ABSTRACT

CHAVEZ, STEPHEN JOHN. Preference and Estimation of Intake in Cattle, Horses, and Pigs. (Under the direction of Dr. Gerald Huntington).

The objectives were to 1) use alkanes to estimate intake of cattle consuming a mixed forage diet and then to use the alkane method to estimate intake of grazing horses and 2) determine preference for feeds with nutritive additives that can influence intake through orosensorial properties. Twelve beef cattle were housed individually with access to six feeding stations and fed a supplement dosed with dotriacontane and hexatriacontane. They were given a blended alfalfa:switchgrass hay flavored with water for control, sucrose as sweetener, or citric acid as sour additive. Intake was estimated from hay and fecal analyses for alkanes. There was no difference ($P < 0.35$) between actual and estimated intake using hentriacontane (C31) as an internal marker. Cattle preferred ($P < 0.01$) the hay with sucrose addition over both control and citric acid addition, while preferring ($P < 0.01$) the control over citric acid.

The alkane procedure previously used in the cattle was used to estimate intake of horses stall-fed or grazing pasture. Oats were dosed with hexatriacontane. Six mature geldings were randomly assigned to either a hay-fed or pasture treatment for 14 d and then switched to the other treatment. All horses were housed in stalls without feed from 0700 to 1300 daily and given hay ad libitum or access to pasture for 1300 to 0700. Horses were supplemented with hexatriacontane alkane-coated oats. Fecal grab samples were collected twice daily during d 8-12 of each period. Actual intake did not differ ($P < 0.49$) from estimated intake in hay-fed horses when C31 or tritricontane (C33) were used as internal markers. However, estimated DMI of pasture with C31 differed ($P < 0.001$) from estimated
pasture intake with C33. Estimated pasture intake with C31 was $11.74 \pm 0.51$ kg DM/d and $13.90 \pm 0.53$ kg DM/d with C33.

Alkanes were used to estimate and compare intake of horses that began their daily grazing in the AM versus PM. Using oats to administer the even-chained alkane, the same six horses were assigned to either initiation of grazing in AM from 0700 to 1500 or PM from 1230 to 2030 in a random switchback design. Horses grazed non-toxic endophyte-infected tall fescue and were housed individually in pens with water during the remainder of the d. There was a tendency for an interaction where horses had higher estimated intakes ($P < 0.07$) in period 2, while intakes were higher in the PM for estimates with C33 as compared to C31. When estimating intake with hentriacontane, estimated intake was higher ($P < 0.03$) in period 2 and tended ($P = 0.10$) to be higher in the PM.

Twenty second parity or greater sows of mixed breed were randomly assigned to a sweet or umami taste group with a nutritive and non-nutritive ingredient taste additive in each group. The sweet group consisted of control (CON), sucrose (SU) or non-caloric sweetener (SW) and the umami group with CON, monosodium glutamate (MSG) or non-nutritive umami additive (UM). The effect of flavoring agent fed to sows on piglet taste preference at weaning was determined by cross-fostering piglets across respective taste groups at parturition. At weaning, pigs were given 3-d double-choice preference tests. The SU and SW pigs preferred ($P < 0.03$) SU over CON, SU and CON pigs preferred ($P < 0.02$) SU over SW, and CON pigs preferred ($P < 0.01$) SW over CON. Pigs in the UM group preferred ($P < 0.02$) MSG over CON, preferred ($P < 0.03$) MSG over UM, but no preference ($P > 0.16$) UM over CON. Piglets preferred the feed with nutritive taste additives.
Preference and Estimation of Intake in Cattle, Horses, and Pigs

by
Stephen John Chavez

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Nutrition

Raleigh, North Carolina
2011

APPROVED BY:

