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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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 *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 *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 *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.

(53 pages)

PUBLIC ABSTRACT

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

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.

LITERATURE REVIEW

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 Gandolfil 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 period. 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 methlyation 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 (Ramboulliet 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).

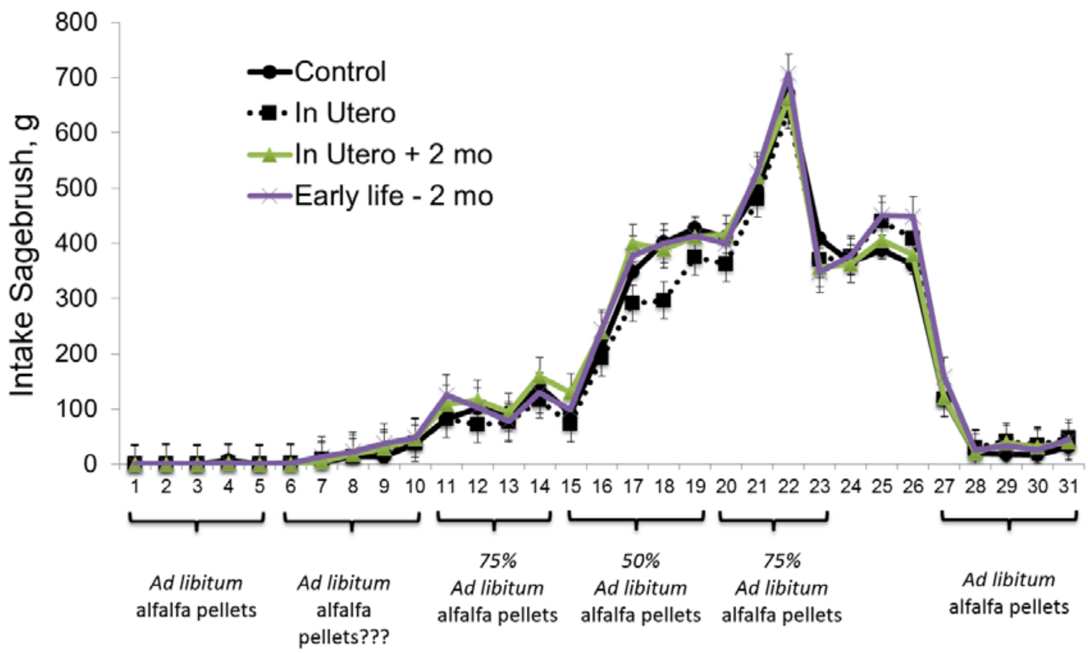

Figure 1. Daily intake of sagebrush by four groups of lambs with different degrees of sagebrush exposure early in life. 1) no early exposure (Control), 2) exposure *in utero*, 3) exposure *in utero* and for the first 2 mo of life, and 4) exposure for the first 2 mo of life. Throughout the trial, the 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.

