Does Experience with Sagebrush In utero and Early in Life Influence the Use of Sagebrush by Sheep?

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DOES EXPERIENCE WITH SAGEBRUSH IN UTERO AND EARLY IN LIFE
INFLUENCE THE USE OF SAGEBRUSH BY SHEEP?

by

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ABSTRACT

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by

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Learning from mother begins early in the developmental process and can have lifelong effects when it comes to forage preferences. Recent research suggests that mothers are a powerful and positive influence before birth. Pregnancy is not an incubation period but a staging period for well-being and disease later in life. Better understanding the developmental processes which take place \textit{in utero} and the effects they have later in life may help us create management plans that utilize grazing animals to their full potential as landscape manipulators.

Using \textit{in utero} and early-life programming as a management tool is a relatively new concept, but offers a faster approach than genetic selection to respond to environmental contingencies in the short-term. Experiences \textit{in utero} and early in life may have marked effects on the ability of herbivores to consume toxin-containing plants such as sagebrush. This is because environmental experiences cause epigenetic alterations in consumers which are translated into neurological, morphological, and physiological
changes that influence foraging behavior. This change in behavior can reduce the competitive ability of toxin-containing plants in the community and allow for greater primary production and diversity. However, information regarding herbivores’ exposure early in life to plant toxins and their subsequent physiological and behavioral responses is limited. Moreover, no information is available on early life experiences to toxin-containing shrubs like sagebrush and their subsequent influence on feeding behavior by herbivores. Thus, the objective of my research was to explore how experience in utero and early in life with sagebrush affected intake of and preference for sagebrush by sheep later in life.
PUBLIC ABSTRACT

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Ashley T. Longmore

Learning from mother begins early in the developmental process and can have lifelong effects when it comes to food preferences. Recent research suggests that mothers are a powerful and positive influence before birth. Pregnancy is not an incubation period but a staging period for well-being and disease later in life. Better understanding the developmental processes which take place in utero and the effects they have later in life may help us create management plans that utilize grazing animals to their full potential as tools in landscape management.

Using in utero and early-life programming as a management tool is a relatively new concept, but offers a faster approach than genetic selection to respond to ever changing environmental conditions. Experiences in utero and early in life may have significant effects on the ability of livestock to eat toxin-containing plants such as sagebrush. This is because environmental experiences cause a change in genetics in the consumers which are translated into neurological, morphological, and physiological changes that influence foraging behavior. This change in behavior can reduce the competitive ability of toxin-containing plants in the community and allow for greater primary production and diversity. However, information regarding herbivores’ exposure early in life to plant toxins and their physical and behavioral response is limited.

Moreover, no information is available on early life experiences to toxin-containing shrubs
like sagebrush and their influence on feeding behavior by herbivores. Thus, the objective of my research was to explore how experience *in utero* and early in life with sagebrush affected intake of and preference for sagebrush by sheep later in life.
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INTRODUCTION

Sagebrush steppe is one of the largest eco-regions in North America, covering millions of hectares of rangeland in the western United States (West, 1993). Over the past 30-40 years, forage production on sagebrush steppe has dramatically declined from approximately 800 pounds of grass and forbs per acre to less than 100 pounds per acre due to decadent stands of sagebrush which outcompete essential understory species (Winward, 1991). In addition to primary production, plant diversity generally declined during the same period of time as woody species, such as sagebrush and juniper, came to dominate the landscape. Several factors have led to this decline including overgrazing by livestock in the 1930’s-1950’s as well as fire suppression policies all of which favor decadent stands of sagebrush (Laycock, 1979; Striby et al., 1987; Winward, 1991). This decline in production and diversity adversely affects sagebrush-steppe ecosystems (Bryant et al., 1991). Nutrient cycling, plant production, and herbivore nutrition are negatively impacted because sagebrush - although abundant and nutritious – contains high concentrations of terpenes, which are plant secondary compounds that are toxic to soil and rumen microbes, and to herbivores (Ngugi et al., 1995, Dziba and Provenza, 2007; Dziba et al., 2007). To reverse the negative trends on production and biodiversity, management strategies must (1) rejuvenate sagebrush stands and (2) favor a mixture of plant species in the understory.

