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Dissertation

Self-Medication in Horses

Submitted by

David Earl Williams

Forest, Rangeland, and Watershed Stewardship Department

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY DAVID EARL WILLIAMS ENTITLED SELF-MEDICATION IN HORSES BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Adviser

Co-Adviser

Department Head/Director

ABSTRACT OF DISSERTATION

Self-Medication in Horses

Horses are known herbivore generalists that must rely upon available plant forage for dietary needs. Diet selection in rangeland herbivores has been shown to be based upon post-ingestive physiological consequences. The basic premise of post-ingestive physiological consequences is the ability of an animal to associate the taste of a particular food with its possible hedonic shift. A negative hedonic shift results in the animal to avoid the taste in future encounters, while a positive hedonic shift results in the animal to seek the taste in future encounters. Thus, taste determines the palatability of plant forages thereby leading to an animal's ability to form a preference for food. Many available plants consumed by horses in natural habitats are known to contain secondary compounds referred to as toxins and all toxins are known to be drugs. Locoweed contains the toxin swainsonine and is known to cause the neurological condition described as locoism in large continuous doses. However, recent studies have shown that swainsonine has medicinal affects in humans and animals. The current study tested four chronically lame horses to examine their ability to form an association of a flavor, either carrots or apples, with a possible post-ingestive physiological consequence induced by a drug. There were two drugs utilized in this study; locoweed that contained swainsonine, and butorphanol tartrate a synthetic opiate analgesic (brand name Torbugesic). The horses were divided into two groups and each group was assigned a respective drug throughout the duration of two separate trials. The first trial associated a flavor with each group's respective drug

treatment and the second trial involved the reversal of flavors while holding the drug treatments constant for each group. Each trial involved a conditioning period followed by test days when horses were challenged to make a decision between the treatment associated flavor or the non-treatment associated flavor. The horses were then challenged with the drugs returned to flavored feeds. The results suggest that horses do have the ability to associate a taste with a post-ingestive consequence induced by a drug. This suggestion gives insight into the horse's possible capability of self-medication.

David Earl Williams
Forest, Rangeland, and
Watershed Stewardship
Fort Collins, CO 80523
Fall 2008

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I would like to dedicate this dissertation to the horse, of which I truly love.

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Introduction

In natural habitats, predator and prey interactions have coevolved adaptive mechanisms to increase their survival. Although there are a few plants that are able to physically avoid predation; there are many that have evolved a number of other techniques to avoid herbivory. Of particular interest to the present study is the common defense of plants that contain toxins. There is a plethora of evidence that toxins affect physiological systems allowing them to be developed and implemented as medicines in both humans and animals. There have been many observational reports of wild animals using natural products medicinally.

It is well known that horses evolved on the ranges of North America and are generalists whose diet consists of a wide spectrum of plant forages. One group of plants that have coevolved with the horse is the locoweed and is known to contain the toxin swainsonine. Swainsonine is produced by a commensal fungus that grows inside the locoweed. Recent studies have shown that swainsonine has medicinal affects in both animals and humans. In large continuous doses, swainsonine causes the condition called locoism. It has even been suggested that locoweed is addicting, intoxicating, or habit forming. However, it has never been examined if horses can consume locoweed in small doses for medicinal effects. All horses in the present study have been deemed chronically lame by a licensed veterinarian. In this thesis, I will take the first steps in examining whether horses use locoweed medicinally. To begin, we will test whether horse's naïve to locoweed can associate the physiological consequences of consumption with a specific taste. We will also begin by testing whether horse's naïve to a synthetic opiate can associate the physiological consequences of consumption with a specific taste.

The Horse

The evolutionary history of the horse, *Equus caballus*, has been well documented (Getty 1975; Janis 1976; MacFadden 1992; Budiansky 1997). Due to the large abundance of bone and especially teeth in the fossil records, the horse is the single most cited paradigm of evolution (Budiansky 1997). In the United States alone there are in excess of a half a million specimens of fossil horses in museums and academic collections. The horse is believed to have been the result of sequential changes in *Hyracotherium*, a dog sized, four toed creature some 55 million years ago. Budiansky (1997) reports that a quarry site in Colorado known as the Castillo Pocket revealed a significant number (24) of *Hyracotherium taperinium* fossils. Today, it is common knowledge that the horse's origin began on the rangelands of North America and continued to evolve for nearly 55 million years. Utilizing mitochondrial DNA analysis, paleontologists now know that *Equus* evolution is full of many branches, blind turns, and dead ends (MacFadden 1992). The modern horse is one of the six remaining species of the genus *Equus*, along with three zebras and two asses.

Like all living organisms, the modern horse is a result of their evolutionary past; mainly attributed to their several-million year diet selection (Janis 1976; MacFadden 1992; Budiansky 1997). It is well known that horses evolved as generalists, cecal fermenters, and specialized in fibrous, low-quality forage, which required vast ranges for ample food supply. These traits allow the horse to eat large amounts of varied plant species both of which change drastically through time and space. As Budiansky (1997) points out; horses "had been victims of their own success". It appears that they were too well adapted to a niche that was disappearing towards the end of the Ice Age (Janis 1976;

MacFadden 1992). Other than the horses that emigrated across the ice laden land bridges spanning the Bering Strait, by 10,000 years ago the horse had become extinct in North America (Budiansky 1997).

In 1494 Christopher Columbus re-introduced the horse to North America (Budiansky 1997); followed by re-introduction of additional horses by the Spanish Conquistadors Cortes in 1519 and Coronado in 1540, respectively. The American Colonists were also known to re-introduce horses from England to the eastern seaboard in 1747. Horses were used mainly for transportation, warfare, speed, and power amplification during the first few hundred years of the United States history. At the end of the Civil War in 1865, with the introduction of the steam engine and the combustion engine to the United States, the usefulness of the horse had dwindled. With the abundance of "jobless" horses remaining many horses were turned out in fenceless pastures, some escaped fenced pens for "greener pastures", and some were simply let loose and set free. These horses eventually migrated to uninhabited areas of little value at the time such as the vast rangelands of the United States. Therefore, after a nearly 10,000 year hiatus the horse had been re-introduced to the land of their origins. Although their origins were the rangelands of the United States the "wild horses" or "mustangs" of today are considered feral.

The environment shapes the organism by placing selective pressure on both the phenotype and the genotype. Over the past 55 million years the horse has endured many different and changing environments thus has changed morphologically (Figure 1). It has been suggested that the morphological changes in the history of the horse can be accounted for by the neo-Darwinian theory of microevolution: genetic variation, natural

selection, genetic drift, and speciation (Futuyama 1986). Along with the many changes in the selective pressures placed upon the horse it would be expected that as the

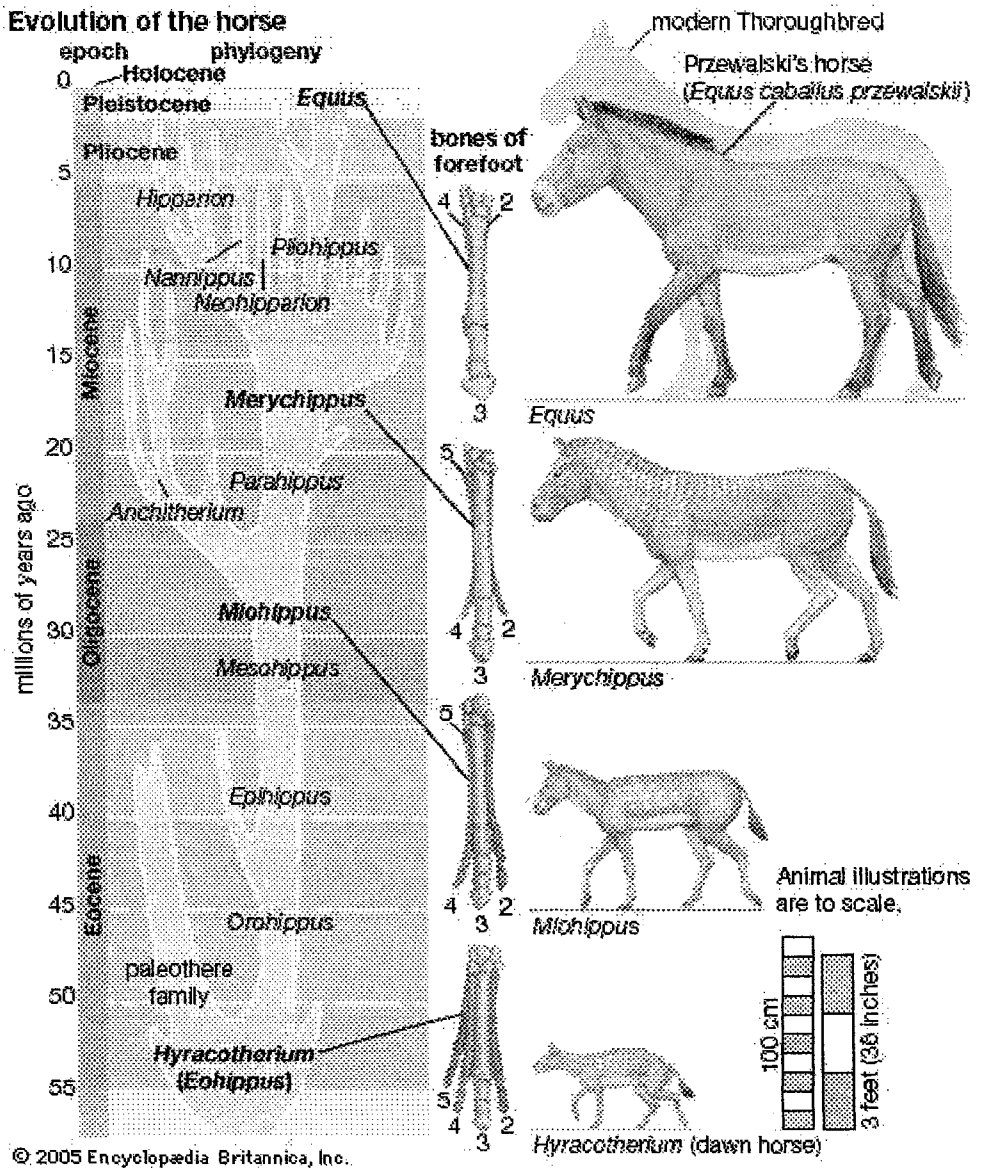


Figure 1. A display showing the evolution of the horse over the past 55 million years. Note the numbered toes in the fore foot illustrations trace the gradual transition from a four-toed to a one toed animal. The horse has changed morphologically and has increased in size.

environment changed the diet selection changed as well. It is well known that behavior is a phenotype and that a phenotype is the result of a genotype. Therefore, as genetic

changes were evolving the behavior; diet selection was changing according to the available forages. What this means is that horses have had 55 million years of practice in evolving diet selection methods. The result of the many environmental changes placed upon the phenotype and genotype is the modern day horse.

Today, the modern wild horses (mustangs) that roam our rangelands are still confronted with constant environmental changes that effect their individual diet selection. The mustangs are constantly faced with hourly changes, weekly changes, seasonal changes, and all these changes can vary from year to year throughout an individual horse's life. In addition to the environmental changes the horse must endure, their individual dietary needs are constantly changing as well. Horse's diets change in response to aging, reproduction, morphology, and physiology which includes illnesses and the overall health of the animal (Howerey 1998; Engel 2002; Huffman 2003; Provenza 1995; Villalba 2006). Therefore, the horse has endured millions of years of dietary selection experience covering a large spectrum of circumstances. The horse, *Equus caballus*, should be an excellent candidate offering a wealth of possibilities for behavioral studies involving self-medication through diet-selection processes. Despite the wealth of possibilities the horse may offer to behavioral studies there is very little systematic research that has emerged from the current literature pertaining to self-medication through diet selection (Cooper 2007).

The Locoweed and Locoism

Taxonomically, locoweeds belong to the genera *Oxytropis* and *Astragalus*, and are phenotypically considered to be weeds. There are reports that have identified 22 species of *Oxytropis* and 300 species of *Astragalus* inhabiting North America (Allison

1984), and most locoweeds are known to be cool season (C3), perennial, and native (Stubbendiek et al. 2003). Generally speaking, locoweeds characteristically germinate and become established during warm, wet, fall seasons. Thereby, allowing the locoweed to remain green over the winter season, and thus enabling an accelerated growth rate during the early spring season. The plants flower during mid- April to early-August; the fruits mature between June and October, followed by the release and germination of seed thus repeating the yearly cycle of reproduction by seed (Stubbendiek et al. 2003). It is very likely that locoweed can be identified on many grazing rangelands throughout the world (Nielsen et al. 1988).

Swainsonine is the known active ingredient of interest in locoweed and is not directly produced by the locoweed. The fungal endophyte *Embelisia* sp. is known to produce swainsonine and this particular endophyte has been isolated from locoweeds (Braun et al. 2003). This particular swainsonine-producing fungal endophyte has been strongly correlated with the locoweed toxicity (Braun et al. 2003; Gardner et al. 2003). When *Astragalus* and *Oxytropis* plants are grown without the fungi it has been shown that they lose their toxicity (Romero et al. 2003). This strongly suggests there may possibly be a symbiotic relationship between locoweed and *Embelisia* sp..