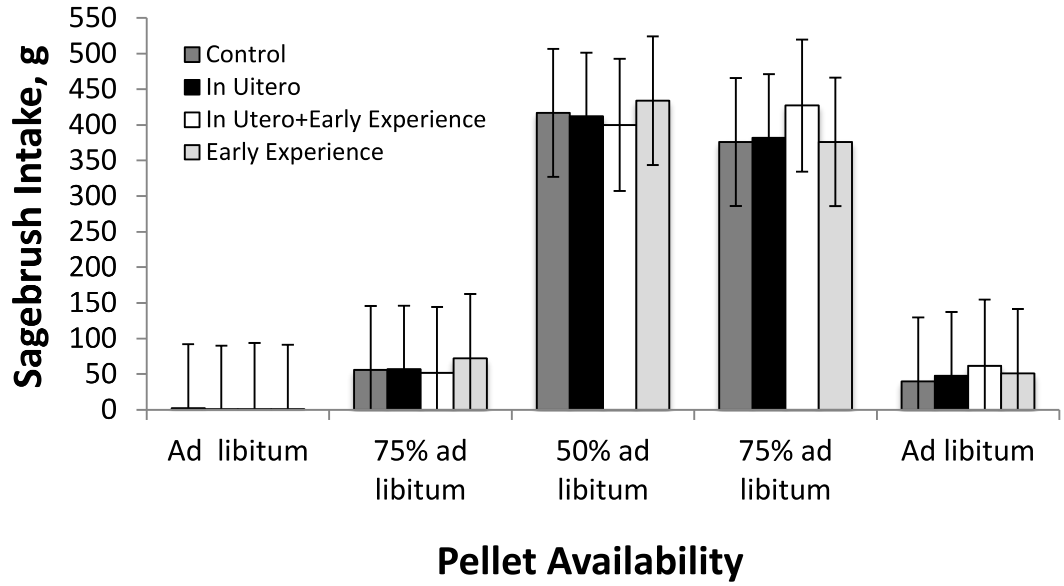

Figure 2. Total average intake of sagebrush offered ad libitum by four groups of lambs with different degrees of sagebrush exposure early in life. 1) no early exposure (Control), 2) exposure *in utero*, 3) exposure *in utero* and for the first 2 mo of life, and 4) exposure for the first 2 mo of life. 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

Anway, M. D., A. S. Cupp, M. Uzumcu, and M. K. Skinner. 2005. Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science. 308:1466-1469.

Asay, K. H., K. B. Jensen, and B. L. Waldron. 2001. Responses of tall fescue cultivars to an irrigation gradient. Crop Sci. 41:350-357.

Barker, D. J. P., C. N. Martyn, C. Osmond, C. N. Hales, and C. H. D. Fall. 1993. Growth *in utero* and serum cholesterol concentrations in adult life. BMJ. 307:1524-1527.

Bilkó, Á., V. Altbäcker, and R. Hudson. 1994. Transmission of food preference in the rabbit: the means of information transfer. Physiol. Behav. 56(5):907-912.

Bossdorf, O., C. L. Richards, and M. Pigliucci. 2008. Epigenetics for ecologists. Ecol. Letters. 11:106-115.

Bryant, J. P., F. D. Provenza, J. Pastor, P. B. Reichardt, T. P. Clausen, and J. T. du Toit. 1991. Interactions between woody plants and browsing mammals mediated by secondary metabolites. Annu. Rev. Ecol. Syst. 22:431-446.

Burdge, G. C. 2006. Homocysteine: a role in fetal programming? British J. Nutr. 96:415-417.

Burritt, E. A., and F. D. Provenza. 1989. Food aversion learning: ability of lambs to distinguish safe from harmful foods. J. Anim. Sci. 67(7):1732-1739.

Catanese, F., R. A. Distel, R. M. Rodriguez Iglesias, and J. J. Villalba. 2010. Role of early experience in the development of preference for low-quality food in sheep. Animal. 4(05):784-791.

Catanese, F., E. Freidin, M. I. Cuello, and R. A. Diste. (2011). Devaluation of low-quality food during early experience by sheep. Animal. 5(06):938-942.

Catanese, F., R. A. Distel, F. D. Provenza, and J. J. Villalba. 2012. Early experience with diverse foods increases intake of nonfamiliar flavors and feeds in sheep. J. Anim. Sci. 90(8):2763-2773.

Chadwick, M. A., P. V. Vercoe, I. H. Williams, and D. K. Revell. 2009a. Programming sheep production on saltbrush: adaptations of offspring from ewes that consumed high amounts of salt during pregnancy and early lactation. Anim. Prod. Sci. 49:311–317.

Chadwick, M. A., P. E. Vercoe, I. H. Williams, and D. K. Revell. 2009b. Dietary exposure of pregnant ewes to salt dictates how their offspring respond to salt. Physiol. Behav. 97(3):437-445.

Chadwick, M. A., I. H. Williams, P. E. Vercoe, and D. K. Revell. 2009c. Feeding pregnant ewes a high-salt diet or saltbush suppresses their offspring's postnatal renin activity. Animal. 3(07):972-979.

Cooney, C. A., A. A. Dave, and G. L. Wolff. 2002. Maternal methyl supplements in mice affect epigenetic variation and DNAmethylation of offspring. J. Nutr. 132:2393S–2400S.

Dearing, M. D., and S. Cork. 1999. Role of detoxification of plant secondary compounds on diet breadth in a mammalian herbivore, Trichosurus vulpecula. J. Chem. Ecol. 25(6):1205-1219.

Demment, M. W., and P. J. Van Soest. 1985. A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. Am. Nat. 125(5):641-672.

DiPietro, J. A., M. M. Ghera, K. Costigan, and M. Hawkins. 2004. Measuring the ups and downs of pregnancy stress. J. Psychosom. Obstet. Gynaecol. 25(3-4):189-201.

Distel, R. A., J. J. Villalba, and H. E Laborde. 1994. Effects of early experience on voluntary intake of low-quality roughage by sheep. J. Anim. Sci. 72:1191-1195.