It is possible that through proper management, the very same animals that contributed to a reduced biodiversity and primary production in sagebrush steppe ecosystems, i.e., livestock, become part of the solution representing one of the most
economical means to accomplish the aforementioned objectives. This is because ungulates can significantly alter ecosystem processes at multiple temporal and spatial scales. They affect plant communities by selective removal of tissue, physical disturbance, and they influence nutrient cycling in soils (Hobbs, 1996). In support of this, fall grazing by sheep with the appropriate supplements increases plant diversity in sagebrush steppe ecosystems (Dziba et al., 2007; Petersen et al., 2014). However, use of sagebrush by livestock is constrained by the presence of terpenes, which reduces the amount of plant tissue that animals can eat each day (Dziba et al., 2007). Supplemental macronutrients (e.g., highly digestible carbohydrates, protein) facilitate detoxification of terpenoids, thus mitigating the negative impact of these toxins (Villalba et al., 2002; Petersen et al., 2014).

Another approach to increase intake of sagebrush by herbivores is to utilize locally-adapted animals which have experience consuming sagebrush early in their lives (Petersen et al., 2014).

While understanding animal adaptations to landscapes is an important aspect of the nutritional ecology of ruminants (Demment and Van Soest, 1985; Hofmann, 1989), land managers have not attempted to put these ideas into practice until recently. Instead, many people in agribusiness and livestock production have emphasized production at the expense of profit, without linking animals ecologically to the landscapes they inhabit. Thus, animals have been selected without concern for their abilities to utilize the forage resources in local environments (Provenza, 2008).
Experiences in utero and early in life have life-long influences on herbivores by causing neurological, morphological, and physiological changes that influence foraging behavior (Distel et al., 1994; 1996; Catanese et al., 2010; 2012). By interacting with the genome during growth and development, social and biophysical environments influence gene expression and behavioral responses in mammals (McCormick et al., 2000; Moore, 2002; Dufty et al., 2002). Thus, while the body influences the structure of experience, experience is at the same time influencing the structure and function of the body (Provenza, 1996). These processes, which enable animals to adapt to local diets and habitats, imply that the “absolute fitness value” or “nutritional quality” for a certain unpalatable food may change as a function of an animal’s early experiences with such food (Villalba et al., 2015).

Learning from mother begins early in the developmental process and can have lifelong effects when it comes to forage preferences. Recent research suggests that mothers are a powerful and positive influence before birth. Pregnancy is not an incubation period but a staging period for well-being and disease later in life (DiPietro et al., 2004; Paul, 2010). Better understanding the developmental processes, which takes place in utero and the effects they have later in life may help us create management plans that use grazing animals to their full potential as landscape manipulators.

The concept of fetal programming was first hypothesized for humans using epidemiological data, which suggested that the uterine environment in undernourished mothers altered the long term development, growth, and susceptibility to disease in their offspring (Barker et al., 1993). The role of environmental early events, probably acting as epigenetic factors, on the “programming” of behavioral responses in mammals was then
unveiled for stress responses in rats (Meaney and Szyf, 2005). Since then it has been shown that management of maternal nutrition in livestock influences fetal organ development, muscle development, postnatal calf performance, carcass characteristics, and reproduction (reviewed by Summers and Funston, 2013).

As a management tool, using in utero and early-life programming is a relatively new concept, but offers a faster approach than genetic selection to respond to environmental contingencies in the short-term and potentially increase the herbivores’ ability to consume unpalatable forages. This effect can reduce the competitive ability of toxin-containing plants in the community and allow for greater primary production and diversity. However, information regarding herbivores’ exposure to plant toxins and their subsequent physiological and behavioral responses is limited (Welch et al., 2012). Moreover, no information is available on early life experiences to toxin-containing shrubs, like sagebrush, and their subsequent influence on feeding behavior by herbivores. Thus, the objective of my research was to explore how experience in utero and early in life with sagebrush affected intake of and preference for sagebrush by sheep later in life.
Plant Secondary Compounds:

Historically, in both ecology and agriculture, we have made a distinction between primary (nutrients) and secondary (antiherbivore defense) compounds. Nutrients are involved in the plant’s primary metabolism while secondary compounds act as chemical defenses (Palo and Robbins, 1991). In agriculture, we have selected against secondary compounds because they limit intake of foods grown and fed in monoculture. In ecology, we came to view secondary compounds as defenses against herbivory (Palo and Robbins, 1991). These views notwithstanding, the distinctions between these two categories are becoming increasingly blurred as we come to realize nutrients in too high doses can be toxic and toxins in moderate doses can be beneficial as they can enhance the health and nutrition of consumers (Provenza and Villalba, 2006; Provenza, 2008). These effects will ultimately depend on the dose of each chemical ingested as well as on the type of foods present in the diet (Villalba et al., 2015).