Many species of the genera *Oxytropis* and *Astragalus* are known to cause the chronic neurological disease described as locoism (McLain-Romero et al. 2004). The two most harmful native rangeland weeds responsible for locoism are woolly locoweed, *Astragalus mollissimus* Torr., used in this study (Figure 2), and silky crazyweed,

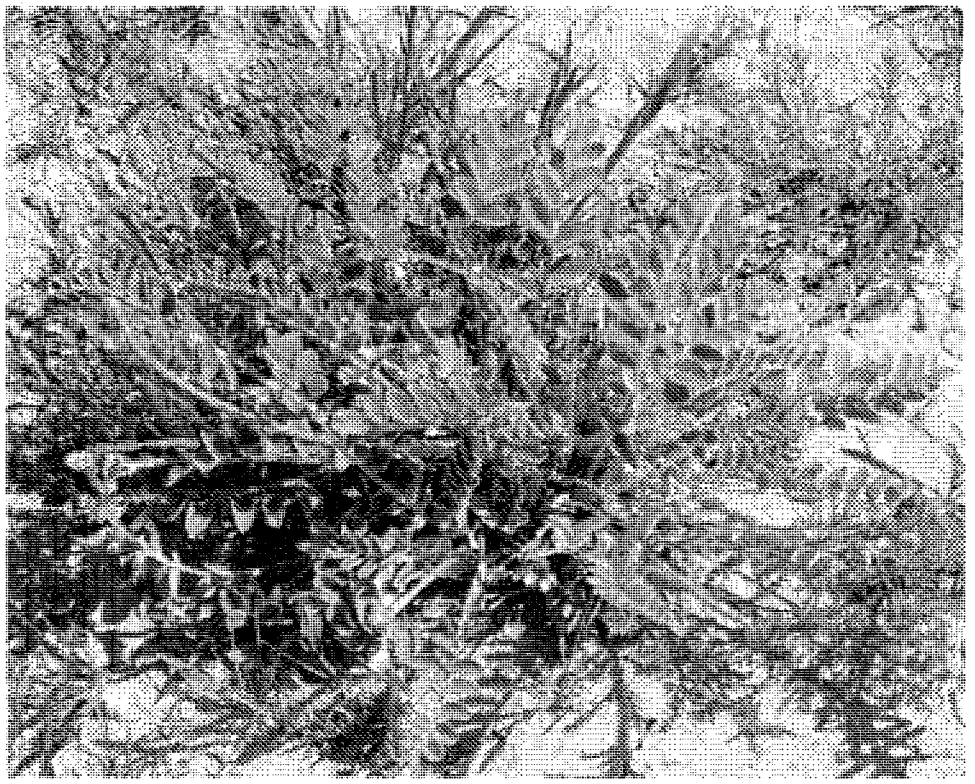


Figure 2. Picture depicting live Woolly locoweed (Astragalus mollissimus var. mollissimus) utilized in this study. The locoweed in this study was dried and cured. *Oxytropis sericea* Nutt. (Formerly referred to as *Astragalus lambertii*) (Thompson et al. 1995).

Locoism is known to occur in domestic livestock including cattle, sheep, goats, and horses. It is also well known that wildlife such as elk, antelope, deer, and feral horses are intoxicated by locoweed consumption (Nielsen et al. 1988). There is very little known why livestock graze locoweed (Ralphs et al. 1986). Stubbendiek et al. (2003) suggests that the forage value of locoweed is poor to worthless for livestock and wildlife; is generally unpalatable and consumed only when other forage is not available. In contrast, Ralphs et al. (1988) report that the mean crude protein (CP) content in locoweed falls

somewhere between 12% and 17%, and the forage value is considered to be good. In addition, Ralphs et al. (1986) adds that livestock will continue to consume locoweed even when good quality green feed is available. In a study of 4 naive horses exposed to locoweed for the first time, the horses ate relatively little dry grass even when it was abundant and as a result of locoweed consumption for 2 weeks displayed depression (Pfister et al. 2003). Pfister et al. (2003) reports that after 6 weeks of exposure 2 horses had to be euthanized and all 4 naive horses exhibited clinical signs of locoism. When the study was finished all horses were severely poisoned, thin, and in poor condition (Pfister et al. 2003).

Plant Adaptations to Herbivores

A very basic cornerstone in Darwin's theory of evolution is the idea that as important resources are utilized they may become critically short in supply (Krohne 1998). Plants have been competing with other plants, both interspecifically and intraspecifically, for limited resources for literally billions of years thus coevolution has occurred. Not only have plants been competing with other plants they have had to deal with the possibility of being consumed by herbivores. Animals have the ability to move to "greener pastures" when resources are low or food selection is lacking. Individual plants do not have this ability to move and must live their entire life with the same view. This implies that all necessary resources that enable a plant to complete lifecycles must be present during establishment or "delivered" to their residence. In addition, because of the sessile nature of plants they are vulnerable to herbivory. Krohne (1998) suggests that in the same manner as carnivorous predators; herbivores constitute an important selective

force on their prey. Predators and prey mutually influence one another's evolution (Purves & Orians 1983). Therefore, plants and the animals that feed on them interact in an evolutionary relationship.

Plants have evolved two basic anti-herbivore adaptation strategies: (1) structural adaptations; and (2) chemical adaptations. A very obvious structural adaptation for deterring grazing is the production of spines and thorns. It is also known that structural elements incorporated in plant tissues deter grazing. For example, McNaughton et al. (1985) suggest that large numbers of grasses incorporate silica into their stem and leaf tissue thus rendering the plant tissue less palatable in addition to wearing the grazers teeth down. A good example of coevolution between a plant and an herbivore is the horse, *Equus caballus*, which in response to grasses incorporating silica into stem and leaf tissue to wear the horse's teeth down, the horse as a species has responded by producing teeth that grow continuously. McNaughton et al. (1985) suggest that this particular plant evolutionary adaptation to herbivory is very precise: populations of grasses that have been subjected to heavy grazing pressures have higher silica concentrations. This specific predator/prey interaction example has become widely referred to by many authors as the "evolutionary arms race"

The other major grazing deterrent put forth by certain plants is in the form of chemical substances known as secondary compounds. Secondary compounds were thought to represent "by-products" or "waste products" of plant biochemical pathways with functions unknown that happen to be toxic to herbivores (Laycock 1978; Krohne 1998; Pfister 1999; Engel 2002). An alternative view was suggested over 40 years ago that these compounds are not derived from metabolic by-products but rather are evolved

de novo in response to grazing as a selection pressure (Ehrlich & Raven 1964). Stamp (1992) suggests that most ecologists have come to accept the Ehrlich & Raven hypothesis most likely because biochemical evidence indicates that these secondary compounds are regularly anabolized and catabolized intracellularly and are not the by-products of other pathways. At present there are approximately one hundred thousand different secondary compounds that have been identified and they form the bulk of nature's pharmacy (Engel 2002).

Plants are known to produce a wide array of compounds and numerous strategies to deliver them to their predators. According to Krohne (1998) these strategies can be broken down into three main groups: (1) repellants; (2) toxins; and (3) hormone/pheromone mimics. Repellents deter predators from feeding on the plant and may even deter insects from laying eggs in the plant. Tannins for example are common secondary compounds utilized by numerous plant species that results in the leaves unpalatable and/or less digestible to herbivores. Toxins take this a step further; rather than simply repelling the herbivore, they prevent grazing by causing mortality of the predator. *Trifolium repens*, a white clover, is a good example of this method. This particular plant's tissues contain cyanogenic glycosides which are essentially sugar compounds bound to cyanide, a known toxin (Krohne 1998). When grazing damages the plant tissue there are two special enzymes released that separate the cyanide from the sugars thus releasing this highly toxic substance into the digestive tracts of the grazer. Swainsonine fits into this group of toxins and its mode of action will be discussed and described later in this paper. Secondary compounds are found not only in angiosperms

and gymnosperms but even in the most primitive plants such as cyanobacteria suggesting a very long history of plants utilizing chemical defense mechanisms against herbivory.

The third group, hormone/pheromone mimics, produce compounds that mimic the compounds of their attackers. An excellent example of this method of defense can be observed in the potato, *Solanum berthaulthii*. The potato synthesizes a component of an alarm pheromone released by aphids when attacked by a predator. The potato simply releases this compound which elicits a flight response in the aphid (Krohne 1998). Another interesting example of plant defenses of herbivory has been suggested by Rhoades (1985) that reports when a single Sitka willow tree, *Salix sitchensis*, in a stand is attacked by tent caterpillars, it and other individuals in close proximity decrease the leaf nutritional value. Rhoades (1985) was able to demonstrate in chamber experiments that when the first plant was attacked other individual plants in close proximity with no physical connection also decreased nutritional quality. Rhoades (1985) attributes a message sent via pheromone release as a possible signal of inter-plant communication.

Coevolution: The Horse / The Locoweed

Although there are many unanswered questions as to the quantity of locoweed consumed, the numbers of individual horses that consume locoweed, or the frequencies of consumption, there should be no doubt that the horse and the locoweed have evolved together on the rangelands of North America; horses eat locoweed, locoweed is eaten by horses. This implies that the horse has placed selective pressures on the locoweed; and the locoweed has placed selective pressures on the horses that consume them thus coevolution. The long-term effects (4-6 weeks) of locoweed consumption on individual horses has become common knowledge; locoism. However, the short-term effect of

either high doses or low doses, at present, has not been thoroughly investigated. It is most likely that many horses that consume locoweed go undetected until full locoism is observed. It is possible that many horses eat small amounts of locoweed over long-term or short-term durations, while some may eat large amounts for short-term durations, without visible changes in physical or behavioral traits, thus going undetected. The effect upon the locoweed as a result of grazing by herbivores is currently unknown and not fully understood.

The lack of any overwhelming evidence that poisonous plants always have an advantage over their predators, and the lack of any overwhelming evidence that herbivores have an advantage by developing mechanisms that always operate to prevent poisoning by these plants, strongly indicate that coevolution has taken place (Laycock 1978). Due to coevolution, the horse nor the locoweed has a clear cut "advantage"

Thirty years have passed since Laycock (1978) recognized that the evolutionary history of poisonous plants, the toxic compounds in the plants, or the animals that graze these plants had received little attention. However, due to the increasing interest in plant-animal interactions there has been an increasing amount of research involving coevolution. For example, Huffman (2003) has recently reported that it was most likely very early in the coevolution of plant-animal relationships that some arthropod species began to use the chemical defenses of plants to protect themselves from their own predators and parasites. This implies self-medication may have a long coevolutionary history.

Swainsonine: The Toxin

It is well known that swainsonine, an indolizidine alkaloid (1,2,8-trihydroxyoctahydroindolizidine) is the chemical structure (Figure 3)

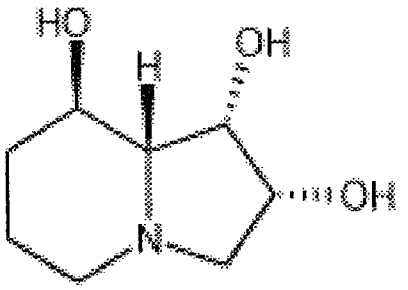


Figure 3. The molecular structure of swainsonine is $C_8H_{15}NO_3$ with a molecular weight of 173.21. Swainsonine is the known substance responsible for locoism. Swainsonine may also have medicinal properties.

responsible for inducement of locoism when consumed by grazing herbivores (Molyneux & James 1982; Tulsiani et al. 1984). According to McLain-Romero et al. (2004) swainsonine is a potent inhibitor of mannosidase, an enzyme known to be responsible for the breakdown of mannose, in both the Golgi apparatus and the lysosome. The swainsonine molecule has the affinity and thus the ability to bind to active binding sites located on the enzyme mannosidase. When this occurs; mannose, the normal substrate for the enzyme, is unable to bind because the active binding site has been blocked by swainsonine and mannosidase can no longer catalyze the break down of mannose. This results in an intracellular accumulation of the sugar mannose. The intracellular vacuoles, where the normal enzymatic break down of mannose occurs, become swollen organelles (lysosomes). When this happens in cells of the Central Nervous System (CNS) the build up of mannose in lysosomes may result in cellular death. When cellular death (neurons)

occurs what is essentially happening is that normal neural transmission has been altered or even stopped. Therefore, whichever neural circuits involved would be compromised and the clinical signs upon presentation could vary dramatically. The clinical signs mostly observed in locoed ungulates include, but are not limited to, ataxia, low head carriage, apparent blindness, salivation, seizures, decreased coordination and mobility, followed by severe weakness in the effected animal (El-Hamidi & Leipold 1989). The severe weakness and the decreased mobility are most likely responsible for the inablity of the animal to fulfill its daily needs (i.e., eating and drinking), thus resulting in death. Locoism is the long term effect of swainsonine as a toxin when consumed by horses.

Other detrimental effects of swainsonine on grazing herbivores has been reported such as birth defects, reproduction problems, congestive heart failure, edema, stunted growth, and a general loss of body condition, however, not all animals are known to die (Nielsen, 1978; Ralphs et al. 1986; Nielsen et al. 1988). Swainsonine is known to cross the blood brain barrier, and the placental barrier indicating further and possibly unknown effects of swainsonine on grazing herbivores. Furthermore, swainsonine has been found in the milk of lactating females thus passing it on to their developing offspring (Taylor & Strickland 2002). At present whether or not these effects of swainsonine are short term or long term is unknown and/or not fully understood.

Swainsonine: The Medicine

Many drugs for human and animal use have been derived from plant substances. Cocaine and opium just to name a couple have made their way into medical history. Medicinal uses for marijuana are currently being investigated. The three most widely

used psychoactive drugs in the United States today are alcohol, nicotine, and caffeine (Julien 2005); all derived from plants. Swainsonine is currently under heavy investigation for medicinal uses in humans (Figure 3). Recall that swainsonine is a known mannosidase inhibitor. It also inhibits mannosidase II, which is an enzyme involved in the processing of glycoproteins on the surface of cancer cells (Pyne 2005). Pyne (2005) suggests this process has been associated with cancer metastasis and thus swansonine analogues are potentially useful anti-metastasis drugs for treatment of cancer.