Distel, R. A., J. J. Villalba, H. E. Laborde, and M. A. Burgos. 1996. Persistence of the effects of early experience on consumption of low-quality roughage by sheep. J. Anim. Sci. 74:965-968.

Dufty, A. M. Jr., J. Clobert, and A. P. Moller. 2002. Hormones, developmental plasticity and adaptation. Trends Ecol. Evol. 17:190-196.

Dziba, L. E., and F. D. Provenza. 2007. Dietary monoterpene concentrations influence feeding patterns of lambs. Appl. Anim. Behav. Sci. 109:49-57.

Dziba, L. E., F. D. Provenza, J. J. Villalba, and S. B. Atwood. 2007. Supplemental energy and protein increase use of sagebrush by sheep. Small Rum. Res. 69:203-207.

Flaherty, C. F., and S. J. Sepanak. 1978. Bidirectional contrast, matching, and power functions obtained in sucrose consumption by rats. Anim. Learn. Behav. 6:313–319.

Freeland, W. J., and D. H. Janzen. 1974. Strategies in herbivory by mammals: the role of plant secondary compounds. Am. Nat. 108(961):269-289.

Fowden, A. L., J. Szemere, P. Hughes, R. S. Gilmour, and A. J. Forhead. 1996. The effects of cortisol on the growth rate of the sheep fetus during late gestation. J. Endrocrinol**.** 151:97-105.

Gicquel, C., A. El-Osta, and Y. Le-Bouc. 2008. Epigenetic regulation and fetal programming. Best Prac. Res. Clin. Endocrinol. Metab. 22:1–16.

Gluckman, P. D., M. A. Hanson, and H. G. Spencer, 2005a. Predictive adaptive responses and human evolution. Trends. Ecol. Evol. 20(10): 527-533.

Gluckman, P. D., M. A. Hanson, and C. Pinal. 2005b. The developmental origins of adult disease. Matern. Child Nutr. 1(3):130-141.

Godfrey, K. M. 2002. The role of the placenta in fetal programming—a review. Placenta. 23(Suppl A):20–27.

Hobbs, N. T. 1996. Modification of ecosystems by ungulates. J. Wildlife Manage. 60(4):695-713.

Hofmann, R. R. 1989. Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia, 78(4):443-457.

Hepper, P. G. 1988. Adaptive fetal learning: prenatal exposure to garlic affects postnatal preferences. Anim. Behav. 36(3): 935-936.

Hepper, P.G., and B. Waldman. 1992. Embryonic olfactory learning in frogs. J. Exp. Psychol. 44(3-4):179-197.

Hepper, P. G., and D. L. Wells. 2006. Perinatal olfactory learning in the domestic dog. Chem Senses. 31:207–212.

Hill, N. S., W. A. Parrott, and D. D. Pope. 1991. Ergopeptine alkaloid production by endophytes in a common tall fescue genotype. Crop Sci. 31(6):1545-1547.

Hulshoff Pol, H. E., H. W. Hoek, E. Susser, A. S. Brown, A. Dingemans, H. G. Schnack, et al. 2000. Prenatal exposure to famine and brain morphology in schizophrenia. Am. J. Psychiatry. 157:1170–1172.

Johnson, A. E., L. F. James, and J. Spillett. 1976. The abortifacient and toxic effects of big sagebrush (Artemisia tridentata) and juniper (Juniperus osteosperma) on domestic sheep. J. Range Manage. 29(4):278-280.

Kelsey, RG, J. R. Stephens, and F. Shafizadeh. 1982. The chemical constituents of sagebrush foliage and their isolation. J. Range Manage. 35:617–622.

Laycock, WA. 1979. Management of sagebrush. Rangeland J. 1(5):207-210.

Link, S. O., W. J. Waugh, J. L. Downs, M. E. Thiede, J. C. Chatters, and G. W. Gee. 1994. Effects of coppice dune topography and vegetation on soil water dynamics in a cold-desert ecosystem. J. Arid Environ. 27(3):265-278.

Lumey, L. H., and A. D. Stein. 1997. *In utero* exposure to famine and subsequent fertility: The Dutch Famine Birth Cohort Study. Am. J. Public Health. 87(12):1962-1966.

McCormick, JA, V. Lyons, M. D. Jacobson, J. Noble, J. Diorio, M. Nyirenda, S. Weaver, W. Ester, J. L. Yau, M. J. Meaney, J. R. Seckl, and K. E. Chapman. 2000. 5'-heterogeneity of glucocorticoid receptor messenger RNA is tissue specific: differential regulation of variant transcripts by early-life events. Mol. Endocrinol. 14:506-517.