A few of the most common classes of secondary compounds are -- phenolics, alkaloids and terpenes -- each with thousands of compounds. Phenolic compounds, such as lignin and tannins, build organic matter in soil and can provide antioxidant and protein binding activities to consumers (Mueller-Harvey, 2006). Alkaloids are nitrogen-containing ring compounds which increase drought tolerance, pest resistance, tiller numbers and biomass, seed mass and numbers, and germination rates in plants (Hill et al., 1991; Asay et al., 2001). Terpenes are a large and diverse class of carbon-based secondary compounds, produced by a variety of plants, particularly woody species, and biosynthetically derived
from units of isoprene. Monoterpenes consist of two isoprene units whereas diterpenes are composed of four isoprene units (Tholl, 2006).

Monoterpene concentrations are estimated to be roughly 2-6% in varying Artemisia tridentata spp (Kelsey et al., 1982). Concentration of monoterpenes vary throughout the growing season, with concentrations being the lowest in the spring and increasing during the summer months until the flowering stage in fall where levels begin to decline and remain low throughout the winter (Kelsey et al., 1982).

Liver biotransformation helps herbivores ingest plants high in secondary compounds through two different detoxification pathways (Freeland and Janzen, 1974; Dearing and Cork, 1999). In mammals, biotransformation usually occurs in two phases. Phase I introduces a reactive group, such as OH, NH2, COOH, or SH, into the structure of the secondary compound, these secondary compound tend to be more hydrophilic and polar. During Phase II, the newly formed compound is conjugated with endogenous molecules or groups – glucuronic acid, amino acids, sulphates, or methyl groups – that are hydrophilic so the compound can be excreted in the urine and bile (Osweiler et al., 1985). Plant secondary metabolites such as terpenes are processed primarily via the Phase I pathway (Sipes and Gandolfi 1986). While these and other detoxification processes are well known, relatively little research has been conducted to assess the influence of experiences in utero and early in life on the ability of herbivores to ingest –and detoxify– foods with high concentrations of terpenes (Welch et al., 2012).
Experiences *In utero* and Early in Life:

While people know that a young animal learns from its mother, we are beginning to understand that learning from mother begins even earlier in life than after birth, as flavors of foods the mother eats are transferred to her offspring *in utero* and through her milk, thus preparing the developing fetus and neonate for foods it will encounter later in life (Nolte and Provenza 1992). Much of what a female encounters in daily life – air, food, water, and chemicals – are shared in some fashion with the fetus and neonate which uses these experiences as information in the developmental process (Paul, 2010). The ability of an animal to correctly predict its future environment and maintain a developmental trajectory that will match that environment is essential for postnatal survival. Predictive adaptive responses (PAR) are defined as experiences at early stages in life, which cause changes neurologically, morphologically and physiologically, and create behaviors to better adapt a fetus to its postnatal life (Gluckman et al., 2005b). These responses provide the fetus with the benefit of developing a phenotype that matches the environment where it will be reared, as long as the mother’s behavior matches that of the post weaning environment and that the environment does not change drastically in the offspring’s lifetime (Ross et al., 2005; Provenza, 2008).

Environmental stimuli can influence the course of development during many stages *in utero*. The stage at which the stimuli are applied is important in determining the biological system in which the changes takes place. Low birth weights in sheep and pigs were associated with excess progesterone and urea levels during the pre-implantation
Nutrient deficiencies early in gestation can lead to abnormalities in cardiovascular, metabolic, and endocrine function (Fowden et al., 1996; Godfrey, 2002; Myatt, 2006).

