

University of Nevada, Reno

**Assessment of the Digestibility and Nutritional Value of Cheatgrass (*Bromus tectorum*) with Energy and Nitrogen Supplementation in Cattle through the use of a Dual Flow Continuous System**

A thesis submitted in partial fulfillment  
of the requirements for the degree of

Bachelor of Arts in Veterinary Science and the Honors Program

by

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**UNIVERSITY  
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RENO**

**THE HONORS PROGRAM**

We recommend that the thesis  
prepared under our supervision by

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entitled

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tectorum*) with Energy and Nitrogen Supplementation in Cattle through the use of a  
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requirements for the degree of

**Bachelor of Veterinary Science**

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## Abstract

Cheatgrass (*B. tectorum*) is an invasive noxious weed that dominates the Great Basin. Cheatgrass changes the fire cycle of sagebrush ecosystems and leads to fire damages across the Western US. A strategy to reduce the amount of cheatgrass is conservation grazing using livestock. This study aims to determine how supplementation of nitrogen (urea), carbohydrate (molasses), or both, affect cheatgrass diets in terms of nutrient flow, microbial protein synthesized, and digestibility through the use of a dual-flow continuous culture system. Eight fermenters were utilized in a 4 x 4 Latin square design using four 10-day periods. Experimental treatments (also called diets), on a dry matter basis, were cheatgrass only (control), cheatgrass plus urea, cheatgrass plus molasses, and cheatgrass plus urea and molasses. The fermenters were given 72 grams of their diet daily. The true digestibility of neutral detergent fiber and acid detergent fiber was not affected by experimental diets. Diets including molasses had higher true digestibility of organic matter, and the true digestibility of crude protein was greater in the molasses only diet. Diets with molasses had more acidic pH and higher concentrations of volatile fatty acids (VFAs). The combined diet led to a higher concentration of VFAs, with propionate concentrating greater and acetate concentration lower. Molasses only diet led to increased branch-chain volatile fatty acids. The urea only diet resulted in a higher concentration of  $\text{NH}_3\text{-N}$  and nitrogen flow, but the other diets without urea led to more non-ammonia and bacterial nitrogen. There was no effect on bacterial efficiency, regardless of diet. These results suggest that adding urea and molasses to a cheatgrass diet could improve the amount of nutrients leaving to rumen and

being available to the animal, especially VFAs and microbial nitrogen. However, supplementation did not appear to enhance cheatgrass digestion in regards to neutral detergent fiber digestion, which is significant since cheatgrass as a plant is primarily fiber.

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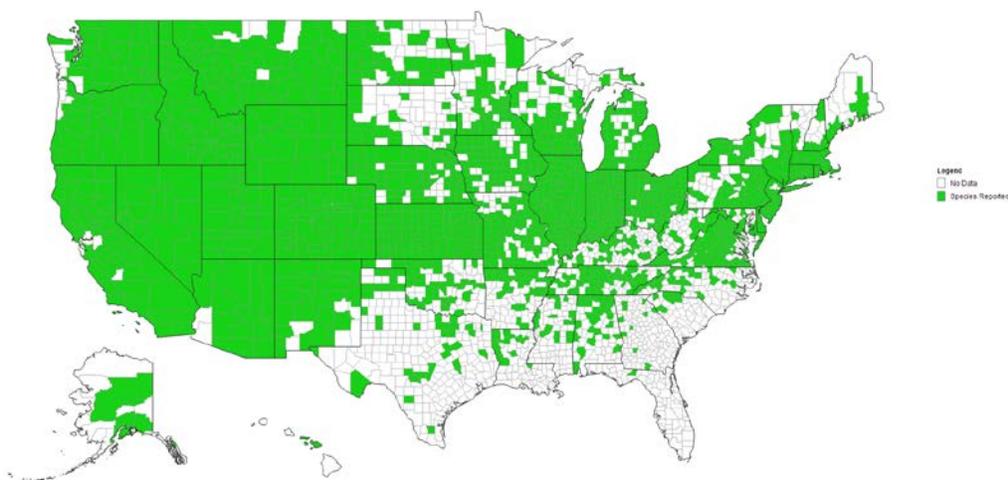
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## Introduction

### The History of Cheatgrass

Cheatgrass (*Bromus tectorum*) is an invasive annual grass species, originating in southwest Asia (Swanson et al., 1987). Cheatgrass is labeled an annual noxious weed, so cheatgrass conflicts with land management goals significantly (DiTomaso, 2000). Today, *B. tectorum* is found across the United States and Canada (Davies et al., 2016), as seen in (Figure 1.), but its influence is most prominent in the Great Basin. The Great Basin is an area consisting of five states (Figure 2.), covering over 390,000 square kilometers (Knapp, 1996). Approximately two-thirds of the Great Basin is in Nevada, with *B. tectorum* being present in almost all of the sagebrush/bunchgrass zones in Nevada (Knapp, 1996).

cheatgrass (*Bromus tectorum*)



Map generated on May 5, 2017

EDD Maps  
Eradication Decision Support System

**Figure 1. Map of Cheatgrass in United States (Swearingen & Barger, 2016).**

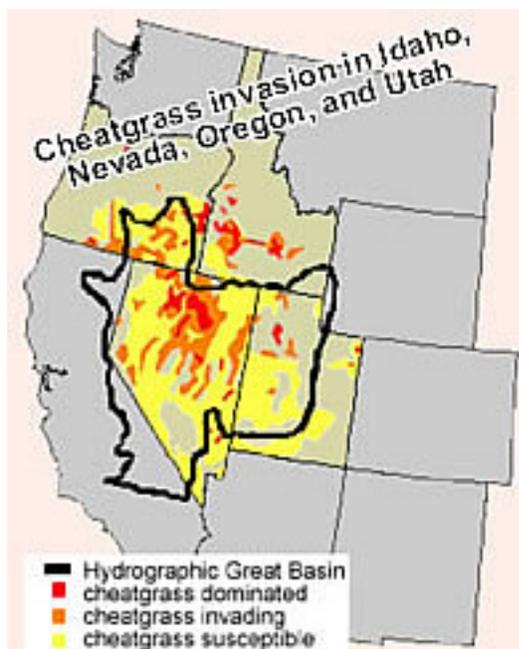


Figure 2. Map of Cheatgrass in the Great Basin (King, 1999).

*B. tectorum* was first brought to North America in approximately 1861, as familiar crop for immigrants traveling to America, and spread through the 1900s (DiTomaso, 2000). *B. tectorum* was found in the Great Basin in 1894, and it was first found in Nevada in 1905 (Knapp, 1996). Cheatgrass spread through the Great Basin due to humans using it as bedding straw for cattle (Knapp, 1996). Large cattle herds became present in the Great Basin in 1864 due to the boom of the Comstock Lode in Virginia City (Knapp, 1996). Historically, the plant biodiversity of the Great Basin did not vary much with grazing activities of the endemic wildlife, but overgrazing of cattle brought to the Great Basin throughout the early 1900s allowed *B. tectorum* to fulfill the niche of a native annual grass (Knapp, 1996). Previously, no native annual grasses were present in the Great Basin (Knapp, 1996), so cheatgrass was able to effectively fit into the ecological niche without competition.

## Ecological and Financial Impacts of Cheatgrass

The over-grazing of the early 1900s had a significant impact on decreasing the number of endemic perennial grasses (such as *Agropyron desertorum*, *Sporobolus airoides*, and *Sporobolus contractus*), which are important plants involved in resisting the invasion of non-native species (Davies et al., 2016). Once *B. tectorum* secured a foothold in the Great Basin, *B. tectorum* was able to maintain dominance while having adverse effects on the native plant species. Twenty percent of the sagebrush/bunchgrass zones in Nevada contain an amount of cheatgrass so great that other native shrubs (*Chrysothamus nauseosus* and *Gutierrezia sarothrae*) can't germinate, or begin growth (Knapp, 1996).

Cheatgrass is able to secure dominance by reproducing quickly. Cheatgrass produces more seeds than previously mentioned native species (Bureau of Land Management, 2013), allowing cheatgrass to secure a stronger foothold in the Great Basin environment with every generation. *B. tectorum* is also able to survive in a wider variety of environmental conditions than native plants (Knapp, 1996); the species is able to withstand environmental changes more readily than native species. Cheatgrass germinates earlier in the spring than native species and achieves its peak growth in the winter, allowing it to grow during a time period when other native species aren't yet able to compete for nutrients (Knapp, 1996). Therefore, cheatgrass is able to outcompete native plants efficiently.