Meaney, M. J., and M. Szyf. 2005. Environmental programming of stress responses through DNA methylation: life at the interface between a dynamic environment and a fixed genome. Dialogues. Clin. Neurosci. 7:103–123.

Mennella, J. A., A. Johnson, and G. K. Beauchamp. 1995. Garlic ingestion by pregnant women alters the odor of amniotic fluid. Chem. Senses. 20(2):207-209.

Mennella, J. A., C. P. Jagnow, and G. K. Beauchamp. 2001. Prenatal and postnatal flavor learning by human infants. Pediatrics. 107(6):e88-e88.

Moore, D. S. 2002. The Dependent Gene: The Fallacy of "Nature vs. Nurture". Henry Holt and Company. New York, NY.

Mueller-Harvey, I. 2006. Unravelling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agr. 86(13):2010-2037.

Myatt, L. 2006. Placental adaptive responses and fetal programming. J Physiol. 572:25–30.

Ngugi, R. K., F. C. Hinds, and J. Powell. 1995. Mountain big sagebrush browse decreases dry matter intake, digestibility, and nutritive quality of sheep diets. J. Range Manage. 48(6): 487-492.

Nolte, D. L., and F. D. Provenza. 1992. Food preferences in lambs after exposure to flavors in milk. Appl. Anim. Behav.Sci. 32(4):381-389.

Nolte, D. L., F. D. Provenza, R. Callan, and K. E. Panter. 1992. Garlic in the ovine fetal environment. Physiol. Behav. 52(6):1091-1093.

Oh, H. K., M. B. Jones, and W. M. Longhurst. 1968. Comparison of rumen microbial inhibition resulting from various essential oils isolated from relatively unpalatable plant species. Appl. Microbiol. 16(1):39-44.

Osweiler, G. D., T. L. Carson, W. B. Buck, and G. A. Van Gelder. 1985. Clinical and diagnostic veterinary toxicology. – Kendall/Hunt. Dubuque, IA.

Palo, R. T., and C. T. Robbins. 1991. Plant defenses against mammalian herbivory. CRC Press.

Paul, A. M. 2010 Origins. New York: Free Press.

Petersen, C. A., J. J. Villalba, and F. D. Provenza. 2014. Influence of Experience on Browsing Sagebrush by Cattle and Its Impacts on Plant Community Structure. Rangeland Ecol. Manage. 67(1):78-87.

Pliner, P. 1982. The effects of mere exposure on liking for edible substances. Appetite. 3(3):283-290.

Porter, R. H., and M. Picard. 1998. Effects of early odor exposure in domestic chicks. Reprod. Nutr. Dev. 38(4):441-448.

Provenza, F. D. 1995. Postingestive feedback as an elementary determinant of food preference and intake in ruminants. J. Range Manage. 48(1):2-17.

Provenza, F. D. 1996. Acquired aversions as the basis for varied diets of ruminants foraging on rangelands. J. Anim. Sci. 74(8):2010-2020.

Provenza, F. D. 2008. What does it mean to be locally adapted and who cares anyway? J. Anim. Sci. 86(14):E271-E284.

Provenza, F. D., and J. J. Villalba. 2006. Foraging in domestic herbivores: Linking the internal and external milieu. En: Bels, V.L (ed.) Feeding in Domestic Vertebrates: From Structure to Function. CABI Publ., Oxfordshire, UK. pp. 210-240.

Provenza, F. D., J. J. Villalba, L. E. Dziba, S. B. Atwood, and R. E. Banner. 2003. Linking herbivore experience, varied diets, and plant biochemical diversity. Small Ruminant Res. 49:257-274.

Reik, W, W. Dean, and J. Walter. 2001. Epigenetic reprogramming in mammalian development. Science. 293(5532):1089-1093.

Roseboom, T., S. de Rooij, and R. Painter. 2006. The Dutch famine and its long-term consequences for adult health. Early Hum. Dev. 82(8):485-491.

Ross, M. G., M. Desai, C. Guerra, and S. Wang. 2005. Prenatal programming of hypernatremia and hypertension in neonatal lambs. Am. J. Physiol-Reg I. 288(1):R97-R103.

Schaal, B., P. Orgeur, and C. Arnould. 1995. Olfactory preferences in newborn lambs: possible influence of prenatal experience. Behaviour. 132(5):351-365.