Exposure to chemosensory stimuli through maternal diet, especially later in gestation, can influence postnatal behaviors by creating behavioral preferences for the particular chemical later in life (Hepper, 1988; Schaal et al., 1995; 2000; Simitzis et al., 2008). Chemosensitization is a process that involves olfactory, taste and nerve receptors, which work together to produce a single sensation or flavor (Simitzis et al., 2008). Flavors and odors from the mother’s diet are transported to the amniotic fluid which is ingested by the fetus (Mennella et al., 1995; Schaal et al., 1995). For instance, the flavors of plants like onions and garlic are transferred this way, which increases the likelihood that young animals will eat onion and garlic when they begin to forage (Nolte et al., 1992). Animals exposed to oregano essential oil via maternal ingestion ate higher quantities of the oregano test feed compared to control lambs, even when given the option of orange-flavored feed which enhances palatability and is generally preferred over oregano flavored feed (Simitzis et al., 2008). Infants who were exposed to carrot juice in either amniotic fluid or breast milk had fewer negative facial expressions when given carrot flavored rice cereal when compared to infants who had never had experience with carrot juice (Mennella et al. 2001). Lambs exposed to saltbush in utero gained more weight, had higher salt excretion, produced more wool and maintained a higher intake of saltbush than animals that had not been exposed (Chadwick et al., 2009a; 2009b; 2009c). In utero exposure to flavors and odors greatly increases preference later in life (Bilko et al., 1994; Hepper and Waldman, 1992; Hepper and Wells, 2006; Porter and Picard, 1998; Sneddon et al., 1998;
Smotherman, 1982), and if correctly matched with the rearing environment, allows for a safe and natural method of adaption and development (Burdge, 2006; Gluckman et al., 2005a; Hepper and Wells, 2006).

Epigenetics:

Epigenetics refers to heritable changes in gene expression that do not change the DNA sequence and are potentially passed down through several generations. There is indication that epigenetic variation is independent of genetic variation and that in fact epigenetic variation can be induced by environmental factors. There are two main ways in which epigenetics variations are activated: DNA methylation and histone modification of the chromatin structure (Bossdorf et al., 2008; Gicquel et al., 2008). DNA methylation refers to addition of a methyl group to a gene that represses the expression of that gene, while removing the methyl group allows for gene expression. Histone acetylation modifies the chromatin structure that is wrapped around the DNA. Tightly wrapped chromatin does not allow for the gene to be expressed while more loosely wrapped chromatin allows for such expression. (Welch et al., 2012; Reik et al., 2001).

Pesticides can influence gene expression. For example, rats exposed to the insecticide methoxychlor or the fungicide vinclozolin decreased sperm production and increased infertility. Four generations of male pups were adversely affected by this exposure even though no additional generations were exposed to the chemicals (Anway et al., 2005).

Availability of maternal nutrients is crucial in the DNA methylation process and a lack of nutrients can cause deactivation of genes important for development (Burdge, 2006;
Cooney et al., 2002; Gicquel et al., 2008; Waterland and Jirtle, 2003). For instance, a 2003 study demonstrated how a methyl group can activate or inactive a gene used in a strain of mice known as agouti mice. These mice have yellow fur, are overweight and prone to diabetes and cancer, all characteristics attributed to the agouti gene. Researchers questioned whether or not this gene could be deactivated through prenatal nutrition. The researchers running the study fed one group of pregnant females a regular diet and a second group (treatment) a diet high in genistein (a phytoestrogen donor of methyl groups). Pups born to the regular diet group mirrored their parents with fat, yellow bodies and had a tendency to develop the same health problems their parents did. However, the group fed the methyl donor food had brown fur, were slender and healthier than the pups from the first group. It seems that prenatal nutrition had indeed deactivated the agouti gene (Waterland and Jirtle, 2003).