While *B. tectorum*'s ability to outcompete native species leads to a loss of biodiversity in the Great Basin, *B. tectorum* causes further problems for the humans living in these areas through its relationship with fire. Cheatgrass burns quickly and recovers from fire more quickly than native species (Bureau of Land Management, 2013;

Swanson et al., 1987). Cheatgrass also serves as a source of fuel for fire; since cheatgrass dies more quickly than native species, burns earlier in the growing season and results in young native species being burned in fires (Swanson et al., 1987). Additionally, *B. tectorum* has the capacity to create instability in ecosystems by increasing the frequency of fires (DiTomaso, 2000). The native species present in the Great Basin are not adapted to frequent fires and, therefore, don't recover quickly like the fire-adapted cheatgrass. Dead cheatgrass gives fires more material to burn and recovers after fire more quickly than native species, leading to further invasion of cheatgrass due to the absence of growing native plants (Davies et al., 2016). *B. tectorum* is ten to five hundred times more likely to start fires than the native bunchgrasses, and the presence of cheatgrass has increased the length of the fire season by up to three months (Knapp, 1996). Additionally, ninety percent of the areas burned by fire over a thirty-one year study in the Great Basin were dominated by cheatgrass (Knapp, 1996). *B. tectorum*'s alteration of the fire cycle has resulted in an increased cost in fire damages. The fire damage of *B. tectorum* added up to approximately ten million dollars for the United States in just the previously mentioned thirty-one year study on the basis of resource losses, prevention costs, rehabilitation costs, and managing fires (Knapp, 1996). Limiting the dominance of cheatgrass in the Great Basin is crucial for maintaining biodiversity and decreasing fire damage. One possible method is using livestock grazing for *B. tectorum* control.

#### Grazing Management as a Solution

Ironically, one of the causes of cheatgrass's invasion has been attributed to over-grazing by cattle and other grazing livestock once colonization of the Great Basin began

in 1864 (Davies et al., 2016; Knapp, 1996). However, more monitored and controlled levels of grazing could be used to decrease the amount of *B. tectorum* in the Great Basin and give native species a chance to re-colonize. How well an invasive species adapts to an area is a product of climate characteristics and interactions between other species (Bansal & Sheley, 2016). Livestock grazing could weaken the stronghold of invasive species by acting on the latter category, interactions with other species. Moderate cattle grazing (where only thirty to forty percent of the forage in the plots was actually eaten) in burned and un-burned plots of land containing *B. tectorum* in the Great Basin resulted in 1.7 times more cheatgrass cover in the ungrazed plots versus the grazed plots (Davies et al., 2016). Therefore, cattle were able to decrease the amount of cheatgrass in those areas. The total cover by native herbaceous plants was 1.2 times more in grazed plots versus ungrazed plots, as was the biomass of native perennial bunchgrasses by 1.9 times (Davies et al., 2016). Endemic (native) species were either not as affected by cattle grazing as *B. tectorum*, or the endemic species were able to gain a foothold once *B. tectorum* was limited in an area. Both scenarios result in an increase in native species and a decrease in the invasive cheatgrass. Additionally, the implementation of moderate grazing by cattle was shown to increase the resistance of endemic plant species to cheatgrass, after a fire had occurred (Davies et al., 2016). Grazing reduces the damage that *B. tectorum* can cause through altering the fire cycle while increasing the fitness of native species. Importantly, the effects of grazing (decreased cheatgrass and increased native species) are still relevant over twenty years after the fire (Davies et al., 2016). Grazing can be used as a long-term method to lessen the amount of *B. tectorum* in an area.

Furthermore, moderate grazing can decrease the amount of soil disturbed, limiting the potential for *B. tectorum* to infest an area initially (DiTomaso, 2000). The amount of seeds produced by *B. tectorum* was fifty percent less in grazed and burned plots compared to plots that were ungrazed and burned, and ungrazed by not burned (Diamond et al., 2012). Decreasing the number of seeds *B. tectorum* produces also decreases the amount of *B. tectorum* plants that will reach maturity. When cheatgrass is unable to secure its growth in an area, a “die-off” occurs and seeds of native species take advantage of the lower pH, higher phosphorous and nitrogen, lower magnesium, and more moisture in the resulting soil (Baughman et al., 2016). Removing cheatgrass from an area gives endemic species a greater chance to thrive in the soil conditions. Moreover, out of almost one hundred different factors (including soil composition, weather conditions, etc) that influence invasive annual grasses, the presence of *B. tectorum* is most correlated with living factors, referring to interactions with other living organisms (Bansal & Sheley, 2016). Cheatgrass is therefore more susceptible to grazing (a living factor) than other abiotic (non-living) methods of control. Perennial grass cover is also not significantly affected due to grazing (Bansal & Sheley, 2016).

“Intensive grazing,” grazing in which cattle consume the majority of plants in an area, can be another useful strategy to reduce fires caused by *B. tectorum* by removing biomass that could be used as a fuel source (Diamond et al., 2012). Intensive grazing can force livestock to utilize all of the feed available in an area, so that invasive species are consumed in the process (DiTomaso, 2000).

Overall, grazing has been shown to decrease the amount of cheatgrass in an area, decrease the ability of cheatgrass to re-grow in an area, and increase the probability that

native species will have favorable soil conditions to grow and survive. Grazing is an effective strategy to reduce the amount of *B. tectorum* in the Great Basin. However, the nutritional value of cheatgrass must also be assessed in order to confirm if conservation grazing against cheatgrass would be viable in terms of the health and productivity of livestock species.

#### Cheatgrass as a Forage Source

Concerning pasture lands, *B. tectorum* is the most substantial source of forage for livestock in Nevada (Swanson et al., 1987). Being able to utilize this major forage source would be economically significant for ranchers in that a very cheap source of feed would be readily available for their livestock to graze on. *B. tectorum* is green and has a higher nutritional value while it is young, but it dies and dries out more quickly than native species (Cook & Harris, 1952). Livestock have a limited time to consume cheatgrass at its highest nutritional value. However, *B. tectorum* also begins growing in the fall and grows throughout the winter, so the plant is green and young while most other native plants (*Agropyron desertorum*, *Sporobolus airoides*, *Chysothamus nauseosus*, *Gutierrezia sarothrae*, etc.) are not (Knapp, 1996). Livestock use cheatgrass as a feed source during a time when other feed sources are not as easily available. The digestibility of dry matter eaten by animals is higher in cows than in sheep (Fraser et al., 2009). Additionally, bacteria collected from cattle species and used for in vitro (non-living animal) experiments digest dry matter more efficiently than the bacteria collected from sheep (Fraser et al., 2009). Cattle therefore are a more efficient choice of livestock, on a nutritional basis, for conservation grazing of cheatgrass.

Cattle are able to use cheatgrass as a forage and derive nutritional benefit. Angus, Hereford, and Angus-Hereford crossbred cattle that were grazed on areas containing *B. tectorum* gained an average of 1.43 pounds per animal daily over the seven months they were actually grazing in a three-year period (Murray & Klemmedson, 1968). Cattle are able to gain weight on diets involving grazing on cheatgrass dominated areas. However, as *B. tectorum* matures, the amount of fat, protein, calcium, phosphorus, and energy (carbohydrates) decreases while the amount of lignin and total minerals increases (Cook & Harris, 1952). Cheatgrass therefore loses dietary requirements and becomes less digestible (due to the increased lignin) as the plant ages. Cheatgrass has the potential to be a nutritional forage source for cattle, but losses nutrients must be addressed.

One way to compensate for the lack of protein and other nutrient in old forage is through diet supplementation. Older forage in general becomes less digestible as the plant begins to die and become lower in the amount of crude protein they provide (Njoya, 1997). Increasing the number of the important amino acids, such as methionine and histidine, in cattle diets results in more productivity on the basis of an increase in milk production, milk protein, and milk fat (Kal'nitskii & Kharitonov, 2010). Cattle become more efficient when amino acids, the basis of proteins, are added to diets in adequate amounts. Therefore, the protein requirement is important for ranching productivity. Cattle on diets with the increased amino acid amounts have less urea and a wider variety of individual amino acids in their milk (Kal'nitskii & Kharitonov, 2010). More amino acids and less urea indicates that the cows use more protein effectively on a diet including protein supplementation. Creating optimal rations around the basis of metabolizable energy (defined as specific fatty acids) and metabolizable protein (specifically the amino

acids methionine, lysine, histidine, and leucine) can raise how effectively an animal uses feed nutrients by five percent (Kal'nitskii & Kharitonov, 2010). It is critical for ranchers to ensure their livestock obtain enough energy and protein requirements to run a productive operation. The positive effects on productivity that results from diets higher in amino acids even persist for two months after changing back to a control diet without added amino acids (Kal'nitskii & Kharitonov, 2010), so the benefits obtained from supplementation have long-term effects as well.