Klein et al. (1999) suggest that swainsonie might effect the sensitivity of human cells to other drugs, making it a useful adjuvant in several types of human chemotherapy. When mice were administered with known lethal doses of the chemo-therapeutic drug doxorubicin, followed by administration of swainsonine, the mice were protected from the doxorubicin-induced lethality (Oredipe et al. 2003). In more recent reports by Oredipe et al. (2003), swainsonine **pre-treatment** to doxorubicin protected mice against death induced by a lethal dose of doxorubicin, resulting in the prolonged survival of mice. Swainsonine allows an increased dosage of the chemo-therapeutic drug doxorubicin to be used thereby increasing the effectiveness of the drug.

The bone marrow stimulatory properties of swainsonine have been well documented (Klein et al. 1999, Oredipe et al. 2003). Klein et al. (1999) demonstrated that swainsonine increased both total bone marrow cellularity and the number of circulating white blood cells in mice treated with doses of AZT (3'-azido-3'-deoxythymidine) a highly used drug in the treatment of acquired immune deficiency syndrome (AIDS).

It is clear that swainsonine may have many medicinal uses yet to be discovered. Previously mentioned effects attributed to swainsonine such as birth defects, reproduction problems, congestive heart failure, edema, stunted growth, and general loss of body condition; the fact that swainsonine readily crosses the blood brain barrier and the placental barrier; plus the well known description of locoism, all point to the varied effects of swainsonine. In strong agreement with Pfister (1999), Stegelmeier (2007 personal communications) reiterates that The difference between a toxin and a medicine is the dosage (Pfister 1999; Stegelmeier 2007; personal communication). Even water can be a lethal toxin at extremely high levels. The varying effects of swainsonine may be a result of high doses or low doses (with duration involvement), possibly dictating whether swainsonine becomes a toxin or a medicine. At present the exact dosages that are responsible for either effect remains unclear. The evidence clearly suggests that swainsonine the drug can affect numerous physiological systems at the cellular level; thus the systems level; hence resulting in physical and/or behavioral effects upon the entire organism thus providing a medicinal affect.

Butorphanol Tartrate: The Drug

Butorphanol tartrate, is also known by the brand name Torbugesic (Figure 4). Butorphanol tartrate is a totally synthetic opiate, centrally acting, narcotic agonist-antagonist analgesic with potent antitussive (cough) activity (Fort Dodge Drug Insert). The antagonist action of butorphanol tartrate is on the mu opiate receptor. Butorphanol tartrate binds to the mu receptor thus "antagonizing" or blocking the action of the receptor (Percio et al. 1976). Butorphanol tartrate as an agonist binds to the kappa and

the sigma opiate receptors thus stimulating the receptors thus producing the analgesic and antitussive properties (Percio et al. 1976). Percio et al. (1976) has demonstrated that

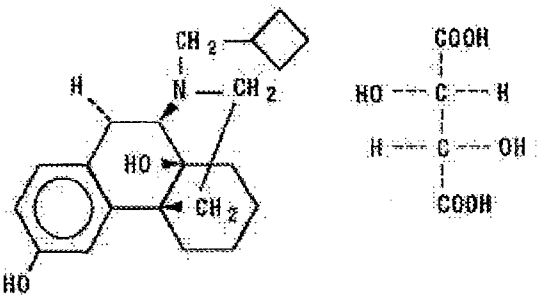


Figure 4. Butorphanol tartrate is a synthetically derived opioid agonist-antagonist analgesic of the phenanthrene series. The chemical name is (–)-17-(cyclobutylmethyl)morphinan-3,14-diol D- (–) - tartrate (1:1) (salt). The molecular formula is $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$, which corresponds to a molecular weight of 477.55. Torbugesic was used in this study.

butorphanol tartrate is up to 4 times more potent than morphine, a well known opiate. Butorphanol tartrate, the injectable form, is widely used in horses for its analgesic and sedative effects (Colburn 2007; personal communication). The tablet form of butorphanol tartrate is mainly used for the analgesic and antitussive effects in dogs (Colburn 2007; personal communication). The tablet form of butorphanol tartrate was used in the present study. The main reason that this particular drug was chosen for the present study is that the dosage is well known, the mode of action is well known, the effect is well known (analgesic), and that it is a synthetic opiate that has been described to induce the common knowledge behavior of addiction. This particular drug is approved for use in horses and is an excellent drug to compare to an unknown drug such as swainsonine.

Diet Selection

In the natural world many animals are faced with a variety of foods some of which they are able, and prepared, to eat (Emmans 1991). Thus diet selection enables a wild animal to utilize the limited range of plant foods to which the specific animal is adapted (Moss 1991). Plant foods may differ in their nutritional value, succulence, fiber contents, chemical content, and morphological features such as spines and thorns, and all these are likely determining factors to palatability (Emmans 1991; Provenza et al. 1992). Howery et al. (1998) suggests that the traditional definition of an animal to "relish" a particular plant as plant forage is *palatability*. Thus palatability is usually called a "plant characteristic" (Provenza 1995, Howery et al. 1998). *Preference* is traditionally defined as an animal's relative consumption of one plant over another when given free choice at a particular time and place (Howery et al. 1998). Preference is referred to by many as an "animal characteristic" (Frost and Ruyle 1993; Howerey et al. 1998). Rangeland herbivores thus have the ability to assess rangeland forages based on palatability, and select a required diet within their particular environment based on preference. Moss (1991) suggests that an individual presumably shows preferences based on archetypes which ensured the genetic survival of its progenitors. This thought by Moss (1991) implies that an animal chooses foods which maximize its genetic factors. Due to the fact that herbivores select forages that meet their needs and avoid forages that do not is remarkable given that palatability and preferences change seasonally and by location, both among and within plant species (Provenza 1995; Howerey et al. 1998). There should be no doubt that the diet-selection problem that an animal in the wild faces is often one of great complexity (Emmans 1991). Diet selection is definitely a very complex problem that the animal has to solve.

Diet selection has been traditionally placed into two categories: *specialists* and *generalists*. An excellent example of a vertebrate specialist is the koala bear (*Phascolarctos cinereus*) which are known to eat only a few species of eucalyptus leaves (Moss 1991; Engel 2002). The eucalyptus leaves are low in nutrients, contain a large proportion of indigestible cellulose and lignin, and are full of toxic chemicals (Moss 1991). As a result the koala does show a "specialized" detoxification mechanism thus a specialist (Moss 1991). In addition, the koala often lives its entire life in areas less than a couple of acres and feed on only a few individual trees in an environment with little environmental change (Engel 2002). Moss (1991) suggests that the main advantage of a specialized diet is that specialists can develop morphological and metabolic means of exploiting defended resources not available to generalists. However, Moss (1991) point out that when environmental conditions change, generalists are better able to adapt, while specialists tend to fall victim to extinction much more easily. As previously stated the horse is a generalist herbivore known to eat a wide variety of foods that change drastically over time and space. A mixed diet may be necessary to produce a balanced diet i.e. one plant providing nutrients which are lacking in another (Moss 1991). Furthermore, a mixed diet may be obligatory for the generalists eating defended forages since the animal's detoxification mechanisms may not be able to cope with a large dose of one toxin (Moss 1991). Thus, no matter how ironic it may sound, a generalist must be "specialized" in selecting certain amounts of certain forages in an ever changing environment especially when considering the diverse plant species available. Emmans (1991) states that from a nutritionist point of view of the complex mixture of such a large number of essential nutrients and potential energy needed by an animal it would require

about fifty dimensions to give it a sufficient description; dimensions a figure could not visualize.

How do animals solve the complex problem of diet selection with the large number of variables involved? Provenza et al. (1992) proposed a process termed *postingestive feedback* to demonstrate how animals regulate forage intake. Animals regulate their forage intake according to whether the postingestive feedback is a "positive feedback" (e.g. macronutrients) or a "negative feedback" (e.g. toxins) (Provenza et al. 1992;Howerey et al. 1998). Animals may change their "preference" for various forages in response to changes in the plants "palatability" (Provenza et al. 1992, 1995). For example, if forages become more or less palatable the forages will become more or less preferred in accordance with postingestive feedback (Howerey et al. 1998). If an animal "adjusts" or "changes" its preference to a food based upon postingestive feedback the animal has "learned" from the consequences of what they eat (Engel 2002, Villalba et al. 2006). Thus, in recent years the term "postingestive feedback" has become synonymous with "postingestive consequences" (Cairns et al. 2002; Engel 2002; Villalba et al. 2006). The process of an animal changing preferences for what they eat is known as a **hedonic shift** (Provenza 1992, 1995; Howerey et al. 1998, Engel 2002).

Provenza et al. (1992) contends that two interrelated systems mediate shifts in food intake through postingestive feedback from the gut to the brain which include: 1) the affective system (subconscious); and 2) the cognitive system (conscious). It is through these two systems that the senses of taste, smell, and sight are linked with postingestive feedback but in functionally different ways (Howerey et al. 1998) (Figure 5).

The affective processes allow the animal to associate the taste of their forages with the positive or negative postingestive effects and form either conditioned preferences or conditioned aversions (Provenza et al. 1992; Howerey et al. 1998). For example, if forage produces a negative postingestive feedback (i.e. nausea, inadequate nutrients, or malaise) the animal may acquire conditioned aversions. On the other hand if the forage produces a positive postingestive feedback (i.e. the sensation of being full or fulfilling a desire) the animal may acquire conditioned preferences.

The cognitive processes allow an animal to integrate the senses of taste, smell, and sight to discriminate among forages that result in the animal to make a "conscious" choice to either select or avoid a food based on prior experience with the food's postingestive affect (Provenza et al. 1992; Howerey 1998) (Figure 5). For example, if a certain food resulted in a negative feedback consequence, such as malaise, its taste becomes undesirable and the animal uses its senses of smell and sight to "avoid" it in the future. On the other hand, if a certain food previously induced a positive feedback consequence (i.e. satiety) the particular taste becomes desirable and the animal uses its senses of smell and sight to "seek" it in the future.

It should be clear that central to the schematic presented by Provenza et al. (1992) (Figure 5) the processes that govern diet selection is taste. All arrows lead directly or

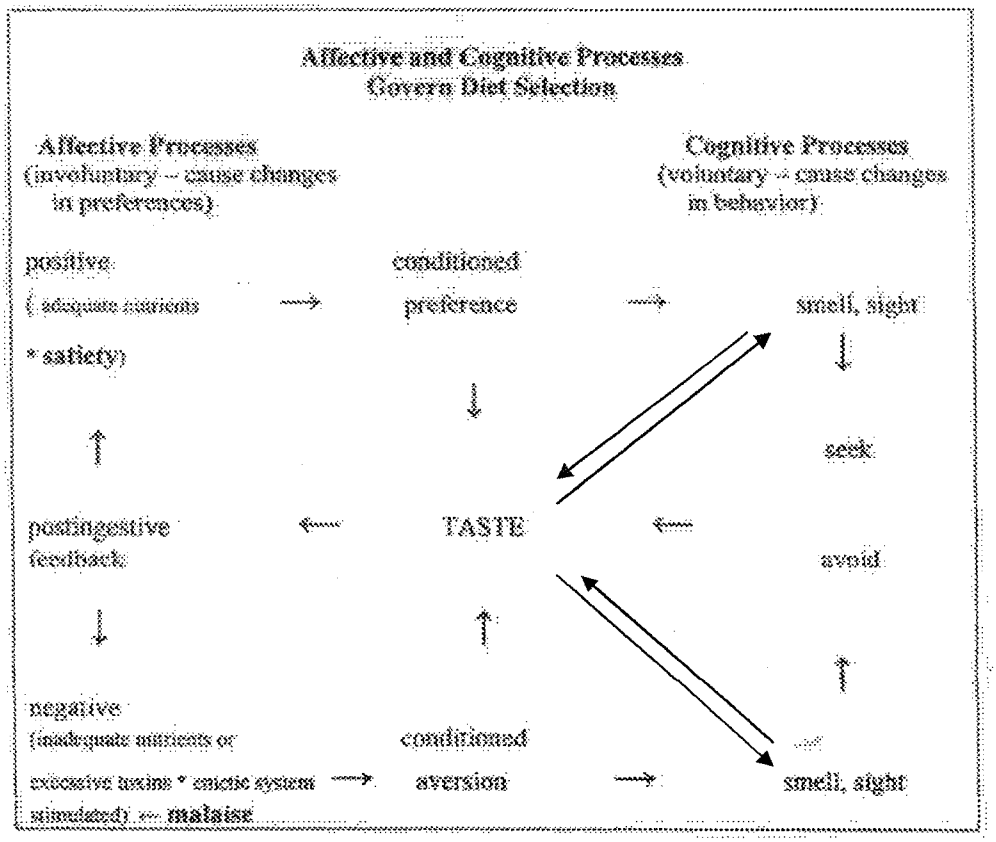


Figure 5. Schematic representation of affective and cognitive processes in diet selection. The affective system links the taste of food with its postingestive feedback (positive and negative). The cognitive system integrates the senses of taste, smell, and sight which animals use to seek or avoid foods in accord with positive or negative postingestive feedback. There is an iterative exchange of information between these systems which allows animals to modify their foraging behavior in response to changing forage conditions, and in response to changing nutritional needs. (Adapted with permission from Provenza et al. 1992).

indirectly to taste. Glendinning (2007) clearly states that the taste system involved in orosensory mechanisms serve as the final arbiter of whether a substance should be swallowed. In natural settings if a substance is not swallowed a postingestive consequence could not be possible. Although not included in the schematic it would be expected that if a drug produced "satiety" utilizing the definition of "fulfilling a desire", the drug would fit into the category of "positive postingestive feedback". Methods utilized by Villalba et al. (2006) support this suggestion in that postingestive

consequences (both negative and positive) are responsible for the self-medicative behaviors in sheep.