During the Dutch famine that occurred during WWII (1944-1945) epidemiological evidence suggests that maternal undernutrition may have had long-term biological effects on the offspring. Adult offspring that were undernourished during pregnancy had higher incidence rates of schizophrenia (Hulshoff Pol et al., 2000). Men from mothers exposed to famine had higher obesity rates whereas women had higher rates of breast cancer (Roseboom et al., 2006). Not only did the under-nutrition affect the offspring but also the grandchildren (Lumey and Stein, 1997). Thus, despite the availability of food after the famine, several generations were still affected by the events that took place during that time. This supports the idea of maternal nutrition causing epigenetic changes, not only immediately but over several generations.
Plant secondary compounds can negatively affect livestock when ingested at high doses (reviewed by Palo and Robbins, 1991). Herbivores detoxify plant secondary compounds through metabolic pathways, which require additional nutrition to allow their body to alter and excrete the toxin to maintain homeostasis. There is great variation between species of livestock as well as individuals within species in their ability to detoxify plant secondary compounds. Individual variation is thought to be caused by changes in the metabolic capabilities of each individual (Provenza et al., 2003). There is some question as to whether exposure to plant secondary compounds in utero may cause epigenetic changes. These changes would be caused by the chemicals crossing the placental barrier and inducing alterations in the methylation patterns of the DNA and/or promoting changes in chromatin structure which create physiological, morphological and behavioral changes that may enable the individual to detoxify larger loads of secondary compounds. Epigenetic changes that last for several generations may result in animals that are well adapted to forages containing plant secondary compounds and are better able to thrive in environments that would otherwise be unsuitable and/or unproductive for animals that are not adapted (Welch et al., 2012). Epigenetic variation is likely to be altered in the fetus by interaction with the environment through the mother. These changes have the potential to increase tolerance and detoxification of plant secondary compounds. Herbivores that are more readily able to detoxify secondary compounds will be able to better utilize otherwise unavailable nutrients in native range forages that contain high concentrations of these compounds like sagebrush.
Objectives

While there has been some work done to better understand the influence that mother has on the offspring’s foraging behavior (Provenza, 2008), my research attempts to shed light on the importance of early life experiences to a toxin-containing plant like sagebrush by sheep. I expect to show that the fetus is a dynamic and active creature that responds and adapts to the environmental conditions experienced inside its mothers’ body and that such experience helps prepare the individual for the conditions in the outside world (Paul, 2010). Thus, I predict that sheep exposed early in life to sagebrush (in utero and after birth) will consume more of the shrub and display greater preferences than individuals lacking such experience.

Fetal experiences can help create livestock that are better suited for the environment they will be born into. This idea will help steer the future of agriculture and natural resource management from water and soils to plant and herbivores and ultimately to humans in a quest for the development of systems that are better adapted to local environments and as a consequence become more efficient. Thus, my specific objective was to determine whether fetal experiences in utero and early in life with sagebrush by sheep enhance intake of and preference for sagebrush later in life.
MATERIALS AND METHODS

Conditioning

Multiparous mature ewes (Ramboulliét x Columbia x Finn) were held in two separate pens at the Utah State University/ARS research site in Richmond, UT (41.9194° N, 111.8103° W). All procedures were carried out in accordance with the Utah State University Animal Care and Use Committee (IACUC 1389). Throughout the study, ewes and their lambs had ad libitum access to water and trace mineral salt blocks.

In late October 2008, 4 mature rams were selected based on breeding soundness evaluation exams and 2 rams were placed in each pen. Rams were painted with an oil-based brisket paint to monitor breeding/cover rates. Immediately following the addition of rams to each of the 2 pens, all animals in one pen were given access to 50-70 lbs of sagebrush, 2-3 times a week after they had been fed their complete basal diet of alfalfa pellets and barley grain. Sagebrush (Artemesia tridentata spp. tridentata) was cut from surrounding foothills and placed in holding pens during mid-morning and re-assessed the next morning to confirm intake by ewes. Animals in the other pen were not offered sagebrush. Thus, pens only varied in exposure to sagebrush. At approximately 8 weeks of gestation, all ewes were ultrasounded to confirm pregnancy and eighty pregnant ewes (40 exposed to sagebrush; 40 without exposure to sagebrush) continued to receive their respective sagebrush exposure.

In January 2009, due to bad weather conditions, animals were moved to the Green Canyon Ecology Center, Utah State University, Logan, UT (41°45'58.5"N 111°47'14.2"W). In April, 2009 ewes began to lamb. At birth lambs were identified by
ear tags, vaccinated, males castrated and tails docked. Ewes and their lambs were placed in individual pens for 3 days following parturition. On day 4, lambs with their mothers were separated into four groups according to prior and subsequent exposure to sagebrush: 1) no exposure (Control), 2) exposure in utero, 3) exposure in utero and for the first 2 mo of life, and 4) exposure for the first 2 mo of life.