Schaal, B., L. Marlier, and R. Soussignan. 2000. Human foetuses learn odours from their pregnant mother's diet. Chem. Senses. 25(6):729-737.

Shaw, R. A., J. J. Villalba, and F. D. Provenza. (2006). Influence of stock density and rate and temporal patterns of forage allocation on the diet mixing behavior of sheep grazing sagebrush steppe. Appl. Anim. Behav. Sci. 100:207–218.

Simitzis, P. E., J. A. Bizelis, S. G. Deligeorgis, and K. Feggeros. 2008. Effect of early dietary experiences on the development of feeding preferences in semi-intensive sheep farming systems—a brief note. Appl. Anim. Behav. Sci. 111(3):391-395.

Sipes, I. G., and A. J. Gandolfi. 1986. Biotransformation of toxicants, pp. 64-98. Casarett and Doull's Toxicology, The Basic Science of Poisons. Macmillan, New York.

Smirnova, L., A. Sittka, and A. Luch. 2012. On the role of low-dose effects and epigenetics in toxicology. Molecular, Clinical and Environmental Toxicology 499-550. Springer Basel.

Smotherman, W. P. 1982. Odor aversion learning by the rat fetus. Physiol. Behav. 29(5):769-771.

Sneddon, H., R. Hadden, and P. G. Hepper. 1998. Chemosensory learning in the chicken embryo. Physiol. Behav. 64(2):133-139.

Striby, K.D., C. L. Wambolt, R. G. Kelsey, and K. M. Havstad. 1987. Crude terpenoid influence on *in vitro* digestibility of sagebrush. J. Range Manage. 40:244-248.

Summers, A.F., and R. N. Funston. 2013. Fetal Programming: Implications for beef cattle production. Paper presented at Range Beef Cow Symposium XXIII. Rapid City, SD.

Tholl, D. 2006. Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. Curr. Opin. Plant Biol. 9(3):297-304.

Villalba, J. J., F. D. Provenza, and R. E. Banner. 2002. Influence of macronutrients and activated charcoal on intake of sagebrush by sheep and goats. J Anim. Sci. 80(8):2099-2109.

Villalba, J. J., F.D. Provenza, F. Catanese, and R. A. Distel. 2015. Understanding and Manipulating Diet Choice in Grazing Animals. Anim. Prod. 55:261-271.

Waterland, R. A., and R. L. Jirtle. 2003. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol. Cell Biol. 23(15):5293-5300.

Welch, K. D., F. D. Provenza, and J. A. Pfister. 2012. Do plant secondary compounds induce epigenetic changes that confer resistance or susceptibility to toxicosis in animals? Animal farming and environmental interaction in the Mediterranean region EAAP-European Federation of Animal Sciences Volume 131. Springer, NY.

West, N. E. 1993. Biodiversity of rangelands. J. Range Manage. 46(1):2-13.

Winward, A. H. 1991. A renewed commitment to management of sagebrush grasslands. Management in the sagebrush steppe. Agr. EXP. Sta. Spec. 880:2-7.

APPENDIX

Model Information	
Data Set	WORK.SAGEBRUSH
Dependent Variable	Intakesage
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	Levels	Values
Treatment	4	C E IU IUE
Animal	21	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Day	31	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31

Dimensions	
Covariance Parameters	2
Columns in X	320
Columns in Z	73
Subjects	1
Max Obs Per Subject	2263

Number of Observations	
Number of Observations Read	2263
Number of Observations Used	2261
Number of Observations Not Used	2

Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	26575.40026639	
1	2	26053.11361303	0.00000000

Convergence criteria met.

Covariance Parameter Estimates							
Cov Parm	Estimate	Standard Error	Z Value	Pr Z	Alpha	Lower	Upper
Animal(Treatment)	5280.80	999.25	5.28	<.0001	0.05	3762.49	7951.69
Residual	12868	412.31	31.21	<.0001	0.05	12096	13716

Fit Statistics	
-2 Res Log Likelihood	26053.1
AIC (smaller is better)	26057.1
AICC (smaller is better)	26057.1
BIC (smaller is better)	26061.7

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	67	1.81	0.1532
Day	30	1946	1.30	0.1271
Treatment*Day	90	1946	1.48	0.0028
Weight	1	1946	17.80	<.0001
Weight*Treatment	3	1946	1.82	0.1409
Weight*Day	30	1946	6.27	<.0001
Weight*Treatment*Day	90	1946	1.55	0.0009