Supplementation of crude protein through cottonseed meal for cattle actively grazing older forage has resulted in an increase in weight and sustaining the weight for a longer period of time than cattle on diets without supplementation (Njoya, 1997). Protein supplementation has been shown to increase the protein digested and absorbed by cattle. A nutrient supplementation approach to a cheatgrass forage based diet has not yet been explored.

## Literature Review

The foundational study on *B. tectorum* nutrition took place over sixty years ago (Cook & Harris, 1952), and the technology surrounding in vitro experiments and nutrient analysis has advanced significantly. The dual-flow continuous system is a method devised to mimic the environment of the rumen (first stomach of cattle when most digestion takes place) in the lab rather than using live animals, as was used in the foundational study (Del Bianco Benedeti et al., 2015). New designs and modifications of the dual-flow continuous system allow parameters such as temperature, pH, and buffers

to be controlled (Del Bianco Benedeti et al., 2015). Fermenters will allow for simulation of how feed and fluids circulate in the rumen, or first stomach where a majority of digestion takes place, while keeping the experiment in a controlled environment (Hannah et al., 1986). Importantly, the bacterial composition, digestibility of organic matter, crude protein degradation, passage of amino acids, degradation of amino acids, and ammonia-nitrogen amounts does not significantly differ between in vivo experiments (using live animals) and the fermenters used in this design (Hannah et al., 1986). The dual-flow continuous system design will permit the rates that solids and liquids leave the fermenters to vary at their own rate (Hoover et al., 1976), allowing for the experiment to more effectively simulate a true cow rumen. All of the previously mentioned benefits of a dual-flow continuous system will help to update the limited in vivo techniques used previously (Cook & Harris, 1952).

The foundational experiment that measured the nutritional value of cheatgrass found that cattle are more likely to graze on mature cheatgrass in the winter, as long as protein supplements and water are available (Cook & Harris, 1952). Protein supplementation in this case hints that the crude protein in *B. tectorum* may be low, a parameter I will measure in my experiment. My experiment will use urea as the nitrogen source. While urea itself does not contain amino acids, the microbes present in the rumen are able to convert urea into microbial amino acids and therefore protein that can be utilized by the cow (Tisch, 2009). Thus, urea will be a suitable nitrogen supplement.

Carbohydrate supplementation acts primarily as an energy supplement (Tisch, 2009). However, other experiments have found that increasing sugars in the diet led to an increase in organic matter (OM) intake and digestibility than controls (Heldt et al., 1999).

Additionally, the digestion of starch (fiber) was significantly increased with carbohydrate supplementation (Heldt et al., 1999), which is especially important for my experiment since cheatgrass is primarily composed of fiber (Cook & Harris, 1952). These results address the lack of literature on how carbohydrate supplementation affects the digestibility of forages like *B. tectorum*. Previous experiments described have focused on the lack of protein in forages like cheatgrass (Kal'nitskii & Kharitonov, 2010; Njoya, 1997).

#### Purpose of this experiment

The experiment described in this thesis aims to measure the digestibility and nutritional value of cheatgrass with supplementation of energy, nitrogen, or both through the use of a dual-flow continuous system and four different diets (treatments) given to fermenters.

## Materials and Methods

### Fermenters and Dual Flow Continuous System Conditions

The experimental design, developed by the Animal Nutrition Lab, consisted of 8 fermenters in 4 x 4 Latin square design, meaning that 8 fermenters (glass containers that mimic the rumen of cattle) were studied with 4 experimental diets over 4 experimental periods (Figure 3). 4 fermenters were used in each period, and each of these 4 fermenters received one of the experimental diets randomly, so that each fermenter was given all of the experimental diets over the course of the experiment. The random assignment of diets ensured that there was no unconscious bias towards any one fermenter.

Period 1		Period 1							
1	A	C	B	5	B	C	A	D	
2	B	D	C	A	6	A	D	C	B
3	C	A	D	B	7	C	B	D	A
4	D	B	A	C	8	D	A	B	C

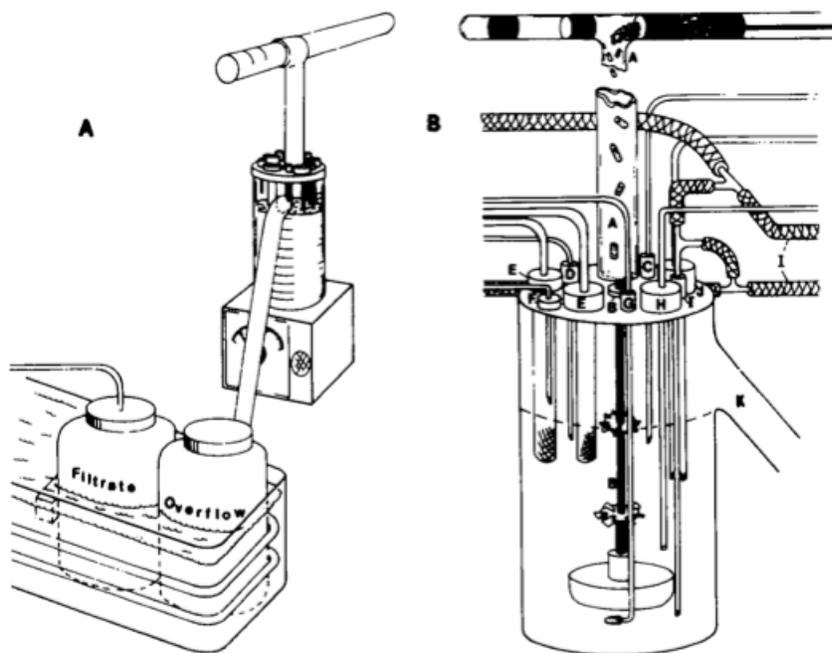
  

Period 2		Period 2							
1	D	B	C	A	5	C	B	A	D
2	C	A	B	D	6	A	D	B	C
3	B	D	A	C	7	B	C	D	A
4	A	C	D	B	8	D	A	C	B

**Figure 3. 4x4 Latin Square Design Schematic.** 1-8 = fermenters in the lab. A-D = different experimental diets. 4 periods were achieved by having fermenters 1-8 run 4 fermenters separately, in regards to the different diets, twice.

Each fermenter had a volume of 1,223mL and was obtained from Omni-Culture Plus, Virtis Co. Inc. The fermenters were put into a dual-flow continuous system,

consisting of the fermenter itself with ports for artificial saliva, feed, and nitrogen (Fig. 4). Both liquid and solid portions of the material exiting the fermenters were able to exit at their own rates (Fig. 4). These portions represent the materials, and thus nutrients, which were digested by microbes and, theoretically, would be available for the cow to absorb in an experiment using live cattle.



**Figure 4. Schematic of a fermenter in a dual-flow continuous system (Hannah et al, 1986).** "A" shows that the liquid (filtrate) and solid (overflow) digesta are collected separately. "B" shows the various inputs for saliva and nitrogen gas.

Fermenters were continuously kept at 39°C. Nitrogen gas (N<sub>2</sub>) was continuously added to each fermenter at 40mL/min. Artificial saliva was added to each fermenter at 2mL/min, and the saliva contained urea in order to mimic how ruminant saliva contains urea due to nitrogen recycling, a natural process. I monitored to rates of saliva flow in the mornings post-feeding the fermenters. The components of artificial saliva follow the methods developed by Weller and Pilgrim (1974), and are listed in Table 1 in the

appendix.