Ewes and their lambs in Groups 3 and 4 were fed their basal diet of alfalfa hay and barley daily along with 50-70 lbs of sagebrush 3 to 4 days a week from April to the end of June. Groups 1 and 2 were kept in a paddock free of sagebrush and fed only alfalfa hay and barley. As animals were group-fed, individual intakes were not recorded. At approximately 8 weeks of age all lambs were weaned. Lambs from all 4 groups were then placed on a common grass pasture until feeding trials began in October 2009.

Testing

The objective of this trial was to determine whether prior exposure to sagebrush by lambs affected intake of sagebrush later in life. Throughout the trial, the amount of alfalfa pellets fed to all lambs was variable across feeding periods while the amount of sagebrush offered to lambs in all 4 groups was presented in ad libitum amounts from 0800 to 1700 daily for 32 days. All sagebrush was collected daily and ground up using a bark shredder. Excess sage was sealed, frozen and used the following day.

Lambs from all groups were moved to individual adjacent pens, measuring 2.4×3.6 m, located outdoors under a protective roof. Lambs, regardless of exposure group, were randomly distributed and assigned individual pens. There were 16, 17, 21, and 19 lambs in the groups 1) no exposure (Control), 2) exposure in utero, 3) exposure in utero and for the
first 2 mo of life, and 4) exposure for the first 2 mo of life, respectively. All lambs were then offered alfalfa pellets in ad libitum as well as ad libitum fresh ground sagebrush for the first 5 days of the trial, from 0800 to 1700. After these 5 days, average individual intake of alfalfa pellets was calculated and for the subsequent 10 days the amount of alfalfa pellets offered was decreased to 75% of the individual average intake per animal. On the following period, the amount of alfalfa pellets was decreased to 50% of the initial intake for 7 days. The amount of alfalfa pellets was then increased to 75% of initial intake and this amount was fed for another 4 days. Pellets were then offered in ad libitum amounts once again for 5 days. Every day at 1700 the refused sagebrush and pellets were weighed and intake calculated and recorded. At day 32 all animals were weighed.

**Statistical Analyses**

Sagebrush and alfalfa intake were analyzed as a split-plot design with lambs (random factor) nested within group. Group (1-no exposure (Control), 2- exposure *in utero*, 3- exposure *in utero* and for the first 2 mo of life, and 4- exposure for the first 2 mo of life) was the between-animal factor and day was the repeated measure in the analysis (fixed factors). Final lamb weight was a covariate in the analysis. All analyses were computed using a mixed-effects model (SAS Inst., Inc. Cary, NC; Version 9.1 for Windows). The variance-covariance structure used was the variance components, which yielded the lowest Bayesian information criterion. The model diagnostics included testing for a normal distribution of the error residuals and homogeneity of variance. Means were analyzed using pairwise differences of least squares means.
RESULTS

Body weights

Lambs had similar body weights by the end of the trial: 44 (SEM = 1.7), 45 (SEM = 1), 44 (SEM = 1), and 43 (SEM = 1.4) kg for the groups 1) no exposure (Control), 2) exposure in utero, 3) exposure in utero and for the first 2 mo of life, and 4) exposure for the first 2 mo of life, respectively.

When lambs’ body weight was used as a covariate in the analyses, no significant effects were observed of the covariate with group (alfalfa intake: group x weight P = 0.27; sagebrush intake: group x weight P = 0.14) suggesting that body weight was similar across groups and that it didn’t bias food intake.

Alfalfa intake

No differences in alfalfa intake were detected among groups of lambs (Group effect; P = 0.24; group x day; P= 0.99). Lambs in groups 1) no exposure (Control), 2) exposure in utero, 3) exposure in utero and for the first 2 mo of life, and 4) exposure for the first 2 mo of life ate on average: 1377 (SEM = 30), 1405 (SEM = 26), 1384 (SEM = 22), and 1362 (SEM = 23) g of alfalfa pellets(SEM = 25 g), respectively. A day effect ( P < 0.0001) was detected as a consequence of the different amounts of alfalfa fed to the lambs during the different feeding periods.