Least Squares Means							
Effect	Treatment	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment	C		170.23	18.8807	67	9.02	<.0001
Treatment	E		182.01	17.7340	67	10.26	<.0001
Treatment	IU		161.86	19.1569	67	8.45	<.0001

Least Squares Means							
Effect	Treatment	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment	IUE		177.71	16.5275	67	10.75	<.0001
Day		1	0.5445	16.1582	1946	0.03	0.9731
Day		2	0.9635	16.1582	1946	0.06	0.9525
Day		3	0.6021	16.1582	1946	0.04	0.9703
Day		4	2.8575	16.1582	1946	0.18	0.8596
Day		5	0.5640	16.1582	1946	0.03	0.9722
Day		6	1.4266	16.1582	1946	0.09	0.9297
Day		7	7.6092	16.2005	1946	0.47	0.6386
Day		8	16.9939	16.1582	1946	1.05	0.2931
Day		9	26.2457	16.1582	1946	1.62	0.1045
Day		10	42.7089	16.1582	1946	2.64	0.0083
Day		11	99.4917	16.1582	1946	6.16	<.0001
Day		12	98.3842	16.1582	1946	6.09	<.0001
Day		13	84.8062	16.1582	1946	5.25	<.0001
Day		14	136.55	16.1582	1946	8.45	<.0001
Day		15	98.2459	16.1582	1946	6.08	<.0001
Day		16	220.86	16.1582	1946	13.67	<.0001

Least Squares Means							
Effect	Treatment	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Day		17	354.22	16.1582	1946	21.92	<.0001
Day		18	371.83	16.1582	1946	23.01	<.0001
Day		19	406.30	16.1582	1946	25.14	<.0001
Day		20	397.31	16.1582	1946	24.59	<.0001
Day		21	506.60	16.1582	1946	31.35	<.0001
Day		22	669.94	16.2005	1946	41.35	<.0001
Day		23	370.82	16.1582	1946	22.95	<.0001
Day		24	370.44	16.1582	1946	22.93	<.0001
Day		25	421.18	16.1582	1946	26.07	<.0001
Day		26	399.20	16.1582	1946	24.71	<.0001
Day		27	128.96	16.1582	1946	7.98	<.0001
Day		28	23.8917	16.1582	1946	1.48	0.1394
Day		29	33.3984	16.1582	1946	2.07	0.0389
Day		30	27.3394	16.1582	1946	1.69	0.0908
Day		31	41.2497	16.1582	1946	2.55	0.0108
Treatment*Day	C	1	0.3859	33.7021	1946	0.01	0.9909
Treatment*Day	C	2	0.9186	33.7021	1946	0.03	0.9783

Least Squares Means							
Effect	Treatment	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment *Day	C	3	0.4450	33.7021	19 46	0.01	0.9895
Treatment *Day	C	4	6.3555	33.7021	19 46	0.19	0.8504
Treatment *Day	C	5	1.0992	33.7021	19 46	0.03	0.9740
Treatment *Day	C	6	1.9028	33.7021	19 46	0.06	0.9550
Treatment *Day	C	7	3.0770	33.7021	19 46	0.09	0.9273
Treatment *Day	C	8	13.4403	33.7021	19 46	0.40	0.6901
Treatment *Day	C	9	13.8526	33.7021	19 46	0.41	0.6811
Treatment *Day	C	10	38.0302	33.7021	19 46	1.13	0.2593
Treatment *Day	C	11	81.9747	33.7021	19 46	2.43	0.0151
Treatment *Day	C	12	101.72	33.7021	19 46	3.02	0.0026
Treatment *Day	C	13	92.3727	33.7021	19 46	2.74	0.0062
Treatment *Day	C	14	141.04	33.7021	19 46	4.18	<.0001
Treatment *Day	C	15	92.1499	33.7021	19 46	2.73	0.0063
Treatment *Day	C	16	207.74	33.7021	19 46	6.16	<.0001
Treatment *Day	C	17	348.32	33.7021	19 46	10.34	<.0001
Treatment *Day	C	18	401.51	33.7021	19 46	11.91	<.0001
Treatment *Day	C	19	426.27	33.7021	19 46	12.65	<.0001