Dr. Gerald Huntington
Committee Chair

Dr. Paul Siciliano

Dr. Eric van Heugten

Dr. C. Scott Whisnant
BIOGRAPHY

Stephen J. Chavez was born on July 28, 1982 in York, Pennsylvania. Spending much of his youth exploring the state forest in Northern Pennsylvania, he became interested in wildlife nutrition and behavior. During high school, Stephen participated in many organizations and received the graduating class award for excellence in the sciences. He had decided there was only one university that he was willing to attend, and he was accepted to the Pennsylvania State University. Majoring in Animal Bioscience, Stephen kept his interest in behavior with a minor in Psychology, where he was inducted in Psi Chi, the International Honor Society in Psychology. Stephen’s animal interests were in horses and poultry. Stephen worked for two years after graduating in 2004 with high distinction so he could save money to continue his education where he attended West Virginia University under the direction of Dr. Eugene Felton. Working to connect behavior data with nutrition in cattle, goats, and sheep, he became friends with many dietetics graduate students. After completing his Master’s of Science in Animal and Nutritional Sciences, he accepted an internship at the Indianapolis Zoological Society under Dr. Jason Williams. Spending the summer researching comparative nutrition in exotic species, he focused on taste research in dolphins. At the end of the internship, Stephen moved to Raleigh, NC to begin his PhD in Nutrition and Animal Science at North Carolina State University.
TABLE OF CONTENTS

LIST OF TABLES.................................................................v
LIST OF FIGURES.............................................................vii
Chapter 1: Literature Review..................................................1
  Introduction..........................................................................2
  Taste.................................................................................2
  Taste receptors.................................................................3
  Hedonic reward.................................................................5
  Early flavor exposure.........................................................6
  Nutrition content and taste..................................................8
  Post-ingestive feedback.......................................................11
  Eating rates........................................................................13
  Total intake.........................................................................14
  Hormonal factors of intake..................................................15
  Taste and hormones............................................................17
  Measuring and estimating intake............................................18
  Alkanes..............................................................................19
  Alkane analysis.................................................................22
  Estimation of intake............................................................23
  Dry matter digestibility.......................................................23
  Summary............................................................................24
  Literature Cited....................................................................24

Chapter 2: Estimation of intake in cattle fed a blended switchgrass and alfalfa hay diet......................................................37
  ABSTRACT..........................................................................38
  Introduction..........................................................................38
  Materials and Methods.......................................................40
  Results................................................................................45
  Discussion............................................................................46
  Conclusion............................................................................48
  Literature Cited....................................................................48

Chapter 3: Intake estimation of horses grazing tall fescue (Festuca arundinacea) or fed tall fescue hay.......................................53
  ABSTRACT..........................................................................54
  Introduction..........................................................................54
  Materials and Methods.......................................................56
  Results................................................................................58
  Discussion............................................................................59
  Literature Cited....................................................................62
Chapter 4: Intake estimation of horses grazing tall fescue (*Festuca arundinacea*) during morning or evening……………………………………………………………………………………………………69
ABSTRACT…………………………………………………………………………………………………………………………..70
Introduction………………………………………………………………………………………………………………………71
Materials and Methods…………………………………………………………………………………………………………72
Results………………………………………………………………………………………………………………………………..75
Discussion…………………………………………………………………………………………………………………………75
Literature Cited…………………………………………………………………………………………………………………79

Chapter 5: Preference of a ground switchgrass and alfalfa hay blend flavored with sucrose or citric acid in cattle………………………………………………………………………………………………………………86
ABSTRACT…………………………………………………………………………………………………………………………87
Introduction………………………………………………………………………………………………………………………..88
Materials and Methods…………………………………………………………………………………………………………89
Results………………………………………………………………………………………………………………………………..91
Discussion…………………………………………………………………………………………………………………………92
Literature Cited…………………………………………………………………………………………………………………94

Chapter 6: Preference in weanling pigs for sweet or umami taste after in utero exposure……………………………………………………………………………………………………………………………101
ABSTRACT…………………………………………………………………………………………………………………………102
Introduction………………………………………………………………………………………………………………………..103
Materials and Methods…………………………………………………………………………………………………………104
Results and Discussion…………………………………………………………………………………………………………106
Literature Cited…………………………………………………………………………………………………………………110