Sagebrush intake

No differences regarding intake of sagebrush were detected among groups when animals had ad libitum access to alfalfa pellets (Fig. 1; P > 0.10). However, a group x day
interaction was observed ($P = 0.003$). When alfalfa pellets were offered at 50% of ad libitum intake, lambs in the group that had only *in utero* experience with sagebrush showed the lowest intakes of sagebrush (days 17-20; $P < 0.05$; Fig. 1). In addition, intake of sagebrush during the second restriction of alfalfa pellets to 75% of ad libitum intake (days 23 to 26) was much greater by all groups of lambs than intake of sagebrush during the first restriction of alfalfa pellets to 75% of ad libitum intake (days 6 to 15; Figs. 1 and 2).

**Figure 1.** Daily intake of sagebrush by four groups of lambs with different degrees of sagebrush exposure early in life. 1) no early exposure (Control), 2) exposure *in utero*, 3) exposure *in utero* and for the first 2 mo of life, and 4) exposure for the first 2 mo of life. Throughout the trial, the amount of alfalfa pellets fed to all lambs was variable across feeding periods (ad libitum; 75% of ad libitum and 50% of ad libitum) while the amount of sagebrush offered to lambs in all 4 groups and for all periods was presented in ad libitum amounts from 0800 to 1700 daily for 32 days.
Figure 2. Total average intake of sagebrush offered ad libitum by four groups of lambs with different degrees of sagebrush exposure early in life. 1) no early exposure (Control), 2) exposure in utero, 3) exposure in utero and for the first 2 mo of life, and 4) exposure for the first 2 mo of life. Intake was measured during different stages of pellet availability 1) Ad libitum pellets, 2) 75% ad lib, 3) 50% ad libitum, 5) 75% ad libitum, and 6) Ad libitum.
DISCUSSION

Early exposure to sagebrush (in utero and for the first 2 months of life) did not have a positive impact on intake of sagebrush by lambs later in life. In fact, lambs exposed to sagebrush in utero showed the lowest intake of sagebrush when availability of alfalfa pellets was restricted to 75% of ad libitum intake. This suggests that in utero exposure to sagebrush decreased sagebrush preference and/or the ability of lambs to consume sagebrush when they were forced to consume the shrub due to a restriction in the amount of alfalfa pellets available. Likewise, sheep that were exposed early in life to a low-quality feed (mature oat hay), later ate less of this feed than sheep that did not experience oat hay early in life (Catanese et al., 2010). Options that are commonly rated as ‘good’ can be perceived as ‘less good’ when experienced closely to access to higher-quality alternatives (Flaherty and Sepanak, 1978). Consistent with this, sheep exposed early in life to an unpalatable feed ‘devalue’ this feed due to continuous comparisons against alternatives of greater quality (Catanese et al., 2011). It is likely lambs exposed in utero to terpenes and other flavors from sagebrush “devalued” this feed when contrasted with ingestion of alfalfa pellets, a feed of much greater quality. Alternatively, exposure in utero to terpenes in sagebrush likely reduced, instead of enhanced, the ability of lambs to detoxify terpenes in sagebrush. Studies in vivo as well as in vitro have shown that toxins are capable of changing the epigenetic pattern in certain cell types, leading to aberrant gene expression profiles in cells and tissues and to disease (Smirnova et al., 2012).

The lack of positive responses of lambs to sagebrush intake as a function of experience could be due to the fact that exposure to sagebrush was not high enough to
cause a permanent change in the animals’ ability to ingest sagebrush. Greater exposure to sagebrush during conditioning (e.g., greater amounts of sagebrush consumed by ewes and lambs) might have enhanced the acceptability of this shrub by lambs later in life. Strong exposure effects have been identified in mammals such that the more frequently a particular food had been tasted, the better it is liked. Thus, the mere exposure effect may play a role in the acquisition and maintenance of food preferences (Pliner, 1982). This idea is supported by results found during testing in this study: Lambs forced to eat sagebrush due to restriction of alfalfa pellets (75% of intake capacity) consumed more sagebrush after the second exposure to that level of restriction than during the first exposure. Ruminants are typically neophobic when offered novel foods but they increase intake of the novel food as they become familiar with such food after a few days of exposure (Burritt and Provenza, 1989; Provenza, 1995). Thus, it appears that exposure to sagebrush during testing and for only a few days days had a more pronounced effect on sagebrush intake than in utero or early in life experiences with the shrub. Nevertheless, an enhancement in sagebrush intake was only observed when the amounts of alfalfa pellets offered were restricted; intake of the shrub was negligible when ad libitum amounts of alfalfa were present either at the beginning or at the end of the study. Similarly, Shaw et al. (2006) observed that when animal density was low and there was high availability of preferred herbs, sheep that were previously conditioned to eat sagebrush due to understory restriction showed similar and very low preference for the shrub as sheep that had only experience with grazing high-quality herbs in the sagebrush understory (Control). However, when the animal density increased and there was a lower probability of encountering the preferred herbs,
conditioned animals displayed a greater selection of sagebrush than animals in the Control group.