### Experimental diets

The four diets implemented were 1) forage only (*Bromus tectorum*), 2) cheatgrass and urea, 3) cheatgrass and molasses, and 4) cheatgrass supplemented with both urea and molasses. Urea was used as the nitrogen supplementation source and molasses was used as the energy supplementation source. The cheatgrass and urea diet contained 1.36% urea and 96.5% cheatgrass. The cheatgrass and molasses diet contained 15.9% molasses and 82.1% cheatgrass. The cheatgrass with both urea and molasses diet consisted of 1.28% urea, 19.3% molasses, and 77.3% cheatgrass. The cheatgrass used in the diets was mature and collected from Reno, Nevada. The proportions of ingredients of these diets were devised by graduate students in the Animal Nutrition Lab. The cheatgrass samples were passed through a 2-mm screen and turned into pellets for feeding. I helped create these pellets through grinding the cheatgrass in a spice grinder. The chemical composition of the molasses and cheatgrass used in these experiments is listed in Table 2 in the appendix. The chemical composition of each diet is listed in Table 3, and this information was used to determine the differences between the nutritional value of the diets themselves and the products of the diets in the fermenters.

All diets were fed to the fermenters on a dry matter basis, meaning that the water content of each feed was removed prior to feeding. Ammonium sulfate was added to diets containing urea at a 9:1 ammonium sulfate to urea ratio, as this is the ratio optimal for nitrogen and sulfur to support growth of the microbes and to ensure that sulfur-containing amino acids (methionine, cysteine) can be synthesized by the microbes. I frequently fed

the fermenters in the morning and ensured that the feed didn't disrupt the flow of other fluids inside the fermenters.

The microorganisms supplied to each fermenter were taken from the rumen of two cannulated Aberdeen Angus steers that had been on a diet of 60% straw, 30% orchard hay, and 2% ionized salt for one week before the samples were taken. The fluid obtained from the rumen was strained using four cheesecloth layers. Each fermenter was given about 10 L of fluid from the rumen, with 5 L coming from one steer and 5L coming from the other steer.

**Table 3. Ingredient list and chemical composition of experimental diets.** <sup>1</sup>Treatments: CON = control diet (98% DM of cheatgrass); URE = cheatgrass plus urea (1.36% DM of urea plus 96.5% DM of cheatgrass); MOL = cheatgrass plus molasses (15.9% DM of molasses plus 82.1% DM of cheatgrass); URE+MOL = cheatgrass plus urea and molasses (1.28% DM of urea, 19.3 % DM of molasses, plus 77.3% DM of cheatgrass). <sup>2</sup>Liquid molasses = obtained from Cerri Feed Co. (Stock, CA). <sup>3</sup>Mineralized salt = fed per kilogram of DM: Zn, 56 mg; Mn, 46 mg; Fe, 22mg; Cu 12 mg; I, 0.9mg; Co, 0.4mg; Se 0.3 mg; vitamin A, 6,440 IU; vitamin D, 2000 IU; vitamin E, 16 IU. <sup>4</sup>NFC = non-fiber carbohydrate. <sup>5</sup>EE = ether extract.

Item	Treatment <sup>1</sup>			
	CON	URE	MOL	URE+MOL
Ingredient, %DM				
Pelleted cheatgrass	98.09	96.5	82.1	77.3
Liquid molasses <sup>2</sup>	0.00	0.00	15.9	19.3
Urea	0.00	1.36	0.00	1.28
Ammonium sulfate	0.00	0.15	0.00	0.14
Mineralized salt <sup>3</sup>	2.00	2.00	2.00	2.00
Chemical composition, % DM				
DM, %	95.3	95.3	91.4	90.6
OM	93.7	92.2	92.8	90.6

CP	4.01	8.24	4.21	8.23
NDF	68.2	67.2	57.1	53.8
ADF	52.2	51.4	43.7	41.1
NFC <sup>4</sup>	19.9	18.1	29.9	30.3
EE <sup>5</sup>	1.13	1.11	1.24	1.25
Ash	6.32	7.77	7.19	8.74

### Periods and Sample Collection

Each period consisted of a 7-day period for adaptation to the diet, and then a 3-day period used for sampling. Each fermenter was given 72g of their diet in 4 equivalent meals at 2:30am, 8:30am, 2:30pm, and 10:30pm. pH meters were used to measure the pH of each fermenter daily. Both liquid and solid portions of digesta overflow from the fermenters were collected and the weights of both portions were individually recorded daily at 8:00am. These portions were removed as waste during the 7-day adaptation period. I frequently measured the pH of each fermenter as well as participated in feeding the fermenters in the morning.

During the 3-day collection period, the liquid and solid portions of digesta overflow (adding up to 500mL) were kept in their containers and put into a 2°C water bath. 25 mL of 50% sulfuric acid was added to each container to stop the microbes in the samples from fermenting any more after collection. The liquid and solid portions collected for each day were added together in a 1,500mL container, and 300mL from each larger container were freeze-dried at -20°C and then manually ground, and then stored. Two 10mL samples from the homogenized digesta overflow (in the 1,500mL

container for each fermenter) were passed through 2 layers of cheesecloth and stored separately every day during the 3-day sampling period. 0.2 mL of 0.2 M sulfuric acid was added. The two 10mL samples were separately centrifuged at 10,000 x g for 10 minutes. The supernatant was taken from these samples and frozen at -20°C.

On day 5 of the adaptation period, the liquid and solid portions of digesta overflow were mixed together by hand. 0.077g of 10.2%  $^{15}\text{(NH}_4\text{)}_2\text{SO}_4$  (which will be referred to as  $^{15}\text{N}$  for short) was added to each fermenter in order to label the bacterial nitrogen in the fluid. 0.077g/L of  $^{15}\text{N}$  was added to the artificial saliva to replace the urea portion as well.  $^{15}\text{N}$  is a marker for  $\text{NH}_3 - \text{N}$ . Infusion of  $^{15}\text{N}$  started on day 5 to ensure the marker will be in a plateau state when sample collection starts on day 7.

On days 7, 8, and 9, each fermenter's pH was recorded every hour for 6 hours after feeding. On day 10 of each period, all of the digesta inside the fermenters was mixed in a blender and passed through 2 layers of cheesecloth, then centrifuged at 5°C at 1,000 x g for 10 minutes. The centrifugation removed any undigested feed. The supernatant was then centrifuged for 20 minutes at 5°C at 10,000 x g in order to separate the microbes from the other components of the fluid, which were found in the solid pellets formed. The pellets were freeze-dried and ground down.

### Chemical analyses

The 300mL consisting of liquid and solid digesta from each fermenter over the 3-day sampling period that were freeze-dried and ground were analyzed on the basis of digestibility. Digestibility analyses were done using methods from the Association of

Analytical Communities, or AOAC (1990), for diet components of dry matter (DM), crude protein (CP), ether extract (EE; fat content), and ash (mineral content). Briefly, DM was measured drying a subsample of the 300mL sample of digesta in an oven to remove the water content via evaporation. I participated in preparing and measuring the DM content of the cheatgrass itself and the DM of the digesta collected from the fermenters. Ash content was measured by burning a subsample of the 300mL sample of digesta in an oven at a higher temperature in order to burn away organic material, leaving only minerals. Briefly, EE was measured by adding ether extract to a subsample of the 300mL sample of digesta to dissolve the lipids (fat). These methods were also used on the feed in the diets, so that the nutritional value of the feed itself could be compared to the nutritional value of the digesta (products of fermentation by microbes in the fermenters). Apparent and true digestibilities were calculated by members of the Animal Nutrition Lab, where apparent digestibility is an underestimate of digestibility that does not include endogenous protein (protein that would be made by the cattle in live experiments). True digestibility accounts for the endogenous protein. The organic matter of the feed and digesta was calculated by subtracting the measured ash from the measured dry matter. Measuring organic matter is important because organic matter represents the portion of the feed that actually provides nutrients. Neutral detergent fiber and acid detergent fiber amounts were measured in sequence, using methods from Van Soest et al (1991) for the Ankom<sup>200</sup> Fiber Analyzer (Ankom Technology, Macedon, NY). Briefly, a subsample from the 300mL sample of digesta was dried, packaged into small pouches, and submerged into the Ankom<sup>200</sup> Fiber Analyzer with neutral detergent fiber for the first measurement, and then acid detergent fiber for the second measurement. Measuring

neutral detergent fiber (NDF) and acid detergent fiber (ADF) amounts is important because they effectively represent the fiber components of the feed, and fiber is an important feed component for ruminants since they have microbes that can digest fiber for energy.