Chapter 7: Conclusion…………………………………………………………………………………………………………125
# LIST OF TABLES

Chapter 2

**Table 2.1.** Alkane composition of hays (mg/kg sample DM)...........................................51  
**Table 2.2.** Alkane concentrations of fecal samples (mean ± SD)...........................................52

Chapter 3

**Table 3.1.** Chemical Composition of feeds.................................................................64  
**Table 3.2.** n-alkane concentrations (mg/kg DM) of feeds consumed in treatments........67  
**Table 3.3.** n-alkane concentrations (mg/kg DM) of feeds consumed in treatments........68

Chapter 4

**Table 4.1.** Nutrient Composition of tall fescue (Festuca arundinacea).............................82  
**Table 4.2.** Alkane concentrations (mg/kg DM) of feeds consumed by horses.................83  
**Table 4.3.** Intake estimates of horses grazing AM and PM in either period 1 or 2........84  
**Table 4.4.** Nonstructural Carbohydrate (% DM)............................................................85

Chapter 5

**Table 5.1.** Nutrient composition of hays and concentrate..............................................96

Chapter 6

**Table 6.1.** Sow gestation diet composition and nutrient composition for crude protein, water soluble carbohydrate, and Na.................................................................114  
**Table 6.2.** Sow lactation diet composition and crude protein, water soluble carbohydrate, and Na.................................................................115  
**Table 6.3.** Nursery diet composition and nutrient content for crude protein, water soluble carbohydrate, and Na.................................................................116
Table 6.4. Preference % for type of sweet additive on weanlings from different sweet gestation and lactation treatments…………………………………………………………………….118

Table 6.5. Preference % for type of umami additive on weanlings from different umami gestation and lactation treatments…………………………………………………………………….119
LIST OF FIGURES

Chapter 1

Figure 1.1: Diagram of a) location of papillae on tongue; b) taste buds in each papilla; c) taste receptor cell within a taste bud…………………………………………………………………34

Figure 1.2: Mechanism of pathways through taste receptor cells leading to activation of neurons and taste discrimination……………………………………………………………………..35

Figure 1.3: Intensity of post-ingestive feedback determines the level of preference or aversion of a given food…………………………………………………………………………………36

Chapter 3

Figure 3.1. Hay intake in individual horses with actual and estimated intake using C31:C36 or C33:C36 alkane pairs…………………………………………………………………………65

Figure 3.2. Hay intake in individual horses with actual and estimated intakes as a % body weight using C31:C36 or C33:C36 alkane pairs……………………………………………………66

Chapter 5

Figure 5.1: Cattle DMI (kg) ± MSE of CON, S100, and CA50 in Experiment 1…………………97

Figure 5.2: Hay intake (kg) ± MSE at each feeding station, Experiment 1………………………98

Figure 5.3: Cattle intake (kg) ± MSE of CON, CA10, and CA25, Experiment 2…………………99

Figure 5.4: Cattle intake (kg) ± MSE of S100, S150, and S25, Experiment 2…………………100

Chapter 6

Figure 6.1. Diagram of cross-fostering piglets with the number of pens represented during preference testing for each gestation/lactation combination…………………………………….117

Figure 6.2. Serum insulin in weanlings across dietary treatments………………………………120

Figure 6.3. Sow serum insulin concentrations before and after feeding across dietary treatments………………………………………………………………………………………….121
Figure 6.4. Sow plasma glucose before and after feeding across dietary treatments……..122

Figure 6.5. Weanling serum leptin across gestation and lactation treatments…………….123

Figure 6.6. Sow serum leptin pre- and post-feeding across dietary treatments………………124
Chapter 1: Literature Review
Introduction

When given the opportunity to select among feeds, mammals will often take time to test their options before choosing a feed to consume. Grazing herbivores select food sources that vary in nutrient composition and must consume the required nutrients independently of one another when quality forages are unavailable (Simpson et al., 2004). This selection of foods to consume varying amounts of nutrients is caused by independent regulation of nutrients and possibly learned. Animals may then select feeds which are more valuable in certain nutrients based on palatability, mainly taste, and post-ingestive feedback. Many factors affect which food is selected, such as taste, texture, and smell (Grovum and Chapman, 1988). Flavors and odors from spices such as anise and garlic are very strong and are known to transfer through milk and amniotic fluid into the human fetus (Hepper, 1988). This method of transmission can alter animal preference during early food choices due to familiarity of taste and smell.