In summary early exposure to sagebrush (*in utero* and for the first 2 months of life) did not have a positive impact on intake of sagebrush by lambs later in life. Exposure to sagebrush during testing had a stronger impact on sagebrush intake than *in utero* and after birth experiences. However, such effect was only evident when the amounts, of a high-quality alternative alfalfa, were restricted. When alfalfa was available ad libitum, lambs displayed negligible values of sagebrush intake regardless of the previous level of sagebrush exposure.
CONCLUSION AND IMPLICATIONS

Many sagebrush communities are in late successional stages dominated by mature even-aged shrubs with little recruitment of young shrubs. Sagebrush transpires year-round with less water available for other plant species (Link et al., 1994). Nutrient cycling, plant production, and herbivore nutrition and welfare all are affected because sagebrush contains high concentrations of terpenes, secondary compounds which are toxic to soil and rumen microbes (Oh et al., 1968) and to ruminants (Johnson et al., 1976).

From the presented analysis it follows that in order to improve diversity and productivity in sagebrush steppe ecosystems, decadent shrub stands must be rejuvenated; young, vigorous, shrub-dominated communities with biological and structural diversity are essential for habitat and foraging opportunities for wildlife and livestock. Browsing sagebrush by livestock is a sustainable way to achieve such management goals as less sagebrush leads to increases in soil moisture and added organic matter from urine and feces helps increase herb production and nutrient content in the rejuvenated sagebrush stands (Petersen et al., 2014). However, use of sagebrush by livestock is constrained by the presence of terpenes, plant secondary compounds or chemical defenses which reduce the amount of plant tissue which animals can consume on a daily basis (Dziba et al., 2007).

My thesis was developed with the aim of exploring the possibility of fashioning systems of management in which locally adapted animals use sagebrush as fall and winter forage more efficiently and to a greater extent than animals which are not locally
or fully adapted to sagebrush steppe communities. There is now evidence that such adaptation in mammals can be achieved through epigenetic mechanisms occurring *in utero* and early in life (Bossdorf et al., 2008). These epigenetic mechanisms are based on a set of molecular processes that can activate, reduce or completely disable the activity of particular genes through different processes such as DNA methylation or chromatin structure remodeling. Consequences of such processes represent physiological and/or behavioral changes in consumers which may enhance their ability to adapt to specific local environments. This may be relevant for sagebrush steppe ecosystems as exposure to sagebrush *in utero* and early in life may cause physiological and behavioral changes in herbivores which may lead to a more efficient and greater use of sagebrush without negatively impacting animal health and productivity. Rather than feeding hay on meadows, which many ranchers do during winter, feeding hay on sagebrush-dominated landscapes during fall and winter can facilitate use of sagebrush by locally adapted livestock while enhancing habitat for wildlife (Petersen et al., 2014).

Despite the potential of *in utero* and after birth experiences with sagebrush, my study showed that early exposure to sagebrush did not influence lambs’ use of sagebrush later in life. The experience with sagebrush gained during testing for a few days appeared more consequential than *in utero* and after birth exposure to sagebrush. Moreover, lambs’ prior experience with sagebrush during testing was only relevant when the amount, of high-quality diet alfalfa hay, was restricted. When alfalfa hay was available ad libitum, prior experience with sagebrush did not reveal any effect on sagebrush intake as the amounts of sagebrush consumed by lambs under those conditions were negligible. The
results from my study then suggest that exposing young lambs for several days to sagebrush while restricting the availability of high-quality forage is a viable option which may enhance utilization of sagebrush.
LITERATURE CITED


APPENDIX
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