The non-fiber carbohydrate (NFC) portions of the DM were calculated using NRC (2001) guidelines and the following formula:

$$\text{NFC} = 100 - (\% \text{NDF} + \% \text{CP} + \% \text{EE} + \% \text{ash})$$

The NFC portions of diets using ammonium sulfate and urea as experimental treatments were calculated using Hall (2000) guidelines and the following formula:

$$\text{NFC} = 100 - [\% \text{CP} - (\% \text{CP from urea} + \% \text{CP from ammonium sulfate}) + (\% \text{urea} + \% \text{ammonium sulfate}) + \% \text{NDF} + \% \text{EE} + \% \text{ash}]$$

Different formulas were used to calculate NFC because the addition of urea and ammonium sulfate altered the nutrient composition of the diet, especially in regards to protein because microorganisms in ruminants can use nitrogen to make microbial amino acids. These formulas were necessary because NFC is not taken as a direct measurement, like DM, OM, EE, CP, NDF, ADF, and Ash were. Instead, it is a calculation based on the measurements of DM, OM, EE, CP, NDF, ADF, and Ash.

The supernatant taken from one of the 10mL samples for each fermenter was analyzed for  $\text{NH}_3 - \text{N}$  content according to Chaney & Marbach (1962) guidelines with a spectrophotometer.  $\text{NH}_3 - \text{N}$  is a representation of the protein that the microorganisms don't convert into microbial protein. In other words, it represents the protein waste to the animal and can therefore be used to evaluate protein degradation and microbial protein efficiency.

The supernatant taken from the other 10mL sample for each fermenter was analyzed for volatile fatty acids (VFAs) via gas chromatography. The equipment used was a Varian Model 3800 with a glass column (dimensions 180cm by 4mm). Nitrogen (N<sub>2</sub>) was the carrier gas used, with a flow rate of 85mL/minute. The oven was kept at 125°C, the injection port at 175°C, and the detector port at 180°C. VFAs are produced by microbes after the organisms take up glucose from the diet. So, the efficiency at which the animal receives usable glucose is determined by what kind/how many each of VFA is produced. The individual VFAs measured in the experiment were: propionate, acetate, butyrate, valerate, and isovalerate. I oversaw the equipment for some, but not all, of the individual VFA analyses for each fermenter.

The digesta from the last collection day and the bacterial pellets were measured on the basis of DM, CP, and ash, as well as for <sup>15</sup>N. A EuroVector model 3028 elemental analyzer was used to measure the isotope amounts present in the samples using the guidelines in Werner et al. (1999).

The nitrogen flow of bacteria was calculated using the following formula:

$$\frac{[\text{non-ammonia nitrogen flow} \times \% \text{ } ^{15}\text{N atoms in digesta outflow}] / (\% \text{ } ^{15}\text{N atoms in bacterial pellet})$$

with the <sup>15</sup>N digesta subtracted from the <sup>15</sup>N enrichment.

The efficiency of the bacteria was calculated using the following formula (Calsamiglia et al, 1996; Soder et al, 2013; Benedeti et al, 2015):

$$\text{Bacterial nitrogen flow (in grams)} / \text{truly digestible OM (in kilograms)}$$

## Statistical Analyses

Statistical analyses were calculated based on the 4 x 4 Latin square arrangement using the GLIMMIX procedure of SAS (release 9.4; SAS Inst. Inc., Cary, NC). The arrangement used the following formula:

$$Y_{ijkl} = \mu + S_i + F(S)_{ij} + P_k + T_l + e_{ijkl}$$

Where  $\mu$  represents the overall mean,  $S_i$  represents the square the fermenter was in (2 placements were possible),  $F(S)_{ij}$  represents the fermenter (F) in the square (meaning the literal placement of the fermenter in the lab),  $P_k$  represents the period,  $T_l$  represents the treatment, and  $e_{ijkl}$  represents the human error involved in measuring the other variables.

LSMEANS/DIFF LINES was the mean method of separation. Data was considered significant at  $P \leq 0.5$ , with trending data being defined at  $0.05 < P \leq 0.10$ . The standard error of mean (SEM), which is equal to the standard deviation divided by the square root of the sample size, show how accurate the mean values calculated are.

pH data was also analyzed using the GLIMMIX procedure of SAS (release 9.4; SAS Inst. Inc., Cary, NC) using the formula:

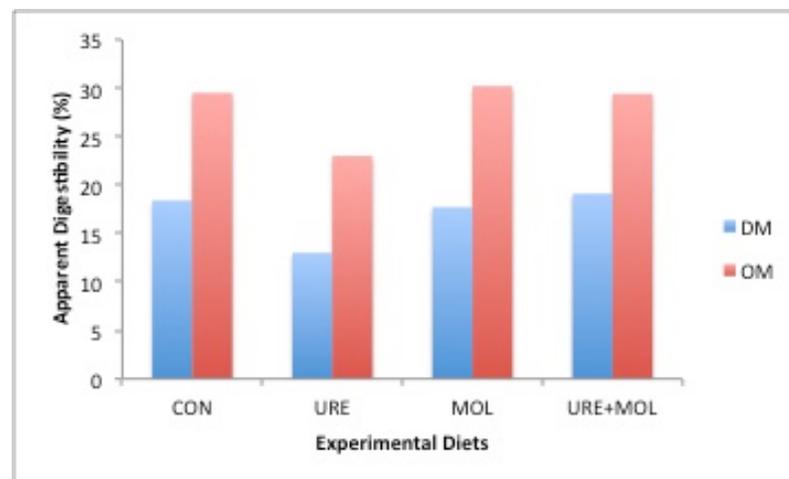
$$Y_{ijklm} = \mu + S_i + F(S)_{ij} + P_k + T_l + Z_m + ZT_{ml} + e_{ijklm}$$

Where  $\mu$  represents the overall mean,  $S_i$  represents square,  $F(S)_{ij}$  represents fermenter (F) in the square,  $P_k$  represents the period,  $T_l$  represents the treatment,  $Z_m$  represents the time,  $ZT_{ml}$  represents the how time and treatment interact, and  $e_{ijklm}$  represents the human error involved in measuring the other variables. This formula is necessary because pH measurements were taken over 6 hours, so the variable of time needed to be accounted for.

## Results

### Digestibility

The addition of urea alone (cheatgrass plus urea diet) tended to decrease the apparent digestibility of dry matter ( $P = 0.10$ ) and organic matter ( $P = 0.06$ ), as shown in Figure 5. The decreased apparently digestibility of DM and OM with diets containing urea could be explained by the different degradation rates of different feed components. Urea alone diets resulting in less apparent digestibility of DM and OM is significant because this means that adding protein via nitrogen supplementation alone leads to lower digestibility of nutrients overall. Diets containing molasses (molasses alone diet and molasses plus urea diet) had increased true digestibility of organic matter ( $P = 0.02$ ), as seen in Figure 5. The urea plus molasses diet resulted in an average of 24.6 g N/kg of organic matter that was truly digestible in the rumen.

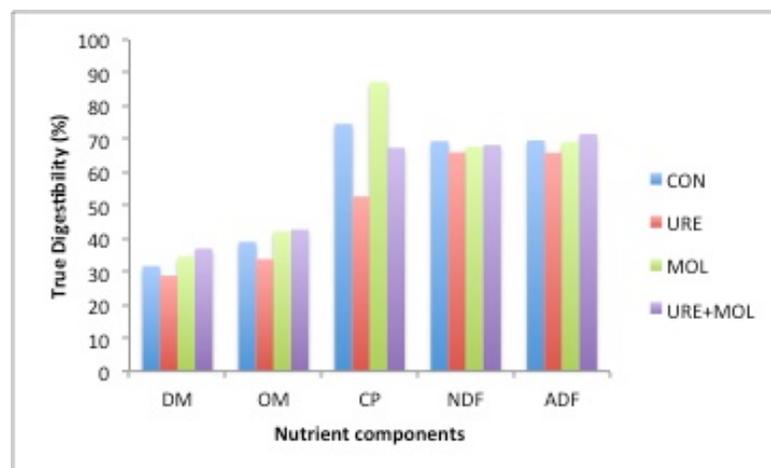


**Figure 5. Effects of experimental diets on digestibility of dry matter (DM) and organic matter (OM).** CON = control diet (98% DM of cheatgrass); URE = cheatgrass plus urea (1.36% DM of urea plus 96.5% DM of cheatgrass); MOL = cheatgrass plus molasses (15.9% DM of molasses plus 82.1% DM of cheatgrass); URE+MOL = cheatgrass plus urea and molasses (1.28% DM of urea, 19.3% DM of molasses, plus 77.3% DM of cheatgrass)

The organic matter value of 24.6 g N/kg is close to the ideal ratio of bacterial efficiency, which is 25g N/kg of OM being truly digestible in the rumen (Czerkawski,

1986), indicating that combination of molasses and urea led to almost optimal digestion of organic matter and, therefore, most nutrients.