Taste

The five basic tastes perceived by humans and many other mammals are sweet, salty, sour, bitter, and umami (Mombaerts, 2004). Salty and sour foods are associated with sodium and hydrogen ions, sweet with sugars, umami with glutamate, and bitter with toxins, such as alkaloids and tannins (Sola-Oriol et al., 2009; Provenza et al., 2000).

When newborn human infants were given flavored solutions to drink, the infants found water to be aversive and limited the intake of water (Desor et al., 1975). When flavors were added to a 0.07 M sucrose solution, citric acid flavor was found to be aversive but
neither the salty or bitter solutions were. The salty and bitter tastes could have been masked or not perceived in the sucrose solution.

Taste receptors

Of the five basic senses, taste and smell are very similar in regards to the type of chemosensory stimulation (Mombaerts, 2004). Taste is one of the five basic senses used by mammals playing a major role in food intake. This basic sense allows animals to identify different feed constituents such as sugars, acids, salts, and amino acids in the oral cavity (Roura et al., 2008). Taste receptors are found on papillae of the tongue and soft palate of humans (Mombaerts, 2000). Papillae are seen in three major types, fungiform, circumvallate, and foliate, which each have many taste buds (Figure 1.1).

Taste receptors develop at different times, beginning in utero and shortly after parturition and innervation at the IX cranial nerve can prevent the full development of vallate taste buds (Hosley et al., 1987). A sensitive period occurs during the first ten days postnatally in the rat. If damage is done later in life, taste bud regeneration is more likely than early in life. The development of taste buds is less likely if damage is done during this sensitive period.

Several taste receptor families exist for vertebrates that correspond to sweet and umami tastes (T1R family) and bitter taste (T2R family) that can affect a vertebrate’s feeding preference (Shi and Zhang, 2006). Both types of receptors are G-protein coupled receptors (Figure 1.2). Sweet taste is found when T1R2 and T1R3 form heterodimers while umami and L-amino acids are perceived with T1R1 and T1R3 heterodimers. When comparing several
vertebrate species, T1R appears to be well conserved while the T2R has more variation. This change in bitter taste receptor cells may reflect toxins or other negative compounds found in different foods that have traditionally been eaten by different vertebrates over the years. Pseudogenes, or unexpressed genes, are highly variable in the T2R across species possibly reflecting evolution and adaptation of methods to avoid bitter or toxic food. Cattle have a higher number of pseudogenes for bitter taste perception possibly from decreased toxic effects with the rumen environment (Li et al., 2005). Very few vertebrate species have been identified that have lost sweet taste perception, most notably the chicken which lost the T1R2 gene. The T1R2 appears to become inactivated in cats, hence the lack of preference for sweet food (Li et al., 2005).

Sour taste is aversive because the taste is associated with acids in foods (Huang et al., 2008). Different acids have different intensities ranging from weaker sour taste in mineral acids to stronger in organic acids. The type of acid influences how quickly the cytosol can be acidified. Type III presynaptic taste bud cells in mice have been found to respond to sour taste with changes in Ca$^{2+}$ influx and a release of serotonin but no effect on Type II cell ATP secretion.

When taste receptor signaling was removed in genetically modified mice, mice still preferred glucose over isocaloric amino acids (Ren et al., 2010). This was independent selection for glucose without the taste signaling on the tongue of mice. This preference may result from post-absorptive metabolic processes with glucose oxidation or glucose transport to the brain affecting dopamine release independent of the nerve pathways for taste. The same taste receptors in the gut were also affected in the knockout mice and did not appear to
have a role in the preference for glucose. Thus the ability of each nutrient to affect glucose oxidation may have caused the increased preference for carbohydrate over a protein.