Least Squares Means							
Effect	Treatment	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment*Day	C	20	410.56	33.7021	1946	12.18	<.0001
Treatment*Day	C	21	495.33	33.7021	1946	14.70	<.0001
Treatment*Day	C	22	671.28	33.7021	1946	19.92	<.0001
Treatment*Day	C	23	409.08	33.7021	1946	12.14	<.0001
Treatment*Day	C	24	364.95	33.7021	1946	10.83	<.0001
Treatment*Day	C	25	387.58	33.7021	1946	11.50	<.0001
Treatment*Day	C	26	361.26	33.7021	1946	10.72	<.0001
Treatment*Day	C	27	118.49	33.7021	1946	3.52	0.0004
Treatment*Day	C	28	19.2987	33.7021	1946	0.57	0.5670
Treatment*Day	C	29	18.3172	33.7021	1946	0.54	0.5868
Treatment*Day	C	30	16.5747	33.7021	1946	0.49	0.6229
Treatment*Day	C	31	31.7379	33.7021	1946	0.94	0.3465
Treatment*Day	E	1	0.5765	31.6553	1946	0.02	0.9855
Treatment*Day	E	2	0.9929	31.6553	1946	0.03	0.9750
Treatment*Day	E	3	0.7145	31.6553	1946	0.02	0.9820
Treatment*Day	E	4	1.5476	31.6553	1946	0.05	0.9610
Treatment*Day	E	5	0.3635	31.6553	1946	0.01	0.9908

Least Squares Means							
Effect	Treatment	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment *Day	E	6	1.4933	31.6553	1946	0.05	0.9624
Treatment *Day	E	7	13.9142	31.6553	1946	0.44	0.6603
Treatment *Day	E	8	22.4441	31.6553	1946	0.71	0.4784
Treatment *Day	E	9	37.2720	31.6553	1946	1.18	0.2392
Treatment *Day	E	10	47.8934	31.6553	1946	1.51	0.1305
Treatment *Day	E	11	125.72	31.6553	1946	3.97	<.0001
Treatment *Day	E	12	103.09	31.6553	1946	3.26	0.0011
Treatment *Day	E	13	76.9158	31.6553	1946	2.43	0.0152
Treatment *Day	E	14	129.17	31.6553	1946	4.08	<.0001
Treatment *Day	E	15	98.7317	31.6553	1946	3.12	0.0018
Treatment *Day	E	16	244.18	31.6553	1946	7.71	<.0001
Treatment *Day	E	17	377.14	31.6553	1946	11.91	<.0001
Treatment *Day	E	18	399.91	31.6553	1946	12.63	<.0001
Treatment *Day	E	19	412.78	31.6553	1946	13.04	<.0001
Treatment *Day	E	20	399.40	31.6553	1946	12.62	<.0001
Treatment *Day	E	21	529.20	31.6553	1946	16.72	<.0001
Treatment *Day	E	22	707.09	31.6553	1946	22.34	<.0001

Least Squares Means							
Effect	Treatment	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment*Day	E	23	347.26	31.6553	1946	10.97	<.0001
Treatment*Day	E	24	377.17	31.6553	1946	11.91	<.0001
Treatment*Day	E	25	450.32	31.6553	1946	14.23	<.0001
Treatment*Day	E	26	448.87	31.6553	1946	14.18	<.0001
Treatment*Day	E	27	157.79	31.6553	1946	4.98	<.0001
Treatment*Day	E	28	25.8730	31.6553	1946	0.82	0.4138
Treatment*Day	E	29	33.7660	31.6553	1946	1.07	0.2862
Treatment*Day	E	30	26.1157	31.6553	1946	0.83	0.4095
Treatment*Day	E	31	44.6201	31.6553	1946	1.41	0.1588
Treatment*Day	IU	1	0.6124	34.1929	1946	0.02	0.9857
Treatment*Day	IU	2	1.0956	34.1929	1946	0.03	0.9744
Treatment*Day	IU	3	0.7676	34.1929	1946	0.02	0.9821
Treatment*Day	IU	4	2.0670	34.1929	1946	0.06	0.9518
Treatment*Day	IU	5	0.3679	34.1929	1946	0.01	0.9914
Treatment*Day	IU	6	1.2676	34.1929	1946	0.04	0.9704
Treatment*Day	IU	7	9.2120	34.5114	1946	0.27	0.7896
Treatment*Day	IU	8	13.8505	34.1929	1946	0.41	0.6855