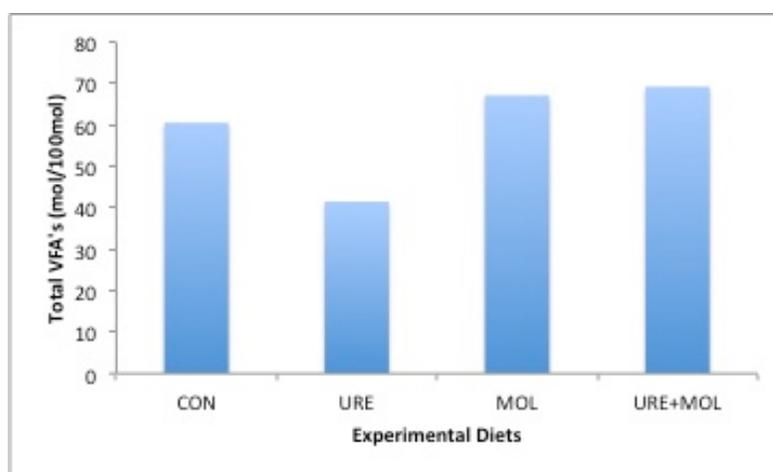
The true digestibility of crude protein (CP) was greater in the molasses only diet ( $P < 0.01$ ), as shown in Figure 6. Increased CP from feeding fermenters the molasses only diet is important because cheatgrass alone is poor in terms of CP (Cook & Harris, 1952). The diet with the combination of urea and molasses resulted in lower CP digestibility than just feeding cheatgrass alone in the control diet ( $P < 0.01$ ), as seen in Figure 6. Since increasing the CP of cheatgrass was one the major goals of this experiment, the decrease in CP in the cheatgrass plus molasses plus urea diet shows that the combination of nitrogen and energy supplementation is not effective in increasing the protein digestibility of cheagrass. The true digestibilities of neutral detergent fiber and acid detergent fiber were not affected by any diet ( $P > 0.05$ ), as shown in Figure 6.



**Figure 6. Effects of experimental diets on digestibility of different nutrients.** CON = control diet (98% DM of cheatgrass); URE = cheatgrass plus urea (1.36% DM of urea plus 96.5% DM of cheatgrass); MOL = cheatgrass plus molasses (15.9% DM of molasses plus 82.1% DM of cheatgrass); URE+MOL = cheatgrass plus urea and molasses (1.28% DM of urea, 19.3% DM of molasses, plus 77.3% DM of cheatgrass). DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber

## pH and Volatile Fatty Acids (VFAs)

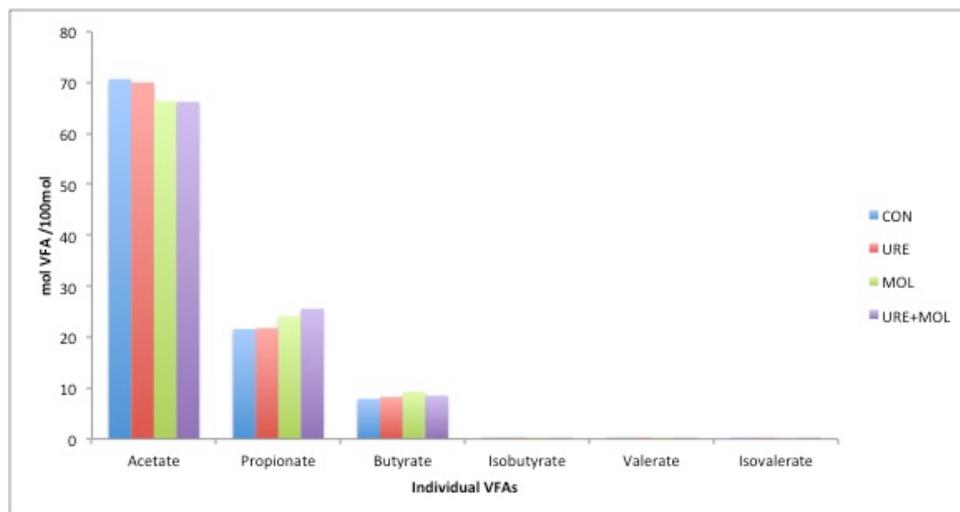
Diets with molasses (molasses alone and molasses plus urea) had decreased pH ( $P < 0.01$ ) and increased VFA concentrations ( $P < 0.01$ ), as shown in Figure 7. The diet containing urea and molasses had an increased VFA concentration ( $P < 0.01$ ) that was greater than the molasses diet alone (Fig. 7) The observation of more VFAs in the diet containing both urea and molasses indicates that this combination allows energy and nitrogen to be used more effectively by microbes.



**Figure 7. Effects of experimental diets on the concentration of total VFAs.** CON = control diet (98% DM of cheatgrass); URE = cheatgrass plus urea (1.36% DM of urea plus 96.5% DM of cheatgrass); MOL = cheatgrass plus molasses (15.9% DM of molasses plus 82.1% DM of cheatgrass); URE+MOL = cheatgrass plus urea and molasses (1.28% DM of urea, 19.3 % DM of molasses, plus 77.3% DM of cheatgrass). VFA = volatile fatty acid.

Propionate concentration was greater ( $P < 0.01$ ) and acetate concentration was less ( $P < 0.01$ ) when the diet contained molasses (cheatgrass plus molasses; cheatgrass plus molasses plus urea) compared to the control (Fig. 8). Increasing propionate resulting from molasses supplementation is favorable because propionate is a precursor to forming glucose in ruminants (Bergman et al., 1968). Butyrate concentration tended to be higher in diets with molasses (cheatgrass plus molasses; cheatgrass plus urea plus molasses),

meaning that the diets containing molasses resulted in less methane production, as butyrate is less likely to result in methane production than acetate (Fig. 8). Diets with molasses (cheatgrass plus molasses; cheatgrass plus urea plus molasses) led to a decreased concentration of isobutyrate ( $P = 0.02$ ) and a smaller acetate to propionate ratio ( $P < 0.01$ ), as shown in Figure 8. Acetate metabolism by ruminants leads to an increase of methane and therefore loss of energy (Tisch, 2009), so the decreased acetate to propionate ratio is indicative of increased glucose utilization and less waste for diets with molasses (cheatgrass plus molasses; cheatgrass plus molasses plus urea). The molasses only diet tended to increase valerate ( $P = 0.07$ ) and isovalerate ( $P = 0.08$ ), as shown in Figure 8. The molasses only diet also had more ( $P = 0.03$ ) total branched-chain VFAs than the other diets. An increased amount of branched-chain VFAs (valerate, isovalerate, and isobutyrate) in the molasses only diet is indicative of increased lipid digestion, since branched-chain VFAs are long, microbe-produced fatty acids (Tisch, 2009).



**Figure 8. Effects of experimental diets on concentrations of individual VFAs.** CON = control diet (98% DM of cheatgrass); URE = cheatgrass plus urea (1.36% DM of urea plus 96.5% DM of cheatgrass); MOL = cheatgrass plus molasses (15.9% DM of molasses plus 82.1% DM of cheatgrass); URE+MOL = cheatgrass plus urea and molasses (1.28% DM of urea, 19.3 % DM of molasses, plus 77.3% DM of cheatgrass). VFA = volatile fatty acid.

The molasses only diet resulted in lower ( $P < 0.01$ ) pH in comparison to the other experimental and control diets (Table 4.) The average pH for the two diets containing molasses was 6.52. The average pH (6.52) of the two diets containing molasses is significant because a pH of 6.4 is considered the prime pH of the rumen for microbes to effectively digest cellulose (Hoover, 1986), a major component of cheatgrass. The urea only diet had significantly higher pH ( $P < 0.01$ ), as shown in Table 4.