Self-Medication

In recent years there has been an increasing amount of evidence that has given great momentum to the study of self-medication in animals referred to as "zoopharmacognosy" (Villalba et.al. 2006; Huffman 2003; Engel 2002; Clayton & Wolfe 1993). It is well known that grazing animals usually select diets composed of the many plant species that are available. Some plants on the rangelands contain only toxins while others are strictly nutritional. There are some plants that contain toxins but also contain varying amounts and kinds of nutrients (Pfister 1999). It is well known that there are many nutrients, substances, and behaviors that are necessary for an animal to achieve homeostasis. The homeostatic mechanisms are known to be physiological thus they involve the processes and functioning of the body. For example, should the external temperature begin to increase a horse may begin to sweat which triggers the capillaries near the surface of the skin to dilate resulting in the cooling of the blood. In addition, evaporation (of the sweat) is known as a cooling process thus the two processes working together may stabilize the internal temperature. However, if the mentioned physiological changes are not successful in the rebalance of the internal temperature the horse may change its behavior by searching for shade, increasing water intake, or even lying in cool water. Although this example is overly simplified it does show how physiology and behavior may interact to maintain homeostasis and thereby maintain health (Engel 2002). There are many other examples of how physiology and behavior interact to maintain health; there is one that is of particular interest and that is what Engel (2002) has termed

the "illness response behaviors". Behavioral changes that are only taken in response to health disruptions that involve the animal's use of a substance not self produced, in such a manner as to rectify malaise, thus defining self-medication (Engel 2002; Villalba 2006).

Clayton & Wolfe (1993) suggest that "not all pharmacists are human". Many research studies involving animal health and welfare have assumed that animals passively endure pathogens, diet, and the varied environmental conditions that they must survive (Engel 2002). However, adaptive behavioral strategies that result in a reduction of physiological health threats from injury, poisons, and pathogens; through natural selection, has been reported (Engel 2002; Hart 1990; Clayton & Wolf 1993). It has been suggested that physiological feedback processes match the animal's dietary choice with consequences (Engel 2002; Pfister 1999). Engel (2002) reported that sheep will increase protein consumption in preference to carbohydrates to compensate for nutritional losses, and are able to learn the consequences of their dietary choices resulting in preferences for foods that previously corrected a nutrient deficiency. Provenza (1995) reported that goats will shift consumption of the same plant (blackbrush) from current season growth with higher nutritional value to older plants that are less nutritional apparently to avoid the higher tannin contents of the current plant growth that adversely affected the animals. Kodiak bears (*Ursus arctos*) chew the roots of *Ligusticum spp.*, spitting the resulting mixture of saliva and juice onto their paws to rub on their fur (Clayton & Wolf 1993). *Ligusticum* is routinely used by humans against viral and bacterial infections (Delgado 1988, as reported in Clayton & Wolf 1993). Navajo legend holds the bear in high regard for teaching them the medicinal powers form the *Ligisticum* root. It has been observed that birds will place ants in their beaks and rub the ants on their skin because the

thermogenic properties of formic acid soothes the skin during feather moult and replacement (Potter 1970), which may suggest that medicinal substances as analgesics may be as common in animals as it is in humans (Clayton & Wolf 1993). Danbury et al. (2000) reports that broiler chicks, which normally avoid bitter tasting medicated food, will consume it when broken limbs produce pain; highly suggesting that gustatory/olfactory tolerances change with health status. Many other reports of wild animal's self-regulating diet selection and utilization of natural conditions to self-medicate have been observed including cattle, mice, elephants, many species of birds, insects, and numerous species of other mammals; including humans (Engel 2002; Julien 2005; Clayton & Wolf 1993). One of the most compelling examples of self-medication emerges from the scientific studies conducted on the African great Apes (Huffman (2003). The great apes consume plants in their diets known to be rich in secondary compounds of a non-nutritional nature, many times containing known toxins, strongly suggesting medicinal properties from their ingestion (Huffman 2003). Huffman (2003) also adds that chimpanzees and humans that co-exist in the sub-Saharan Africa are both known to ingest the bitter pith of *Vernania amygdalina* for the control of nematode infections. In support of this report, phytochemical studies have demonstrated a wide array of biologically-active properties in this medicinal plant species (Huffman 2003). Most reports of self-medication in animals provide evidence by observing sick animals seen consuming substances that are usually not part of their normally ingested diets that contain substances that may be capable of improving the animal's health (Engel 2002; Huffman 2003; Villalba et. al. 2006). Lozano (1998) suggests that while the many

observational and correlative studies reported are consistent with the self-medication hypothesis they do not establish cause and effect.

Of particular interest is a more recent investigational study performed by Villalba et al. (2006). In this study, Villalba et al. (2006) examined whether sheep were able to select specific protective chemical substances to recuperate from ingesting known malaise-inducing food substances. Villalba et al. (2006) randomly chose three treatment groups of lambs and placed each group into separate pens. Each of the three treatment groups were conditioned to consume foods and toxins (grain, tannins, oxalic acid) that led to known distinctive negative internal states. The lambs in each group were then allowed to eat a substance known to rectify each state (sodium bentonite, polyethylene glycol, and dicalcium phosphate, respectively). Control lambs ate the same foods and medicines but were disassociated temporarily so as not to recuperate from the induced sickness. After the conditioning period was complete each individual treatment group of lambs were fed the same illness inducing foods and toxins as before, however, in this trial the lambs were offered all three medicines at once. Each treatment group of lambs were able to choose the correct substance that attenuated their specific sickness. The control animals never changed there pattern of use of the medicine. Thus, Villalba et.al. (2006) investigation supports the self-medication hypothesis with more rigor and numerical evidence thereby clearly demonstrating cause and effect. Villalba et al. (2006) have clearly shown that when sheep are induced with specific illnesses of known origins (cause) the sheep were able to select the appropriate substance known to rectify the illness through postingestive consequences (effect).

Although there are many reports of self-medication in numerous other species there has yet to emerge any reports addressing the possibility that horses may be self-medicating. According to Engel (2002), "Animals do not need to 'know' what is missing from their diet in order to remedy that deficiency." For example Engel (2002) points out that when rats are deprived of the amino acid thiamine that they don't seek out thiamine specifically but sample many different foods until they find what makes them feel better. Provenza (1996) adds that "a positive feedback results in animals seeking out particular plants (e.g."ice-cream plants"), whereas negative feedback causes animals to avoid specific plants." This strongly suggests that there must be an orosensory mechanism of pairing a taste to a post-ingestive consequence (Cairns et al. 2002). In addition, recall that horses are generalists and through evolutionary time have most likely sampled many, many species of grasses and forbs that reside on our rangelands in North America both temporally and spatially. It is most likely that through time horses have sampled all species of grasses and forbs (including locoweed) that are native to the rangelands. Hence, if Provenza et al. (1992), Provenza (1995, 1996), Pfister et al. (1997, 1999), Howery et al. (1998), Engel (2002), and Villalba et al. (2006) are correct that animals can "learn" by consequences; and Rittenhouse (2005 personal communications) is correct that "Diet selection is not random", then it is very possible that horses may be self-medicating during diet selection through the process of postingestive consequences.

Taste Associations

It would be expected that if an animal has the ability to self-medicate that the animal must know what taste is associated with a specific physiological consequence.

This strongly suggests that the animal must learn and remember which plant resulted in which consequence thereby involving learning and memory. As previously described, post-ingestive consequences most likely play a major role in diet selection of herbivores (Provenza et al. 1992; Howerey et al. 199;, Engel 200;, Villalba et al. 2006).

It should be clear that many animals have the ability to associate a taste with a hedonic shift; both negative (avoidance) and positive (preference). This has been demonstrated experimentally by Garcia et al. (1955). Garcia et al. (1955) added saccharin, a sweet tasting artificial sweetener, to the water of rats. The rats were offered one drinking bottle with plain tap water while simultaneously being offered one drinking bottle with tap water containing saccharin. The rats showed a marked preference for the saccharin containing water. The rats were then exposed to gamma radiation which was/is known to cause gastrointestinal disturbances and allowed to drink only the saccharin containing water. After the conditioning period was completed associating saccharin with irradiation treatment, the rats were offered both the plain tap water and the saccharin containing water. The treatment rats changed their previous preference to saccharin to avoidance of saccharin. The non-irradiated control rats retained their preference to saccharin containing water. This experimental procedure clearly demonstrated that rats can 1). form a preference based upon a positive feedback taste, and 2). reverse their preference to avoidance by the negative feedback caused by known gastrointestinal disturbances induced by gamma irradiation. Today, this phenomenon has become known as "The Garcia Effect".

Since Garcia et al. (1955) demonstrated an animal's ability to form taste preferences, followed by a reversal to avoidances induced by physiological

consequences, there have been numerous articles published in scientific journals (Laycock 1978). The phenomenon is also commonly referred to as "conditioned taste aversion" or "learned food aversion". The very basic premise of this phenomenon as proposed by Gustavson (1977) is that when an animal consumes a flavored food and subsequently becomes ill, that particular animal will avoid or drastically reduce consumption of that flavor upon later encounters. Due to the plethora of publications on the topic of food selection and aversive conditioning, an entire book (Barker et al. 1977) has been published and a complete discussion cannot be presented here.

Cairns et al. (2002) have clearly demonstrated that horses have the ability to associate a taste with a post-ingestive physiological consequence based upon energy densities of the feed thus leading to a preference. In this particular study Cairns et al. (2002) began by feeding two groups of horses (n=6/group) a choice of an iso-energetic pellet mixture with either mint flavor or garlic flavor added. Both groups were then manipulated by pairing one flavor with a high energy density feed and the other flavor with a low energy density feed; opposite flavors and densities for both groups. During a brief seven day period all the horses were returned to the iso-energetic feed with no flavors added. Then Cairns et al. (2002) reversed both the flavors and energy levels of both groups. As a result, all horses in both trials, regardless of the flavor, preferred the flavor, that had been associated with the high energy density feed when offered the iso-energetic feed with a cue flavor. Cairns et al. (2002) study strongly suggests and provides experimental evidence that horses have the ability to associate a taste with post-ingestive consequences induced by differing energy levels. In addition, the study clearly

demonstrates that horses can change their preferences for foods as their associated energy levels are altered.

Redgate et al. (2006) have further added evidence that horses can change their preferences due to post-ingestive consequences. In this study horses were fed diets rich in one of three energy sources: protein, hydrolysable carbohydrate or fat. The results of the first trial displayed equal intake of the three energy sources. However, the results of the second trial showed a shift away from the fat rich diet. Selection of the three diets were altered after a period of consumption to each diet; strongly suggesting that horses have the ability to modify their diet on the basis of post-ingestive feedback.

Self-Medication in Horses ?

Once again, despite the large number of reports of self-medication in numerous other species there has yet to emerge any reports addressing the possibility that horses may be self-medicating on locoweed, especially when considering reports that horses are more prone to eating locoweed and are particularly more susceptible to its intoxicating effects than other species (Pfister et al. 2003). During the early 1900's, Marsh (1909) described various animals including mules, pigs, and antelope to be "addicted" to locoweed. Lewin (1931) suggested that livestock became "addicted" to locoweed much in the same fashion as "the morphinist his morphia." In addition, Lewis (1931) suggested animal addictions to the Australian plant *Swainsona* long before it was known that swainsonine was the same "toxin" in both *Swainsona* and locoweed, and declared locoweed as the most famous of the "addictive" plants. Recall that Pfister et al. (2003) introduced four horses, naïve to locoweed, into a pasture where locoweed was present.

The horses had began eating locoweed in the study area by the second day and generally increased locoweed consumption over time even when there was an abundance of dry grasses. The horses showed a clear preference for locoweed, and by the fifth week all four horses were anorectic and behaviorally unstable. Is locoweed addictive? At present, the answer to this question is unknown and/or not fully understood.

Many authors generally refer to addiction as an animal's craving for a particular plant or compound (Pfister et al. 2003). Psychologist often prefer the term as "self-administration" to describe the behavior of animals pursuing a chemically-enhanced sense of well being in the pharmacological sense. With the abundance of toxin containing plants available to many animals it is likely that animals sometimes self-administer these plants for the pharmacological effect (Siegel 1979). In contrast, it has been reported by Ralphs et al. (1990) that dried, ground locoweed was not addictive, however, animals did habituate or become accustomed to eating the plant. In summary, locoweed has been described as addictive, intoxicating, and habit forming. These reported behavioral properties of locoweed add creedence to the choice of butorhanol tartrate, a synthetic opiate, as an excellent choice for a comparative examination.

Hypothesis

Recall that all toxins are drugs and that the dosage is the determining factor as to whether a chemical substance is categorized accordingly. Do horses self-medicate by consuming locoweed? It would be expected that if horses have the ability to self-medicate that the horse must be able to associate a taste with a post-ingestive physiological consequence induced by a drug. There have been no previous studies

addressing the possibility that horses have the ability to self-medicate by consuming a drug. The current study will begin testing whether a horse has the ability to associate a taste (both apple and then reversed to carrot or vice-versa) with a substance of unknown short term effects or dosages (locoweed). The current study will also begin testing whether a horse has the ability to associate a taste (both apple and reversed to carrots or vice-versa) of the well known synthetic opiate Torbugesic (butorphanol tartrate). A comparative examination of the two drugs, swainsonine and butorphanol tartrate, will then be presented.

Hypothesis: Horses have the ability to associate a taste with a post-ingestive physiological consequence induced by a drug.

Null Hypothesis: Horses do not have the ability to associate a taste with a post-ingestive consequence induced by a drug.