Least Squares Means							
Effect	Treatment	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment*Day	IU	9	25.0550	34.1929	1946	0.73	0.4638
Treatment*Day	IU	10	37.7538	34.1929	1946	1.10	0.2697
Treatment*Day	IU	11	81.5540	34.1929	1946	2.39	0.0172
Treatment*Day	IU	12	71.9780	34.1929	1946	2.11	0.0354
Treatment*Day	IU	13	75.6992	34.1929	1946	2.21	0.0270
Treatment*Day	IU	14	116.81	34.1929	1946	3.42	0.0006
Treatment*Day	IU	15	72.9464	34.1929	1946	2.13	0.0330
Treatment*Day	IU	16	191.66	34.1929	1946	5.61	<.0001
Treatment*Day	IU	17	291.47	34.1929	1946	8.52	<.0001
Treatment*Day	IU	18	296.54	34.1929	1946	8.67	<.0001
Treatment*Day	IU	19	374.81	34.1929	1946	10.96	<.0001
Treatment*Day	IU	20	363.02	34.1929	1946	10.62	<.0001
Treatment*Day	IU	21	479.69	34.1929	1946	14.03	<.0001
Treatment*Day	IU	22	640.24	34.5114	1946	18.55	<.0001
Treatment*Day	IU	23	370.80	34.1929	1946	10.84	<.0001
Treatment*Day	IU	24	376.54	34.1929	1946	11.01	<.0001
Treatment*Day	IU	25	440.52	34.1929	1946	12.88	<.0001

Least Squares Means							
Effect	Treatment	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment *Day	IU	26	408.19	34.1929	1946	11.94	<.0001
Treatment *Day	IU	27	118.27	34.1929	1946	3.46	0.0006
Treatment *Day	IU	28	29.6507	34.1929	1946	0.87	0.3860
Treatment *Day	IU	29	41.8945	34.1929	1946	1.23	0.2206
Treatment *Day	IU	30	34.9472	34.1929	1946	1.02	0.3069
Treatment *Day	IU	31	48.3371	34.1929	1946	1.41	0.1576
Treatment *Day	IUE	1	0.6033	29.5017	1946	0.02	0.9837
Treatment *Day	IUE	2	0.8470	29.5017	1946	0.03	0.9771
Treatment *Day	IUE	3	0.4813	29.5017	1946	0.02	0.9870
Treatment *Day	IUE	4	1.4599	29.5017	1946	0.05	0.9605
Treatment *Day	IUE	5	0.4254	29.5017	1946	0.01	0.9885
Treatment *Day	IUE	6	1.0427	29.5017	1946	0.04	0.9718
Treatment *Day	IUE	7	4.2338	29.5017	1946	0.14	0.8859
Treatment *Day	IUE	8	18.2407	29.5017	1946	0.62	0.5365
Treatment *Day	IUE	9	28.8033	29.5017	1946	0.98	0.3290
Treatment *Day	IUE	10	47.1584	29.5017	1946	1.60	0.1101
Treatment *Day	IUE	11	108.72	29.5017	1946	3.69	0.0002

Least Squares Means							
Effect	Treatment	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment *Day	IUE	12	116.75	29.5017	1946	3.96	<.0001
Treatment *Day	IUE	13	94.2371	29.5017	1946	3.19	0.0014
Treatment *Day	IUE	14	159.18	29.5017	1946	5.40	<.0001
Treatment *Day	IUE	15	129.16	29.5017	1946	4.38	<.0001
Treatment *Day	IUE	16	239.86	29.5017	1946	8.13	<.0001
Treatment *Day	IUE	17	399.95	29.5017	1946	13.56	<.0001
Treatment *Day	IUE	18	389.37	29.5017	1946	13.20	<.0001
Treatment *Day	IUE	19	411.32	29.5017	1946	13.94	<.0001
Treatment *Day	IUE	20	416.28	29.5017	1946	14.11	<.0001
Treatment *Day	IUE	21	522.16	29.5017	1946	17.70	<.0001
Treatment *Day	IUE	22	661.14	29.5017	1946	22.41	<.0001
Treatment *Day	IUE	23	356.13	29.5017	1946	12.07	<.0001
Treatment *Day	IUE	24	363.08	29.5017	1946	12.31	<.0001
Treatment *Day	IUE	25	406.32	29.5017	1946	13.77	<.0001
Treatment *Day	IUE	26	378.49	29.5017	1946	12.83	<.0001
Treatment *Day	IUE	27	121.28	29.5017	1946	4.11	<.0001
Treatment *Day	IUE	28	20.7443	29.5017	1946	0.70	0.4820

Least Squares Means							
Effect	Treatment	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment *Day	IUE	29	39.6158	29.5017	1946	1.34	0.1795
Treatment *Day	IUE	30	31.7202	29.5017	1946	1.08	0.2824
Treatment *Day	IUE	31	40.3035	29.5017	1946	1.37	0.1721