30% juniper, which is a quantity of intake identified through previous research as eliciting differences in juniper intake between breeding groups (Campbell et al., 2007).
    - Comment: It is unclear how 30% juniper intake was calculated from data presented by Riddle et al. (1996). Others have shown that maximum intake can exceed 56% of DM (Riddle et al., 1999).
    - Comment: Campbell et al. (2007): During Trial 1 (pen trial), intake by breed did not differ and juniper DMI, on a DM basis and as % of total diet was 77.3%. During Trial 2, maximum juniper intake as percentage of total diet was 31.4%, but breed differences were not apparent.
- Methods: Authors reported that the volatile oil dose "provided (0.270 g/kg BW)."
    - Comment: If the volatile oil content in ***J. ashei*** = 2.15% (DM basis; Owens et al., 1998), then daily consumption of *J. ashei* leaves (as % of DMI) by a 23.64-kg goat would have to be = **296.87 g**/goat [(0.27 g oil/kg BW × 23.64 kg BW)/0.0215] = **12.56 g**/kg BW [296.87/23.64]
      *Assuming total daily DMI of this 23.64-kg goat = 591 g [0.25 BW × 23.64 kg BW × 1000 g], then % juniper in diet = **50.2%** (DM basis) [(296.87/591) × 100]
    - Comment: If volatile oil content in ***J. pinchotii*** = 1.83% (DM basis; Owens et al., 1998), then daily consumption of *J. pinchotii* leaves (as % DMI) by a 23.64-kg goat would have to be = **348.8 g**/goat [(0.27 g oil/kg BW × 23.64 kg BW)/0.0183] = **14.76 g**/kg BW [348.8/23.64]
      *Assuming total daily DMI of this 23.64-kg goat = 591 g [0.25 BW × 23.64 kg BW × 1000 g], then % juniper in the diet = **59%** (DM basis) [(348.8/591) × 100]
- Discussion: Authors state, "Anti-herbivory properties of monoterpenes in juniper appear to be associated with negative postingestive consequences specifically related to central nervous system triggers for cessation of feeding behavior or satiety.

- Comment: As reported in this proposal, the link between juniper consumption and post-ingestive consequences is not warranted.

- Discussion: Authors state, “Monoterpenes have been identified as initiating satiety-based feeding cessation in … and domestic sheep (Dziba et al., 2006)
  - Comment: Cessation is defined as “the stopping of an action.” Dziba et al. (2006) reported that during the first week of the trial, average feeding time was less for lambs dosed with 1,8-cineole vs. lambs not dosed; however, lambs were only observed for 1 hr immediately after ruminal dosing with 1,8-cineole dose, which does not represent “feeding cessation.” During the second week of the trial, DMI was not different among the lambs. In addition, the control animals in Dziba et al. (2006) were not dosed at all (not even with water) because they stated that “we expected no effect of vegetable oil on feeding behavior based on results of Dziba and Provenza (2006). Two issues arise from not dosing the control animals: 1) handling and dosing livestock can affect behavior and feed intake, especially within the first hour of observation and 2) Dziba and Provenza (2006) did not evaluate effects of dosing vs. not dosing with vegetable oil on DMI.
  - Comment: It should also be noted that *J. ashei* and *J. pinchotii* contain very little to no 1,8-cineole (Adams, 2011).
  - Comment: It should also be noted that Dziba et al. (2006) stated that the rumen was dosed with 1,8-cineole (in vegetable oil), which provided 125 mg of 1,8-cineole/kg BW. However, this dose was actually **156.3 mg** of 1,8-cineole/kg of BW [0.625 mg solution/kg BW × 250 mg of 1,8-cineole/ml solution]. This ruminal dose is between the low and medium doses of 135 and 190 mg of 1,8-cineole/kg of BW evaluated by Dziba and Provenza (2006), which did not result in feeding cessation. Intake of diets dosed with 4 terpene compounds decreased, but intake of diets that did not contain terpenes also decreased.
  - Comment: The “medium dose” evaluated by Dziba and Provenza (2006) resulted in 9.35 g of 1,8-cineole being consumed (within a given day). Assuming that sagebrush leaves contained between 0.3 to 1.1% 1,8-cineole (DM basis; Personius et al., 1987), grazing lambs (BW = 49 kg) would have to consume 3,117 g to 850 g of sagebrush leaves/d [DM basis; 9.35/0.003 or 9.35/0.011], which is impossible (e.g., 6.36% of BW, DM basis)
- Discussion: Authors state, “In retrospect, reducing absorption rather than increasing elimination could be a more adaptive response because monoterpenes in juniper cause mild hepatic injury in the form of lipid vacuolation at small dosages (0.18 g oil/kg of BW) and hepatic cellular necrosis at greater dosages (0.36 g oil/kg BW; Straka, 2000).
  - Comment: As reported in this proposal, the Straka (2000) did not use any control animals, thus there is no way to determine if juniper or some other factor caused hepatic insult.
- Discussion: Authors state, “Macronutrient intake and BC can affect disposition of phytotoxins in livestock such as …monoterpenes in goats (Campbell et al., 2007; Frost, 2005).
  - Comment: Results from Campbell et al. (2007) do not support this statement because (1) goats in the negative control group were fed only juniper and (2) goats in the corn group were fed only corn when juniper was offered, which does not represent traditional supplementation practices.
  - Comment: Discussion: Authors discuss high-protein diets affecting detoxification, etc.; however, the study was not designed to evaluate detoxification mechanisms; the main effect of the treatment diets, as discussed by the authors, were probably mainly due to rumen physiology and not toxicology.
- No adverse effects on animal health were reported when dosed with 0.27 g/kg terpenes.