Materials & Methods

This pilot study was conducted at the Vessels Stallion Farm located at 5280 West Lilac Road in Bonsall, CA. 92003. Four chronically lame horses, housing, and feed were supplied by the Vessels Stallion Farm. The attending Veterinarian was Dr. Steven V. Colburn D.V.M. of Creekside Veterinary Services located at 8751 Old Castle Road, Escondido, CA. 92026. Dr. Steven V. Colburn is a licensed veterinarian in the State of California. This pilot study was approved by the Institutional Animal Care and Use Committee (IACUC) at Colorado State University, Fort Collins, Colorado.

Four chronically lame horses of which two were Quarter horses registered with the American Quarter Horse Association (AQHA), one Thoroughbred registered with the American Jockey Club (AJC), and one Paint horse registered with the American Paint Horse Association (APHA). All horses were randomly lead (in random order) to the test location and each horse was placed in a 24ft. by 24ft. test corral temporarily. Each horse had a ranch neck tag identification number upon arrival to the test area. All assignments for horse number, group number, corral assignments, and treatment were made by writing numbers on index cards and drawing them from a container. Using this method, horses and treatments were randomly assigned. When this was completed each horse was referred to as Horse #1, Horse #2, Horse #3, and Horse #4 (Table 1).

Table 1. Horses used in the current study.

Horse & Corral Number	Group Number	Horse Breed	Horse Age	Horse Sex
Number 1	Group 1	Quarter Horse	16	Gelding
Number 2	Group 1	Thoroughbred	17	Mare
Number 3	Group 2	Quarter Horse	15	Gelding
Number 4	Group 2	Paint Horse	14	Gelding

Each corral was clearly posted with the number. Therefore, Horse #1 and Horse #2 are referred to as Group # 1 the locoweed treatment group and Horse #3 and Horse #4 are referred to as Group #2 the Torbugesic treatment group (Figure 6 and Figure 7). All random assignments were for both Trial # 1 and Trial # 2.

A random toss of the coin was performed as to which 5 gallon feed tub was to be used for the treatment: the left 5 gallon feed tub or the right 5 gallon feed tub. The result of the coin toss was that the treatment was to be fed in the left 5 gallon feed tub and referred to as Feed Tub #1; the non-treatment was to be fed in the right 5 gallon feed tub and referred to as Feed Tub #2. There was only one coin toss for feed tub side so all four horses were to receive their treatment in the left placed feed tub throughout both trials. The right placed feed tub was used throughout the procedure as the non-treatment feed tub.

Two flavors were chosen for this pilot study: grated carrots and grated apples. These flavors were chosen because most horses are known to like both flavors. Since there were two flavors and two groups a toss of the coin was used. Group #1 received

carrots with the locoweed treatment and apples for the non-treatment during Trial #1. Group #2 received apples for the Torbugesic treatment and carrots for the non-treatment during Trial #1. During Trial # 2 the flavors were reversed from Trial # 1, while holding the treatments constant for both groups. Group #1 received locoweed treatment throughout both trials and Group # 2 received Torbugesic treatment throughout both trials.

A coin was then tossed as to whether the treatment was to be administered on Day #1 or if the non-treatment was to be used on Day #1. The result was that the treatment was to be used on Day #1 and this was to be for both groups for both Trial #1 and for Trial #2 (see Figure 6,Figure 7, Figure 8,and Figure 9).

Basal Diet and Housing

All horses were fed orchard grass ad libitum, and each horse had an automatic drinking water supply container that was checked and cleaned on a daily basis, insuring all test horses free access to both orchard grass and water throughout the entire experiment. Each horse was fed approximately 10 pounds of alfalfa twice a day; once in the morning and once in the evening. Each horse had an above ground permanent feeder placed in their individual pen. During conditioning days and test days the morning alfalfa feeding was withheld until approximately two hours after consuming their treatment and non-treatment grain/flavor mixtures. This was done for two reasons 1) to prevent any possible adverse effects such as inability to chew, salivate, choke, or swallow that would have endangered the horses safety induced by the drug treatments, and 2) to allow

enough time as to not associate the alfalfa with any possible hedonic shift from the treatment/non-treatment. Between Trial #1 and Trial #2 when no grain or treatments were fed, the morning alfalfa feeding was done at approximately the same time as the conditioning days and tests days, for consistency of the time throughout the entire experiment. All four horses were free to move around their pens and exercise freely as each individual horse desired. All horses were housed in outside corrals and were at least 200 meters away from any other animals. No one was allowed to enter the test area except me and the attending veterinarian Dr. Steven V. Colburn D.V.M.. All care was provided by me.

Trial Number 1: Treatments & Non-Treatments

Locoweed Treatment: A standard 1 gallon coffee can was used to mix the locoweed, the grain mixture, and the flavor. Locoweed was finely ground by hand (mostly leaves) and filled the bottom 1/4 of the can. Then the grain mixture was added as to leave approximately one inch empty at the top of the can. The flavors (either carrots or apple) were grated by hand on a standard kitchen cheese grater. This usually took four regular sized carrots or three medium sized apples both purchased from a local fruit market about every three days. The top inch of the can was then filled with the grated flavor selected. The full can mixture was then poured into individual 5 gallon feed tubs and mixed by hand. This procedure insured that each locoweed horse received the same total volume of feed daily whether it was carrots or apples. Each horse had a new 5 gallon feed tub, black, and used the same tub throughout the two trials. Each 5 gallon

feed tub was rinsed and cleaned after every feeding by hand with only clean water and air dried.

Locoweed non-treatments: A standard 1 gallon coffee can was filled 3/4 full with the grain mixture and then approximately one inch of grated flavor was added either carrots or apples depending on the day. The mixture was then poured into a new 5 gallon non-treatment feed tub, black, and identical to the 5 gallon treatment feed tub. The mixture was then mixed by hand. This procedure insured that each horse received the same volume for the non-treatment day. Each 5 gallon feed tub was washed and cleaned after every feeding with only clean water and air dried. **NOTE**: The amount of the grain in the non-treatment feedings was slightly more than the treatment feedings. The amount of flavor added to the treatment and non-treatment grain feedings was held constant. The reason for this slight grain adjustment difference was to compensate for the difference in total volume due to the volume of locoweed displaced on non-treatment days.

Torbugesic treatment: A standard 1 gallon coffee can was used to mix the Torbugesic, the grain mixture, and the flavor. The grain mixture was added as to leave approximately one inch from the top empty. The flavors (either carrots or apples) were grated by hand on a standard kitchen cheese grater. This usually took four regular sized carrots or three medium sized apples both purchased from a local fruit market about every three days. The top inch of the can was then filled with the grated flavor selected. The full can mixture was then poured into the individual horses 5 gallon feed tub. Then two 5mg tablets of Torbugesic were powderized in a wooden mortar and pestle and added to the mixture in the feed tub. The mixture was then mixed by hand until the the Torbugesic was believed to be completely and evenly mixed into the feed. This

procedure insured that each Torbugesic horse received the same total volume of feed daily whether it was carrots or apples. Each horse had a new 5 gallon feed tub, black, and used the same tub throughout the two trials. Each 5 gallon feed tub was cleaned after every feeding by hand with only clean water and air dried.

Torbugesic non-treatments: A standard 1 gallon coffee can was filled to within approximately one inch from the top with the grain mixture and then approximately 1 inch of grated flavor was added either carrots or apples depending on the day. The mixture was then poured into a new 5 gallon non-treatment feed tub, black, and identical to the 5 gallon treatment feed tub. The mixture was then mixed by hand. This procedure insured that each horse received the same volume for the non-treatment day. Each 5 gallon feed tub was washed and cleaned after every feeding with only clean water and air dried.

Feeding Procedures

Preparation for both the treatment feed and the non-treatment feed was performed out of sight, out of sound, and out of olfactory range of test horses. The 5 gallon feed tubs were then placed in an enclosed vehicle and delivered to the test horse corrals. Each day the two 5 gallon feed tubs/horse were placed directly in front of the pens corresponding to the Horse number. Each 5 gallon feed tub was clearly labeled on the bottom side of the tub. The 5 gallon feed tubs were randomly placed each day in a different order so as horses were not fed in the same order every day. This was done daily by placing numbers on index cards and drawing from a container. However, the order of placement was the order of feeding so as each horse had to wait the same amount of time

before being fed as the others did depending on the placement order. For example, if the placement order was so that the tubs were placed in front of Horse #3, then #2, then #1, then #4, they were then fed in that order. This insured that no horse was fed first each day or last and the waiting period every day for feeding was randomly assigned. .

Each feeding day each horse was caught and led to the middle of the front of their corrals by a standard lead rope and temporarily restrained by laying the lead rope over the top of the fence. The 5 gallon feed tubs were then put into place approximately 12 feet apart on the back portion of the corral. The 5 gallon feed treatment tub was placed on the left side and the 5 gallon non-treatment feed tub was placed on the right side. After the 5 gallon feed tubs were in place I went outside the corral, secured the gate closed and returned to the head of the horse. The horse was then centered at the front of the corral with the head facing in the opposite direction of the 5 gallon feed tubs. The horses were carefully centered equidistant from the 5 gallon feed tubs and straightened so as not to be heading either left or right and held still until steadied and straight. Then the horse was released and allowed to travel to the 5 gallon feed tub of choice. This procedure was followed for both Group #1 and Group #2 both during the 12 conditioning days and the test days.

On test days the same procedure above was performed except after the 5 gallon feed tub placement, before release, each horse was led to the 5 gallon feed tub # 2, then directly to bucket #1, and allowed to smell and view; but not taste the contents of either bucket. Which bucket to be smelled and viewed first was decided by the toss of the coin. All horses were led to the right non-treatment bucket first then to the left treatment

bucket. Then the horse was returned to the front of the corral, centered, steadied, and released to allow the horses to travel to the bucket of choice.

Feeding Schedules

All feeding schedules for Trial # 1 are displayed in Figure 6. and Figure 7. including the Test Day. All feeding schedules for Trial # 2 are displayed in Figure 8. and Figure 9., including the Test Days 13, 14,and 15.

Conditioning Day	Feed Tub #1	Feed Tub #2
Day #1	G / C /L	E
Day #2	E	G / A
Day #3	G / C /L	E
Day #4	E	G / A
Day #5	G/ C / L	E
Day #6	E	G / A
Day #7	G /C / L	E
Day #8	E	G / A
Day #9	G / C/ L	E
Day #10	E	G / A
Day #11	G / C/ L	E
Day #12	E	G / A
Day #13 Test Day	G / C	G / A

Figure 6. A display of feeding schedules for Trial #1, Group #1. Each horse received 6 treatment pairings and 6 non-treatment pairings using the two flavors. In the case of Trial #1/Group #1 the treatment of locoweed was paired with the carrots and the non-treatment was paired with apples. On day # 13 of Trial #1 both horses in Group #1 were offered only a flavor and grain with the treatment removed. G = Grain Mixture, E = Empty, A = Apple, C = Carrots, L = Locoweed.

Conditioning Day	Feed Tub #1	Feed Tub #2
Day #1	G / A / T	E
Day #2	E	G / C
Day #3	G / A / T	E
Day #4	E	G / C
Day #5	G / A / T	E
Day #6	E	G / C
Day #7	G / A / T	E
Day #8	E	G / C
Day #9	G / A / T	E
Day #10	E	G / C
Day #11	G / A / T	E
Day #12	E	G / C
Day #13 Test Day	G / A	G / C

Figure 7. A display of feeding schedules for Trial #1, Group #2. Each horse received 6 treatment pairings and 6 non-treatment pairings using the two flavors. In the case of Trial #1 and Group #2 the treatment of Torbugesic was paired with the apples and the non-treatment was paired with the carrots. On Day #13 both horses in Group #2 were offered only a flavor and grain with the treatment removed. G = Grain Mixture, E = Empty, A = Apple, C = Carrots, T =Torbugesic

Between Trials

After Trial #1 was completed all four horses; the two in Group #1 and the two in Group #2 were given seven days of no grain at all, no treatment, or any flavors and returned to their basal diets.

Trial Number 2: Treatments & Non-Treatments

On the eighth day Trial # 2 began. The exact materials and procedures were utilized in Trial #1 as in Trial #2 with the only difference being that for each individual group the flavors were reversed. In Trial #2 the locoweed treatment was paired with apple for Group #1 and the Torbugesic treatment was paired with carrots for Group #2. In Trial #2 the non-treatment flavor was reversed with non-treatment pairings for Group #1 being carrots and the non-treatment pairings for Group #2 being apple (see Figure 8. and Figure 9.)

Conditioning Day	Bucket # 1	Bucket # 2
Day # 1	G / A / L	E
Day # 2	E	G / C
Day # 3	G / A / L	E
Day # 4	E	G / C
Day # 5	G / A / L	E
Day # 6	E	G / C
Day # 7	G / A / L	E
Day # 8	E	G / C
Day # 9	G / A / L	E
Day # 10	E	G / C
Day # 11	G / A / L	E
Day # 12	E	G / C
Day # 13 Test Day	G / A	G / C
Day # 14 Test Day	G / A / L	G / C
Day # 15 Test Day	G / C / L	G / A

Figure 8. A display of feeding schedules for Trial #2, Group #1. Each horse in Group #1 received 6 treatment pairings and 6 non-treatment pairings using the two flavors. In the case of Trial #2, Group #1 the treatment was paired with apples and the non-treatment was paired with the carrots. On Day #13 both horses in Group #1 were offered only a flavor and grain with the treatment removed. Then on Day #14 both horses were offered the treatment and the flavor and grain only; at the same time. Then on Day #15 of Trial #2 both horses in Group #1 were offered the treatment from Trial #1 and the non-treatment from Trial #1 at the same time. G = Grain Mixture, E = Empty, A = Apple, C= Carrot, L = Locoweed.