**Table 4. Effects of experimental diet on the average pH inside the fermenters.** CON = control diet (98% DM of cheatgrass); URE = cheatgrass plus urea (1.36% DM of urea plus 96.5% DM of cheatgrass); MOL = cheatgrass plus molasses (15.9% DM of molasses plus 82.1% DM of cheatgrass); URE+MOL = cheatgrass plus urea and molasses (1.28% DM of urea, 19.3 % DM of molasses, plus 77.3% DM of cheatgrass).

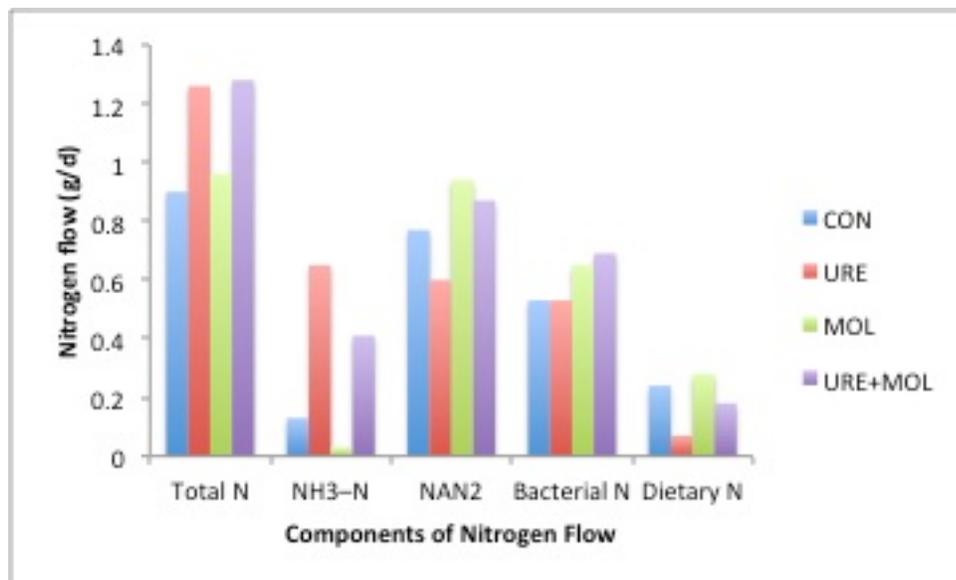
	CON	URE	MOL	URE+MOL
pH	6.99	7.50	6.45	6.59

#### Metabolism of Nitrogen

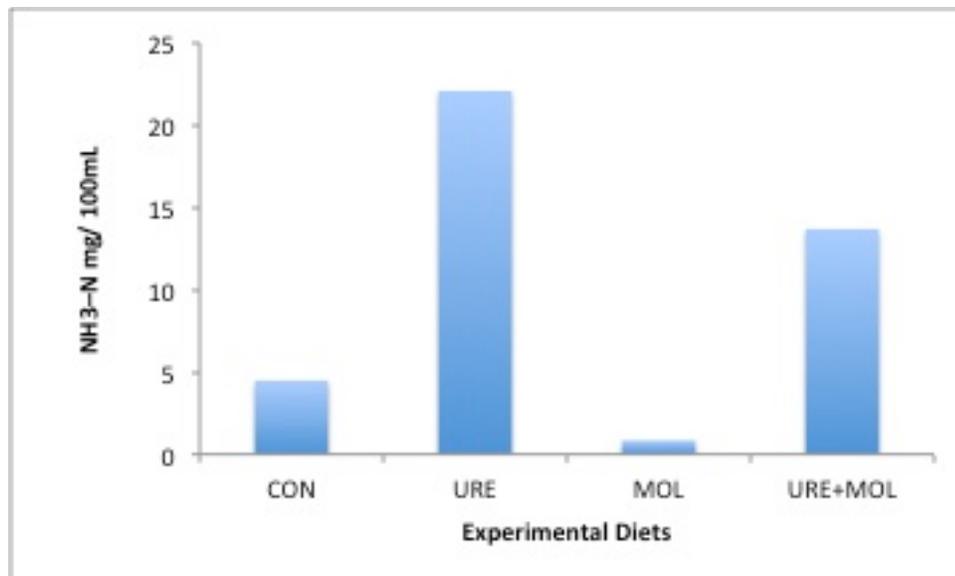
Diets containing urea (urea alone and urea plus molasses) resulted in increased  $\text{NH}_3 - \text{N}$  concentration, and this concentration was higher when urea was supplemented alone (Fig. 9). Higher  $\text{NH}_3 - \text{N}$  concentration in the urea only diet indicates that the urea only diet led to less utilization of nitrogen (urea) by the microbes. The  $\text{NH}_3 - \text{N}$  concentration was lower ( $P < 0.01$ ) in the urea plus molasses diet in comparison to the urea only diet (Fig. 9). Less  $\text{NH}_3 - \text{N}$  in the cheatgrass plus urea plus molasses diets indicates that the combination of nitrogen (urea) and energy (molasses) supplements increases the how effectively microbes use nitrogen. Diets containing molasses had lower ( $P < 0.01$ )  $\text{NH}_3 - \text{N}$  concentration in comparison to the control diet and urea only diet (Fig. 9). The molasses only diet had the smallest ( $P < 0.01$ )  $\text{NH}_3 - \text{N}$  concentration and

higher ( $P < 0.01$ ) bacterial nitrogen flow in comparison to the urea only diet (Fig. 9). The cheatgrass plus molasses only diet resulting in the lowest  $\text{NH}_3 - \text{N}$  suggests that the addition of energy supplementation (molasses) alone led to the most nitrogen metabolism by microbes.

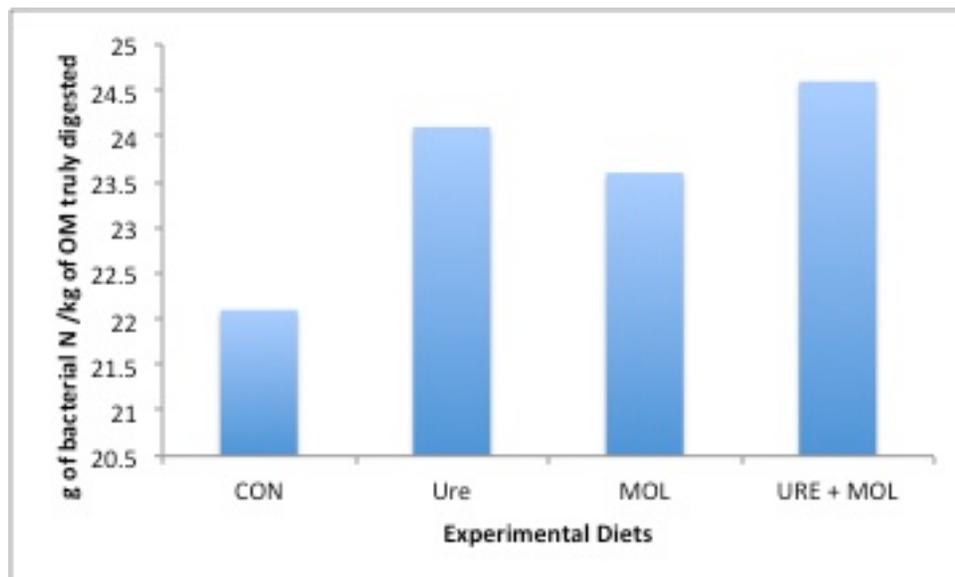
The flow of nitrogen and  $\text{NH}_3 - \text{N}$  was higher in the diets containing urea ( $P < 0.01$ ), as shown in Figure 10. Diets containing molasses resulted in more non-protein nitrogen ( $P = 0.04$ ) and bacterial nitrogen ( $P < 0.01$ ) in the outflow digesta than the other diets (Fig. 9; Fig 11). None of the experimental diets affected bacterial efficiency significantly ( $P = 0.83$ ). While the effect was not statistically significant, bacteria efficiency was marginally higher in the urea plus molasses diet.



**Figure 9. Effects of experimental diets on different components of nitrogen flow.** CON = control diet (98% DM of cheatgrass); URE = cheatgrass plus urea (1.36% DM of urea plus 96.5% DM of cheatgrass); MOL = cheatgrass plus molasses (15.9% DM of molasses plus 82.1% DM of cheatgrass); URE+MOL = cheatgrass plus urea and molasses (1.28% DM of urea, 19.3 % DM of molasses, plus 77.3% DM of cheatgrass). N = nitrogen;  $\text{NH}_3 - \text{N}$  = representation of protein waste;  $\text{NAN}^2$  = non-ammonia nitrogen.