Conditioning Day	Bucket # 1	Bucket # 2
Day # 1	G / C / T	E
Day # 2	E	G / A
Day # 3	G / C / T	E
Day # 4	E	G / A
Day # 5	G / C / T	E
Day # 6	E	G / A
Day # 7	G / C / T	E
Day # 8	E	G / A
Day # 9	G / C / T	E
Day # 10	E	G / A
Day # 11	G / C / T	E
Day # 12	E	G / A
Day # 13 Test Day	G / C	G / A
Day # 14 Test Day	G / C / T	G / A
Day # 15 Test Day	G / A / T	G / C

Figure 9. A display of feeding schedules for Trail #2, Group #2. Each horse in Group #2 received 6 treatments pairings and 6 non-treatment pairings using the two flavors. In the case of Trial #2 Group #2 the treatment was paired with carrots and the non-treatment was paired with apples. On Day #13 both horses in Group #2 were offered only a flavor and grain with the treatment removed. Then on Day #14 both horses were offered the treatment and the flavor and grain only; at the same time. Then on Day #15 of Trial #2 both horses in Group #2 were offered the treatment from Trial #1 and the non-treatment from Trial #1 at the same time.G = Grain Mixture, E = Empty, A = Apple, C = Carrot, T =Torbugesic.

Locoweed

The locoweed for this experiment was supplied by Mr. David Graham of the New Mexico County Extension Service located in Clayton, New Mexico. The first batch of locoweed was cut wet and green and shipped May 11, 2007. The second batch was cut wet and green and shipped May 18, 2007. The locoweed was cut at Gladstone, New Mexico: GPS Address36 degrees21.747N- 104 degrees00.487W at an elevation of 6029 feet. Upon arrival the locoweed was allowed to air dry in the sun until the locoweed simulated hay that was ready to bale. The locoweed was then bagged and stored at a temperature of 65-75 degrees Fahrenheit. Each batch was labeled Batch #1 or Batch #2 then there was a toss of the coin to decide which batch to use for which trial. Batch #1 was used for Trial #1 and Batch #2 was used for Trial #2.

Torbugesic

The Torbugesic used in this study was purchased from Dr. Steven V. Colburn of Creekside Veterinary Service located at 8751 Old Castle Road, Escondido, CA. 92026. The Torbugesic (Fort Dodge brand name for butorphanol tartrate) was supplied in 5 mg tablets and kept in Dr. Colburns Mobile Veterinary Clinic and dispensed at feeding time for preparation to feeding trials.

Lameness Exams

Four subjective lameness exams were scheduled on all four test horses for the current study including one before and after Trial # 1, and one before and after Trial # 2. The lameness exams were performed by Dr. Steven V. Colburn D.V.M. the resident and attending veterinarian. The subjective lameness exam scores are based upon:

0 = Normal

1 = Slight subtle lameness

2 = Moderate easily recognizable lameness

3 = Sever definite lameness

4 = Extreme with little weight bearing

5 = Non weight bearing

Post-experimental Swainsonine Analysis

When the experiment was finished a sample of the volume of locoweed used in Trial #1 and Trial #2 (Group # 1 treatment) was sent to Dr. Dale Gardner-Research Chemist at the Poisonous Plant Research Laboratory located at 1150 E. 1400N. Logan , UT 84341. The two trial samples were analyzed to confirm the presence and/or quantity of the locoweed toxin swainsonine. The total sample from both samples were separately weighed and an aliquot taken for analysis utilizing liquid chromatography mass spectrometry (LC-MS) for the mass of swainsonine (see Gardner et al. 2001). The dose of swainsonine was then simply the % in the sample aliquot(s) multiplied by the total weight of the sample(s). To confirm the identity of the toxin a second aliquot of the extract was then analyzed by gas chromatography/mass spectrometry (GC/MS) (see Gardner et al. 2001).

Results

Lameness Exams: All four horses in the current study underwent a subjective lameness exam before Trial # 1 began and after Trial # 1 was finished. The exam was performed by Dr. Steven V. Colburn D.V.M., the resident veterinarian for the Vessels Stallion Farms and the attending veterinarian for the current study (Table 2).

Table 2. Results of Lameness Exams

		Exam #1	Exam #2
Horse #1	16 year old gray gelding - AQHA -	2 rating	2 rating
Horse #2	17 year old bay mare - Thoroughbred Mare- AJC	4 rating	4 rating
Horse #3	15 year old Palomino gelding- AQHA-	3 rating	3 rating
Horse #4	14 year old paint gelding- APHA-	2 rating	2 rating

Rating Scale for Lameness Exams:

0= Normal, 1= Slight subtle lameness, 2=Moderate easily recognizable lameness, 3= Severe definite lameness, 4= Extreme lameness with little weight bearing, 5= Non weight bearing.

After Trial #1 was finished a second Lameness Exam was performed resulting in the same identical scores. Before starting Trial #2 horses were to be evaluated again however under the discretion of the attending Veterinarian, Dr. Steven V. Colburn D.V.M. advised that all four test horses were chronically lame and that any further lameness exams requiring the horses to ambulate at a trot would cause undue and unnecessary pain. Therefore, scheduled Lameness Exam # 3 and scheduled Lameness Exam # 4 were canceled.

Swaisonine Analysis: When the experiment was finished, a sample of the volume of locoweed used in Trial #1 and Trial #2 was sent to Dr. Dale Gardner-Research Chemist at the Poisonous Plant Research Laboratory located at 1150 E. 1400N., Logan , UT 84341. The two trial samples were analyzed for the locoweed toxin swainsonine which was found in both samples. The total sample from both samples were separately weighed and an aliquot taken for analysis (Gardner et al. 2001). The dose of swainsonine received was then simply the % in the sample multiplied by the total weight of the sample. To confirm the identity of swainsonine a second aliquot of the extract was analyzed by gas chromatography/mass spectrometry (GC/MS)(Gardner et al. 2001)(Appendix A).

Table 3. Assay Results for Swainsonine in Trial Samples

Sample	% Swainsonine (dry weight)	Sample weight	Swainsonine Dose
Trial # 1	0.12	62 g	0.074 g
Trial # 2	0.15	77g	0.116 g

Experimental Results

Trial # 1, Group # 1: The results indicate that both horses (Horse #1 and Horse #2) in the locoweed treatment group (Group #1) preferred the flavor of apple, the non-treatment associated flavor, when simultaneously offered both flavors and grain, separately, with no treatment on Day 13, the Test Day (Figure 11). Both horses went directly to their non-treatment bucket without hesitation and consumed the entire contents of the non-treatment bucket without interruption. Since both horses displayed identical feeding behaviors the results have been reported as Group # 1 for simplicity. The current results are based upon a post-experimental swainsonine analysis indicating that each

locoweed treatment in Trial # 1 consisted of approximately 62 grams of locoweed containing 7.4 milligrams of swainsonine.

Trial # 1, Group # 2: The results indicate that both horses (Horse #3 and Horse #4) in the Torbugesic treatment group (Group #2) preferred the flavor of carrot, the non-treatment associated flavor, when simultaneously offered both flavors and grain, separately, with no treatment on Day 13, the Test Day (Figure 12). Both horses went directly to their non-treatment bucket without hesitation and consumed the entire contents of the non-treatment bucket without interruption. Since both horses displayed identical feeding behaviors the results have been reported as Group # 2 for simplicity. The current results are based upon each Torbugesic treatment containing 10 milligrams of butorphanol tartrate.

Conditioning Day	Feed Tub #1	Feed Tub #2
Day #1	G / C / L	E
Day #2	E	G / A
Day #3	G / C / L	E
Day #4	E	G / A
Day #5	G/ C / L	E
Day #6	E	G / A
Day #7	G / C / L	E
Day #8	E	G / A
Day #9	G / C / L	E
Day #10	E	G / A
Day #11	G / C / L	E
Day #12	E	G / A
Day #13 Test Day	G / C	**G / A (Preference)**

Figure 11. Both horses in Group # 1, the locoweed group, on Test Day 13 showed a clear preference for the non-treatment feed of apples and grain as clearly indicated by bold. The color red represents apple. G = Grain Mixture, E = Empty, A = Apple, C = Carrot, L = Locoweed.

Conditioning Day	Feed Tub #1	Feed Tub #2
Day #1	G / A / T	E
Day #2	E	G / C
Day #3	G / A / T	E
Day #4	E	G / C
Day #5	G / A / T	E
Day #6	E	G / C
Day #7	G / A / T	E
Day #8	E	G / C
Day #9	G / A / T	E
Day #10	E	G / C
Day #11	G / A / T	E
Day #12	E	G / C
Day #13 Test Day	G / A	**G / C (Preference)**

Figure 12. Both horses in Group # 2, the Torbugesic group, on Test Day 13 showed a clear preference for the non-treatment feed of carrots and grain as clearly indicated by bold. The color orange represents carrot. G = Grain Mixture, E = Empty, A = Apple, C = Carrot, T = Torbugesic

Trial # 2, Group # 1: The results indicate that both horses (Horse # 1 and Horse # 2) in the locoweed treatment group (Group # 1) preferred the flavor of carrot, the non-treatment associated flavor, when simultaneously offered both flavors and grain, separately, with no treatment on Day 13, the Test Day (Figure 13). On Test Day 14, when the locoweed treatment was added to the apple flavor, both horses in Group # 1 chose the non-treatment associated with the carrot flavor. On Test Day 15, when the flavors were reversed and the carrot flavor was added to the locoweed treatment both horses preferred the non-treatment flavor of apple (see Figure 13.) The current results are based upon a post-experimental swainsonine analysis indicating that each locoweed treatment in Trial # 2 consisted of approximately 77 grams of locoweed containing 11.6 milligrams of swainsonine.

Trial # 2, Group # 2: The results indicate that both horses (Horse # 3 and Horse # 4) in the Torbugesic group (Group # 2) preferred the flavor of carrot, the Torbugesic treatment associated flavor, when simultaneously offered both flavors and grain, separately, with no treatment on Day 13, the Test Day (Figure 14.). On Test Day 14, when the Torbugesic treatment was added to the carrot flavor, both horses in Group # 2 chose the treatment associated with the carrot flavor. On Test Day 15, when the flavors were reversed and the apple flavor was added to the Torbugesic treatment both horses preferred the treatment flavor of apple. (see Figure 14.). The current results are based upon each Torbugesic treatment containing 10 milligrams of butorphanol tartrate.

Conditioning Day	Feed Tub # 1	Feed Tub # 2
Day # 1	G / A / L	E
Day # 2	E	G / C
Day # 3	G / A / L	E
Day # 4	E	G / C
Day # 5	G / A / L	E
Day # 6	E	G / C
Day # 7	G / A / L	E
Day # 8	E	G / C
Day # 9	G / A / L	E
Day # 10	E	G / C
Day # 11	G / A / L	E
Day # 12	E	G / C
Day # 13 Test Day	G / A	**G / C (Preference)**
Day # 14 Test Day	G / A / L	**G / C (Preference)**
Day # 15 Test Day	G / C / L	**G / A (Preference)**

Figure 13. Both horses in Group # 1, the locoweed group, on Test Day # 13 showed a clear preference for the non-treatment feed of carrots and grain as clearly indicated by bold and colored preference denoted by the color orange On Test Day 14, both horses in Group # 1, showed a clear preference when the locoweed treatment was added back, to the non-treatment feed of carrots and grain as clearly indicated by bold and colored preference denoted by the color orange. On Test Day # 15, when the locoweed treatment was added to the carrots and grain both horses preferred the non-treatment of grain and apple. G = Grain Mixture, E = Empty, A = Apple, C = Carrot, L = Locoweed.

Conditioning Day	Feed Tub # 1	Feed Tub # 2
Day # 1	G / C / T	E
Day # 2	E	G / A
Day # 3	G / C / T	E
Day # 4	E	G / A
Day # 5	G / C / T	E
Day # 6	E	G / A
Day # 7	G / C / T	E
Day # 8	E	G / A
Day # 9	G / C / T	E
Day # 10	E	G / A
Day # 11	G / C / T	E
Day # 12	E	G / A
Day # 13 Test Day	**G / C (Preference)**	G / A
Day # 14 Test Day	**G / C / T (Preference)**	G / A
Day # 15 Test Day	**G / A / T (Preference)**	G / C

Figure 14. Both horses in Group # 2, the Torbugesic group, on Test Day # 13 showed a clear preference for the treatment feed of carrots and grain as clearly indicated by bold and colored preference denoted by the color orange. On Test Day # 14, both horses in Group # 2 showed a clear preference when the Torbugesic was added back for the treatment feed of carrots and grain as clearly indicated by bold and colored preference as denoted by the color orange. On test Day # 15, when the Torbugesic treatment was added to the apple and grain both horses preferred the treatment of grain and apple. G = Grain Mixture, E = Empty, A = Apple, C = Carrot, T = Torbugesic.

Statistical Analysis, Trial # 1: There was no statistical analysis performed for Trial # 1. A descriptive analysis reveals that both horses, 100%, in Group # 1 the locoweed treatment horses, on Test Day # 13 preferred the non-treatment associated flavor of apple. A descriptive analysis reveals that both horses, 100%, in Group # 2 the Torbugesic treatment horses, on Test Day # 13 preferred the non-treatment associated flavor of carrot. All four horses (100%) preferred the non-treatment associated flavor when given a choice on Test Day # 13.

Statistical Analysis, Trial # 2: The Binomial Test was used to calculate the probability of obtaining the observed results strictly due to chance. In Trial # 2 the study produced a dichotomous measurement for each horse at each time point (each feeding). In statistical language the Binomial Test refers to the dichotomous outcomes as either success or failure. There is a probability assigned to success. Thus, based upon mathematical formulas for the binomial distribution the probability of obtaining X number of successes from N number of trials can be calculated. The classic example is the coin toss. Consider heads a success and tails a failure. A fair coin is equally likely to land heads or tails resulting in the probability of a success (heads) as 0.50. For example, tossing a coin 10 times ($n = 10$) the outcome would be expected to result in 5 heads and 5 tails. The question can now be asked as to what the probability would be for the outcome to produce 10 heads all 10 times? The binomial distribution shows the probability to be $p = 0.0010$ suggesting it is very highly unlikely to get 10 straight heads. By analogy, success can be defined as "the horse selects the feed tub with the treatment". If there is no treatment effect, which feed tub the horse chooses should be purely random,

producing a 50% chance the horse would choose the feed tub with the treatment. Since Group # 1 had 2 horses, each of which had 3 measurements with no horse-to-horse variability, the result is a sample size of 6 (analogous to 6 coin tosses). Both horses avoided the treated feed tub at all 3 time points resulting in 0 out of 6 successes in Group # 1, Trial # 2. The binomial distribution shows that if the probability of success is 0.50, the probability of 0 successes out of 6 trials is $p = 0.016$. This means that there is less than 2% chance that the treatment of locoweed would be avoided 6 out of 6 times if in fact the treatment had no effect. Therefore, there is very strong evidence to show the treatment had an effect.

Similarly, in Group # 2, Trial # 2, there were 2 horses with 3 measurements apiece with no horse-to-horse variability. In this case the Torbugesic treated feed tub was selected 6 out of 6 times. Again, if it is assumed there is no treatment effect, it would be expected the choice of feed tub to be purely random and the probability of success (choosing the treated feed tub) would be 0.50. The binomial distribution shows that if the probability of success is 0.50, the probability of 6 successes out of 6 trials is $p = 0.016$. Therefore, the probability of 6 successes out of 6 trials is 1.6%, which is less than the alpha level of 0.05. In that case, both Group # 1, the locoweed treatment group; and Group # 2, the Torbugesic treatment group in Trial # 2, showed statistically significant evidence of a treatment effect.

Discussion

All four horses in the current study were considered chronically lame, and as a result of the lameness exam performed, were verified lame at the beginning and during the entire study. All four horses in the current study were known to be naïve to locoweed consumption and naïve to Torbugesic consumption through dietary intake. This insured that the taste, and any possible hedonic shift induced by either substance, would be novel to each lame horse. In addition, this provided an unbiased pool of test horses with no prior experience to any post-ingestive physiological consequences as a result of the drugs used in this study.

The results from Trial #1, which began with all four horses being naïve to their respective treatments either locoweed or Torbugesic, clearly show that all four horses chose the flavor associated with their respective non-treatment feed. During the twelve day conditioning period which presented every-other-day treatment/non-treatment feedings and respective flavor associations, only one feed tub contained feed. At no time during Trial # 1 did any horse in either group refuse, hesitate, or fail to consume the entire conditioning feed. Due to this observation during the current pilot study, it was decided that on test day both feed tubs would remain in the corrals and the horse's diet selection behaviors observed. On test day, Day 13, when both feed tubs contained grain and their respective flavors only, the horses were challenged to make a decision. After completely consuming their non-treatment associated feed flavor; all four horses went directly to the treatment associated flavor and grain mixture and began consuming its contents. Therefore, the results of Trial # 1 clearly demonstrate that, 1).all horses showed a preference for their non-treatment associated flavor on test day, 2).all horses consumed

all test feeds throughout Trial # 1, 3).no horse displayed aversion to any test feed, and 4).all horses displayed a preference order; non-treatment associated flavor was the first preference, while the treatment associated flavor was the second preference.

When Trial # 1 was completed all horses remained on their basal diets with all grain, treatments, and flavors removed from their diets for a time period of seven calendar days. There were no feed tub placements during this in- between-trial period. The horses remained in their respective corrals and there was no change in their overall care. When Trial # 2 began, all horses had been introduced to their respective treatments and flavors thereby resulting in horses that were no longer naïve to their respective treatments. Additionally the flavors, respective treatments, grain mixtures, and any possible post-ingestive physiological consequences induced by the drugs were no longer novel to the test horses.

The results from Trial # 2, after the 12 day conditioning flavors were reversed from Trial # 1, clearly demonstrate that the locoweed treatment group did not change their preference. On test day, Day 13, both horses chose the flavor associated with the non-treatment feed when given the choice of the grain and flavor only. What this means is that the horses were able to associate the flavor of the non-treatment feed, prefer it when given a choice, regardless of the flavor. On the following day when the locoweed treatment was added back to the same flavor both horses preferred the non-locoweed treated feed. Then on the following day, the final test day, there was an attempt at deception. On the two previous days the horses had chosen carrots when associated with the non-locoweed treatment with grain and a flavor only; and when the locoweed treatment was added back the horses chose carrots again which was the non-locoweed

treated flavor. On the final day, Day 15, the carrots were then added to the locoweed treatment and the apple was added to the non-treatment. Both horses chose the non-treatment flavor of apple. During Trial # 2 both locoweed treatment horses were allowed to consume their feed of choice with both feed tubs remaining in place for observation of further diet selection behaviors. Upon choosing the non-treatment associated flavor, and upon choosing the non-treated flavor, both horses went directly to the other feed tub and began consuming its contents. Therefore, the results of Trial # 2, Group # 1, clearly demonstrate that 1).both horses showed a preference for their non-treatment associated flavor on test day regardless of the flavor, 2).both horses consumed all test feeds throughout Trial # 2, 3) no horse displayed aversion to any test feed, and 4).both horses displayed a preference order; non-treatment associated flavor and/or non-treated flavor feed was the first preference, while the treatment associated flavor and/or treated flavor feed was the second preference regardless of the flavor.

The results of Group # 1, the locoweed group, should be clear; both horses preferred the non-treatment of locoweed throughout the entire study. However, Trial # 1 cannot be compared to Trial # 2. The first reason is that there was an increase in the dosage. The results of the post-experimental swainsonine analysis did confirm the presence of swainsonine, but also revealed that the dosages were higher in Trial #2 verses Trial # 1. Whether the difference in the dosage of swainsonine affected the two horse's diet selection or not is currently unknown. There is currently no dosage response curve available pertaining to dosages that may apply to post-ingestive physiological consequences. There is no available information in the current literature addressing dosages in conjunction with duration. Stegelmeier (2007; personal communications)

suggests that serum levels of swainsonine at .04 mg/ml induced in laboratory administration over a six week period will induce lesions in the horses brain, however, not all horses exhibit locoism at this serum level. It is unknown what dosage level of swainsonine ingested by locoweed consumption is needed to achieve certain serum levels. Gardner (2007; personal communications) suggests that horses have been known to consume large amounts of locoweed known to contain swainsonine but will be undetected in laboratory analysis the next day. This is one reason that an every-other-day administration of locoweed was chosen in an attempt to eliminate any possible residual affect present between alternate locoweed treatment days. There is very little known about swainsonine dosages and/or their effects upon horses. The second reason that Trial # 1 cannot be compared to Trial # 2 is that before Trial # 1 the horses were naïve to locoweed and any possible post-ingestive physiological consequences would be novel to the horses. Before Trial # 2 began both horses were no longer naïve to locoweed and any possible post-ingestive physiological consequence would no longer be novel to the horses. Therefore, any statistical analysis comparing Trial #1 to Trial #2 would be inappropriate. There was a Binomial Distribution test performed on the locoweed treatment group horses from the results of Trial # 2. The results of the binomial test provide evidence, and strongly suggest that the locoweed treatment had a statistically significant treatment effect ($p = .016$).

The results from Trial # 2, after the 12 day conditioning flavors were reversed from Trial # 1, clearly demonstrate that the Torbugesic treatment group changed their preference. On test day, Day 13, both horses chose the flavor associated with the treatment feed when given the choice of the grain and flavor only. What this means is

that the horses were able to associate the flavor of the treatment feed, prefer it when given a choice, regardless of the flavor. On the following day when the Torbugesic treatment was added back to the same flavor both horses preferred the Torbugesic treated feed. Then on the following day, the final test day, there was an attempt at deception. On the two previous days the horses had chosen carrots when associated with the Torbugesic treatment with grain and a flavor only; and when the Torbugesic treatment was added back the horses chose carrots again which was the Torbugesic treated flavor. On the final day, Day 15, the apple flavor was added to the Torbugesic treatment and the carrot flavor was added to the non-treatment. Both horses chose the treatment flavor of apple. During Trial # 2 both Torbugesic treatment horses were allowed to consume their feed of choice with both feed tubs remaining in place for observation of further diet selection behaviors. Upon choosing the treatment associated flavor, and upon choosing the Torbugesic treated flavor, both horses went directly to the other feed tub and began consuming its contents. Therefore, the results of Trial # 2, Group # 2, clearly demonstrate that 1).both horses showed a preference for their treatment associated flavor on test day regardless of the flavor, 2).both horses consumed all test feeds throughout Trial # 2, 3) no horse displayed aversion to any test feed, and 4).both horses displayed a preference order; treatment associated flavor and/or treated flavor feed was the first preference, while the non-treatment associated flavor and/or non-treated flavor feed was the second preference regardless of the flavor, and 5). both horses changed their preferences from Trial # 1.

The results of Group # 2, the Torbugesic group, should be clear; both horses preferred the non-treatment associated flavor during Trial # 1, however, both horses changed their preference to the Torbugesic treatment associated flavor and the

Torbugesic treated feed during Trial # 2. This suggests that before Trial # 1, when the horses were naïve to Torbugesic and the post-ingestive physiological consequence was novel, the horses preferred the non-treatment associated flavor. This suggestion is based upon the possibility of a hedonic shift from a negative feedback to a positive feed back as previously introduced into this thesis. After sampling Torbugesic during Trial # 1, being removed from the treatment during the seven day in-between period, and re-introduced to the post-ingestive consequence albeit with a reversal of flavor, the horses "learned" to prefer the Torbugesic. This learned preference was demonstrated in the current pilot study regardless of the flavor. This would be expected from a known synthetic opiate utilizing a known dosage to alleviate pain in the lame horses. It is also consistent with the current literature that horses, as generalist, sample many forages and form a preference based upon post-ingestive physiological consequences and can change the preference when physiological changes occur. Still further, this result is consistent with the current literature in that it has been shown by Cairns et al. (2002) and Redgate et al. (2006) that horses can associate two flavors with different energy densities based upon post-ingestive physiological consequences. In addition, both Cairns et al. (2002) and Redgate et al. (2006) have demonstrated that horses can change their preferences based upon post-ingestive physiological consequences. There was a Binomial Distribution test performed on the Torbugesic treatment group horses from the results of Trial # 2. The results of the binomial test provide evidence, and strongly suggest that the Torbugesic treatment had a statistically significant treatment effect ($p = .016$).

The results of the entire current pilot study has, upon examination of the produced data, provided much needed information into the possibility of self-medication in horses

based upon post-ingestive physiological consequences induced by a drug. In regards to both treatment groups all feeds and both flavors were palatable resulting in a preference. In both groups the horses were able to associate the flavor, associated with the preference, even when the treatment was removed. In reference to the locoweed, the horses demonstrated that after sampling they were able to retain their preference and the locoweed horses were able to order their preferences. In the case of the locoweed the dosage responsible for forming a preference is currently unknown. The duration of a dosage needed is currently unknown. The locoweed was fed in its natural state but was shown to contain the drug swainsonine. However, many other substances are contained in locoweed that may have nutritional properties and cannot be covered here. As Huffman (2003) so eloquently states;food and medicine may greatly overlap in the diet, sometimes making the difference difficult to perceive. It is possible that over long periods of time nutrients and/or swainsonine contained in locoweed may have medicinal properties and can not be ruled out as a medicine. Longer term studies are needed to investigate this possibility. In reference to Torbugesic, the horses demonstrated that after sampling they were able to change their preferences and were able to order their preferences. In the case of the Torbugesic, the dosage is known and the full effects are known and tested. The effect of this synthetic opiate was specifically designed as a pain killer for horses and is used readily in many veterinary practices for this purpose (Colburn 2008; personal communications).

The aim of the current study was to begin examining the possibility as to whether horses have the ability to associate a taste with a post-ingestive physiological consequence induced by a drug. The results clearly demonstrate that diet selection on

test days in the horses in the current study was not random ($p < .05$). Both groups have clearly demonstrated that their respective treatments did have an effect on their diet selection ($p=.016$). Therefore, through experimental evidence produced by the current pilot study, I must reject the null hypothesis and accept the hypothesis that horses have the ability to associate a taste with a post-ingestive physiological consequence induced by a drug. This ability of horses to associate a taste with a post-ingestive physiological consequence may give insight into possible self-medication in horses; however, due to the small sample size no conclusions can be drawn. Further studies utilizing larger sample sizes over longer periods of time is strongly suggested.

Literature Cited

Allison, C.D. 1984. Locoweeds and livestock poisoning. NM Coop Ext Serv. Pub. 400, B-15.

Barker, L.M., M.R. Best, and M. Domjans (eds.). 1977. Learning Mechanisms in food Selection. Baylor University Press 632 p.

Braun, K., J. Romero, C. Liddell, and R. Creamer. 2003. Production of swainsonine by fungal endophytes of locoweed. Mycol. Res. 107;980-988.

Budiansky, S. 1997. The Nature of Horses. New York: the Free Press.