**Figure 10. Effects of experimental diets on NH<sub>3</sub> – N concentration.** CON = control diet (98% DM of cheatgrass); URE = cheatgrass plus urea (1.36% DM of urea plus 96.5% DM of cheatgrass); MOL = cheatgrass plus molasses (15.9% DM of molasses plus 82.1% DM of cheatgrass); URE+MOL = cheatgrass plus urea and molasses (1.28% DM of urea, 19.3 % DM of molasses, plus 77.3% DM of cheatgrass). NH<sub>3</sub> – N = representation of protein waste.



**Figure 11. Effects of experimental diets on bacterial nitrogen.** CON = control diet (98% DM of cheatgrass); URE = cheatgrass plus urea (1.36% DM of urea plus 96.5% DM of cheatgrass); MOL = cheatgrass plus molasses (15.9% DM of molasses plus 82.1% DM of cheatgrass); URE+MOL = cheatgrass plus urea and molasses (1.28% DM of urea, 19.3 % DM of molasses, plus 77.3% DM of cheatgrass). N = nitrogen; OM = organic matter.

## Conclusion

### Overall

How efficiently nutrients are utilized by microbes in the rumen shows how effective the addition of multiple supplements is for ruminants (Hersom, 2008). Measurements of how well nutrients are working together in a diet include total VFAs, individual VFA concentrations, pH, and  $\text{NH}_3 - \text{N}$  concentration (Hersom, 2008). The urea plus molasses diet resulted in the greater true digestibility of OM, total concentration of VFAs, pH,  $\text{NH}_3 - \text{N}$  concentration, and flow of bacterial nitrogen. These results are all evidence that the supplementation of carbohydrates and nitrogen together, through molasses and urea, resulted in increased fermentation by microbes. This urea plus molasses diet was especially effective in terms of VFA concentration and microbial nitrogen, which are correlated with a better environment in the rumen for bacteria.

Interestingly, NDF digestion was not improved by supplementation. NDF corresponds to the true fiber fraction of feed. Ruminants contain microbes that are able to produce the enzymes necessary to digest fiber, resulting in more energy, higher production levels, microbial growth, and a better rumen environment (Tisch, 2009). Future experiments involving nitrogen and energy supplementation should focus on discovering how supplementation may affect NDF digestibility, since fiber is a huge component of forages like *B. tectorum*.

### Discussion

Different feed supplements have been shown to have different levels of solubility, rates of degradation, and chemical properties than the main forage used in the diet

(Hersom, 2008). These differences can affect how well some of the nutrients are digested (Hersom, 2008). Since cheatgrass and urea degrade at different rates, this may be why the urea only diet tended to decrease the apparent digestibilities of DM and OM.

The higher digestibility of CP with the molasses only diet suggests that the energy from molasses was utilized to release the protein found in the cheatgrass, which would increase the overall CP digested. However, the urea plus molasses diet resulting in a lower true digestibility of CP could be explained by a previous study that found adding supplements can complicate the feed either a beneficial or harmful way (Moore et al., 1999). So, the molasses and urea may have interacted in a way that lowered the CP amounts compared to cheatgrass alone.

The observation diets containing molasses diet resulted in more VFAs than the control and urea only diet could be explained by conclusions from previous experiments (Mould et al., 1983), which state that adding supplements may improve microbes in the rumen and increase the amount of VFAs produced. Additionally supplemented carbohydrates that are degraded by microbes in the rumen lead to more VFAs produced and lowered pH (Bargo et al., 2002; Kennedy & Bunting, 1992).

The greater concentration of propionate and lesser concentration of acetate in molasses-containing diets could be explained the bacterial populations being changed by the different fermentation patterns that resulted from differences in the material being fermented (Ribeiro et al., 2005). Carbohydrates have also been observed to increase the proportion of propionate to other VFAs (Kellogg & Owen, 1969). The smaller acetate to propionate ratio observed in diets with molasses could also be explained by these diets containing more ( $P < 0.01$ ) propionate on a molar level before entering the fermenters.

The higher concentration of butyrate in the molasses-containing diets may have been the result of changing how fermentation is occurring through providing more hydrogen from the sugar in molasses (Piwonka & Firkins, 1996). The addition of carbohydrates that are soluble in the rumen to diets has also been shown to increase the amount of propionate and/or butyrate (in moles), while decreasing the amount of acetate (Chamberlain et al., 1985; Khalili & Huhtanen, 1991a)

. The higher pH observed in the urea only diet could have been caused by the increased  $\text{NH}_3 - \text{N}$  also observed in the urea only diet (Haaland et al., 1982). Also, urea diets result in ammonia formation in the rumen, which is the primary base for ruminants (Owens et al., 1998), would make the pH basic and therefore high on the pH scale.

The higher concentration of  $\text{NH}_3 - \text{N}$  in diets with urea could be explained by the urea degradation rate being greater than fermentation of carbohydrates supplied by the cheatgrass and the rate of microbes using urea to form other products. This is because ammonia (from the urea) can be a nitrogen source that microbes can use for growth when carbohydrates are present to use for energy (Allison, 1969). The  $\text{NH}_3 - \text{N}$  concentration being lower in the urea plus molasses diet could be explained by previous findings that there is evidence that the concentration of  $\text{NH}_3 - \text{N}$  in the rumen is inversely correlated to factors that support use of  $\text{NH}_3 - \text{N}$  by microbes (Kolver & de Veth, 2002; Petit & Veira, 1994). So as these factors (availability of energy and nitrogen) increase, the concentration of  $\text{NH}_3 - \text{N}$  decreases. This also explains why the diets containing molasses had lower concentrations of  $\text{NH}_3 - \text{N}$ . Additionally, there is evidence that as the concentration of easily fermented carbohydrates increases, the concentration of  $\text{NH}_3 - \text{N}$  decreases (Stern

et al., 1978). This is most likely because microbes are able to more effectively utilize nitrogen as more carbohydrates are added.

Higher non-protein nitrogen (NPN) and bacterial nitrogen in the digesta outflow of diets with molasses could be due to ammonia being responsible for over half of the microbial nitrogen produced (Nolan, 1975). The observation that none of the experimental diets affected bacterial efficiency could be explained by the procedure used to find bacterial efficiency. Bacteria could have been lost during isolation or there could have been an error in measuring how organic matter was digested in the molasses only and urea only diets.

This experiment has shown that supplementation of urea plus molasses is an effective strategy to increase the digestibility of DM, OM, and important VFAs such as propionate. Also, this diet increased total nitrogen and bacterial nitrogen, meaning that microbes were able to create microbial protein more effectively. With proper supplementation, cheatgrass could become useful forage for cattle, providing nutritional benefits as well as conservation benefits. The hold of *B. tectorum* on native species could be alleviated while also providing cattle more easily digestible nutrients. Further experiments should focus on finding the appropriate ratio of urea to molasses to cheatgrass to optimize nutritional benefits.

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## Appendix

**Table 1. Artificial saliva components (Weller & Pilgrim, 1974).** <sup>1</sup>anhydrous = containing no water.

Item	Per 15 L
Na <sub>2</sub> HPO <sub>4</sub> (anhydrous <sup>1</sup> )	26.4 g
NaHCO <sub>3</sub>	75.0 g
KCl	9.0 g
MgSO <sub>4</sub> · 7 H <sub>2</sub> O	1.88
KHCO <sub>3</sub>	24.0
NH <sub>2</sub> CONH <sub>2</sub>	6.0
Distilled Water	15.0

**Table 2. Chemical composition of components of experimental diets.** <sup>1</sup>Molasses = Liquid molasses from Cerri Feed Co. (Stock, CA). <sup>2</sup>NFC = non-fiber carbohydrates. <sup>3</sup>EE = ether extract

Item	Ingredient	
	Cheatgrass	Molasses <sup>1</sup>
DM %	94.6	70.9
OM, %DM	95.2	90.4
CP, % DM	4.09	5.40
NDF, % DM	69.6	N/A <sup>4</sup>
ADF, % DM	53.2	N/A <sup>4</sup>
NFC <sup>2</sup> , %DM	20.3	83.1
EE <sup>3</sup> , % DM	1.15	1.86
Ash, %DM	4.80	9.63