Cairns, M.C., J.J. Cooper, H.P.B. Davidson, and D.S. Mills. 2002. Association in horses of orosesory characteristics of foods with their post-ingestive consequences. Anim. Sci. 75, 257-265.

Clayton, D. H., and N.D. Wolfe. 1993. The Adaptive Significance of Self-Medication. Tree Vol.8 No.2, 60-63.

Colburn, S.V. 2007. Creekside Veterinary Services. Escondido. CA.

Cooper, J.J. 2007. Equine learning behaviour: Common knowledge and systematic research. Behavioural Processes. 76, 24-26.

Danbury, T.C., C.A. Weeks, J.P. Chambers, A.E. Waterman-Pearson, and S. Kestin 2000. Self-selection of the analgesic drug carprofen by lame broiler chickens. Vet. Rec. 146, 307-311.

Delgado,G. 1988. Heterocycles 27, 1305-1311.

Ehrlich, P.R. and P.H. Raven. 1964. Butterflies and plants: A study in coevolution. Evolution 18: 586-608.

El-Hamidi, M., and H.W. Leipold. 1989. Poisoning of sheep by *Astragalus lustanicus* in Morocco field and Experimental Studies. J.Vet Med Ser A 36(2): 115-121.

Emmans, G.C. 1991. Diet selection by animals: theory and experimental design. In Proceedings of the Nutrition Society. 50, 59-64.

Engel, C. 2002. Wild Health: How Animals Keep Themselves Well and What We Can Learn from Them. Weidenfield & Nicoloson: London

Frost, B. and G.B Ruyle. 1993. Range management terms and definitions. Arizona Ranchers' Management Guide. Arizona Cooperative Extension.

Futuyma, D.J. 1986. Evolutionary Biology. Sinauer Associates. P.409

Garcia, J., D.J. Kimedorf, and R.A. Koellino. 1955. Conditioned aversion to saccharin resulting from exposure to gamma radiation. Science. 122:157-158. No. 3160.

Gardner, D.R., J. Romero, M.H. Ralphs, and R. Creamer. 2003. Correlation of an endophyte fungus (*Alternaria* sp.) with the presence of swainsonine in Lambert locoweed (*Oxytropis lambertii*). In Poisonous Plants and Related Toxins. T. Acamovic, C. S. Stewart and T. Pennycott, ed. CABI publishing. MA.

Gardner, D.R., R.J. Molyneaux, and M.H. Ralphs. 2001. Analysis of Swainsonine: Extraction Methods, Detection, and Measurement in Populations of Locoweeds (Oxytropis spp.). J. Agric. Food Chem. 49,4573-4580.

Getty, R.G. 1975. Sisson and Grossman's The Anatomy of the Domestic Animals. 5th ed. Vol.1. W.B. Saunders Company, Philadelphia, PA.

Glendinning, J.I. 2007. Coping with defensive chemicals. Biol. Bull 213:252-266.

Gustavson, C.R. 1977. Comparative and field aspects of learned food aversions. In: Learning Mechanisms in Food Selection, L.M. Barker, M.R Best, and M. Domjan (eds), Baylor University Press p. 23-43.

Hart, B.L. 1990. Behavioral adaptations to pathogens and parasites: Five strategies. Neuroscience and Biobehavioral Reviews 14, 273-294.

Howerey, L.D., F.D. Provenza, G.D. Ruyle, and N.C. Jordan. 1998. How Do Animals Learn if Rangeland Plants are Toxic or Nutritious? Rangelands 20(6) December.

Huffman, M.A. 2003. Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. In Proceedings of the Nutrition Society. 62, 371-381.

Janis, C. 1976. The evolutionary strategy of the equidae and the origins of rumen and cecal digestion. Evolution 30. 757-774.

Julien, R. M. 2005. A Primer for Drug Action. New York, Worth Publisher, Inc.

Klein, J-L D., J.D. Roberts, M.D. George, J. Kurtzberg, P. Breton, J-C Cherman, and K. Olden. 1999. Swainsonine protects both murine and human haematopoietic systems from chemotherapeutic toxicity. British Journal of Cancer. Vol. 80:87-95.

Krohne, D.T. 1998. General Ecology. Wadsworth Publishing Company.

Laycock, W.A. 1978. Coevolution of poisonous plants and large herbivores on rangelands. J Range Manage. 31:335-342.

Lewin, L. 1931. Phantastica: Narcotic and Stimulating Drugs. Kegan Paul, Trench Trubner. London.

Lozano, G.A. 1998. Parasitic stress and self-medication in wild animals. In: Advances in the Study of Behavior, Vol.27. Stress and Behavior.Chapter 6 (Ed. By A.P. Meller, M.Milinski and P.J B. Slater), pp. 291-317. London: Academic Press.

Marsh, C.D. 1909. The loco-weed disease of the plains. USDA Bull. 112. Washington, D.C.

MacFadden, B.J. 1992. Fossil Horses: Systematics , Paleobiology, and the Evolution of the Family Equidae. New York: Cambridge University Press.

McLain-Romero, J., R. Creamer, H. Zepeda, J. Strickland, and G. Belt. 2004. The toxicosis of *Embellisia* fungi from locoweed (*Oxytropsis lambertii*) is similar to locoweed toxicosis in rats. J. Anim. Sci. 82: 2169-2174.

McNaughton, S.J., J.L Torrents, M.M. McNaughton, andR.H. David. 1985. Silica as a defense mechanism against herbivory and a growth promoter in African grasses. Ecology 66: 528-535.

Molyneux, R.J., and L.F. James. 1982. Loco intoxication: Indolizidine alkaloids of spotted locoweeds (*Astragalus lentiginosus*). Science 216: 190-191.

Moss, R. 1991. Diet selection: an ecological perspective. In: Proc. Nutr. Soc. 50:71-75.

Nielsen, D.B. 1978. The economic impact of poisonous plants on the range livestock industry in the 17 western states. J. Range Manage. 31 325-328.

Nielsen, D.B., N.R. Rimbey, and L.F. James. 1988. Economic considerations of poisonous plants on livestock. pp. 515. in The Ecology and Economic Impact of Poisonous Plants on Livestock Production. (Eds.) L.F. James, M.H. Ralphs, and D.B. Nielsen. Westview Press, Boulder , Colorado.

Oredipe, O.A., S.L. White, K.Grzegorzewski, B.L.Gause,J.K. Chan, V.A. and K. Olden. 1991. Protective effects of swainsonine on mutine survival and bone marrow proliferation during cytotoxic chemothereapy. J. Natl. Cancer Inst. 83: 1149-1156.

Oredipe, O.A.,P.M. Furbert-Harris, I. Laniyan, W.R. Warren, W.M. Griffin, and R. Sridhar. 2003. Mice primed with swainsonine are protected against Doxorubicin-induced lethality. Cell. Mol. Biol. 49(7), 1089-1099.

Percio, A.W. 1976. The Pharmacology of Butorphanol. Arch Int Pharmacodyn Ther 220 (2): 231-257.

Pfister, J.A., F.D. Provenza, G.D. Manners, D.R. Gardner, and M.R. Ralphs. 1997. Tall larkspur ingestion: can cattle regulate intake below toxic levels? Journal of Chemical Ecology 23, 759-777.

Pfister, J.A. 1999. Behavioral Strategies for Coping with Poisonous Plants. Presented in "Grazing Behavior of Livestock and Wildlife" 1999. (Eds.) K.L. Launchbaugh, K.D. Sanders, J.C. Mosley.

Pfister, J.A., B.L. Stegelmeier, D.R. Gardner, and L.F. James 2003. Grazing of spotted locoweed (*Astragalus lentiginosus*) by cattle and horses in Arizona. J. Anim. Sci. 81:2285-2293.

Potter, E.F. 1970. Auk. 87, 692-713.

Provenza, F.D., J.A. Pfister, and C.D. Cheney. 1992. Mechanisms of learning in diet selection with reference to phytotoxicosis in herbivores. J. Range Manage. 45:36-45.

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

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

Purves, W.K., and G.H. Orians. 1983. Life: The Science of Biology. Grant Press, Boston,MA.

Pyne, S.G. 2005. Recent Developments on the Synthesis of (-) Swainsonine and Analogues. Current Organic Synthesis. 2: 39-57.

Ralphs, M.H., L.F. James, and J.A Pfister. 1986. Utilization of White Locoweed (*Oxytropis sericea* Nutt.) By Range Cattle. J. Range Mange. 39: 344-347.

Ralphs, M.H., L.V. Mickelson, D.L. Turner, and D.B. Nielsen. 1988. Control of white locoweed (*Oxytropis sericea*). Weed Sci. 36: 353-358.

Ralphs, M.H., K.E. Panter, and L.F.James. 1990. Feed preferences and habituation of sheep poisoned by locoweed. J. Anim. Sci. 68:1354-1362.

Redgate, S.E., S. Hall, J.J. Cooper, P. Eady, and P.A. Harris, 2006. Post-ingestive feedback on diet selection in horses (Equus cabellus); dietary experience changes feeding preferences. In: Proceedings of the 40th ISAE Congress Bristol 2006.

Rhoades, D.F. 1985. Offensive-defensive interactions between herbivores and plants: Their relevance in herbivore population dynamics and ecological theory. American Naturalist. 125: 205-238.

Rittenhouse, L.R. 2005. Professor Emeritus. Warner College of Natural Resources. Colorado State University, Ft. Collins, CO. Personal communications.

Romero, J., R. Creamer, M.H. Ralphs, and D.R. Gardner. 2002. Association of a fungal endophyte with seed tissue and locoweed toxicity. (Abstr.) Am. Phytopath. Soc. Meeting, Milwaukee, WI.

Siegel, R.K. 1979. Natural animal addictions: an ethological perspective, p. 29-60. In: J.D. Keehn (ed.) Psychopathology in Animals. Academic Press, N.Y.

Stamp, N.E. 1992. Theory of plant-insect herbivore interactions on the inevitable brink of re-synthesis. Bulletin of the Ecological Society of America. 73: 28-34.

Stegelmeier, B.L. 2007. USDA Poisonous Plant Laboratories, Logan, Utah. Veterinary Pathologist.

Stubbendieck, J., S.L. Hatch, and L.M. Landholt. 2003. North American Wildland Plants. University of Nebraska Press, Lincoln.

Taylor, J.B., and J.R. Strickland. 2002. Appearance and disapearance of swainsonine in serum and milk of lactating ruminants with the nursing young following a single dose exposure to swainsonine (locoweed; *Oxytropis sericea*). J. Anim. Sci. 80:2476-2484.

Thompson, D.C., J.L. Knight, T.M. Sterling, and L.W. Murray. 1995. Preference for specific varieties of wooly locoweed by a specialist weevil, *Cleonidius trivittatus* (Say). Southwestern Entomologist. 20(3): 325-333.

Tulsiani, D.R., H.P.Broquist, L.F. James, and O. Touster. 1984. The similar effects of swainsonine and locoweed on tissue glycosidases and oligosaccharides of the pig indicate that the alkaloid is the principle toxin responsible for the induction of locoism. Arch. Biochem. Biophys. 264: 607-617.

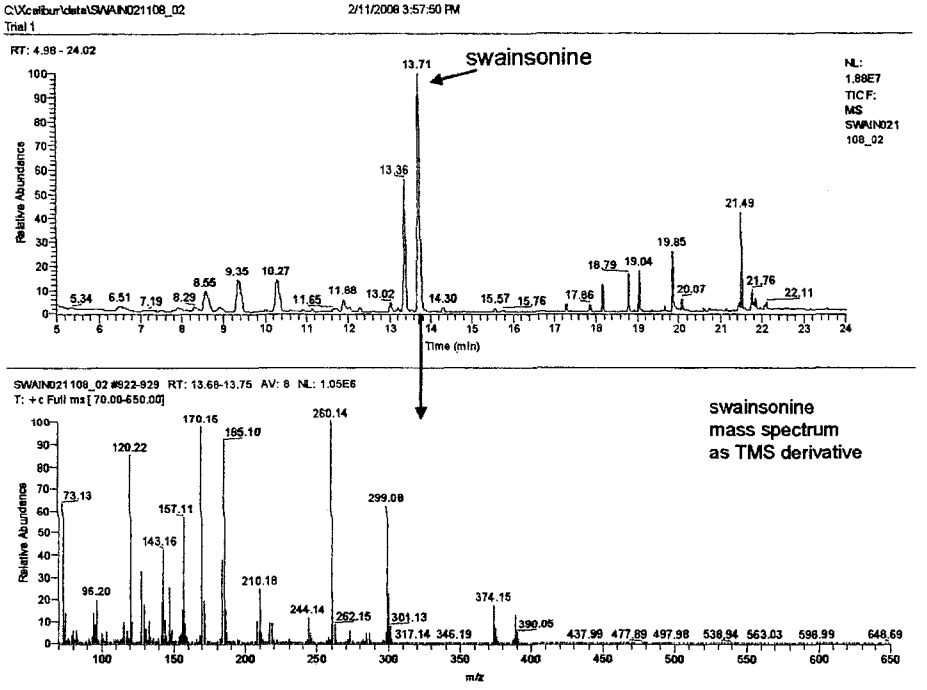
Villalba, J.J., F.D. Provenza, and R.Shaw. 2006. Sheep self-medicate when challenged with illness-inducing foods. Animal Behaviour. 71, 1131-1139.

APPENDIX A

The GC chromatograms and the mass spectrums identifying the peak as swainsonine for Trial #1 and Trial #2. The identifying peaks are clearly labeled as swainsonine, indicated by arrows, designating the presence of swainsonine in both samples of locoweed Batch # 1 for Trial # 1, and Batch # 2 for Trial # 2, utilized in the current study.

GC/MS of Trial Samples

Trial #1



Trial #2