Nutrient content of foods influences caloric intake (Simpson et al., 2004). Because many people are affected by diseases such as diabetes and are required to lower their sugar intakes, artificial sweeteners are increasingly popular. Frank et al. (2008) compared sucrose and sucralse (artificial sweetener) and both had high correlations for sweetness. Although both sucrose and sucralse bind to the sweet taste receptors, brain activation pathways differ with sucrose activating ten anatomical regions and sucralse only activating three. The main primary taste cortex, the frontal operculum and anterior insula, had similar activations from both sucrose and sucralse, but sucrose activating more brain regions may be why sucrose initiates a different brain response without humans identifying a major difference in sweetness (Frank et al., 2008).

**Hedonic rewards**

Stressful situations can often cause an animal to seek calming or familiar stimuli, such as a familiar location or food. Handling of young rats is a stressful stimulus and resulted in depressed accumbens dopamine metabolism and decreased preference for sweet foods (Silveira et al., 2010). Young rats typically have a strong preference for sweet foods and non-handled rats had more hedonic responses to 1 M sucrose solution than handled rats with no difference in aversive reactions.

When considering the taste of food, the intensity can also influence human preference (Havermans et al., 2009). The strength of the flavor can influence what is called sensory-
specific satiety. Sensory-specific satiety is when the pleasantness of the food decreases and a novel food would be preferred. Havermans et al. (2009) found no difference in the ratings of individuals given various intensities of strawberry lemonade and concluded that the hedonic processing of the various intensities may be found in a different location of the brain as that determining preference based on the intensity of the food.

**Early Flavor Exposure**

In utero exposure to certain feed components may alter later preferences from taste and odor. Many foods have a distinct odor, and when consumed, can change the odor of amniotic fluid, such as seen in humans (Mennella et al., 1995). The consumption of garlic not only alters the odor of amniotic fluid, but can change the taste and odor of breast milk in women. Women that consumed anise flavor during pregnancy had infants that preferred the odor of anise during the first several days after birth (Schaal et al., 2000). Babies not exposed to the anise flavor in utero had an aversive or neutral response to the odor.

Prenatal exposure in pigs when a sow was fed anise-flavored feed decreased stress behaviors compared to unexposed pigs during gestation (Oostindjer et al., 2009). Morrow-Tesch and McGlone (1990) found that piglets have well-developed olfactory abilities to differentiate between novel odors. The piglets preferred being near scents from the mother’s feces or skin secretions and avoided the mother’s urine. Crying time in newborn infants was decreased when infants were separated from their mother but provided the odor of the mother’s amniotic fluid (Varendi et al., 1998). Crying time was not different when the babies were provided with the smell of the mother’s breast milk or control.
Feed selection in many species can be influenced by the mother-offspring relationship. Orphaned lambs were fed a milk replacer flavored with either onion or garlic and were given preference tests to determine if the early exposure in milk would alter their preference when transitioned to solid feed (Nolte and Provenza, 1992). Lambs preferred the feed flavored with the same treatment as in the milk replacer and consumed more than 50% of total intake of that feed. Lambs recognized these flavors without having a social aspect to learning which feeds are nutritious from their mother.

Each flavor has a different capacity to cross into milk from blood during lactation making the composition of milk diverse in flavors and intensity (Hausner et al., 2008). Volatiles in the human mother’s diet are clearly transferred into the milk, but non-volatiles such as sugars, glutamate, and urea may also affect early exposure to flavors. Sucrose infusion (2.5%) into the ovine fetus resulted in increased swallowing while saline infusion did not (El-Haddad et al., 2005). Early exposure can also affect intake after weaning in pigs (Langendijk et al., 2007). Sows fed diets with anise and garlic increased post-weaning intake of piglets weaned at six weeks of age. King (1979) fed flavored diets to sows and found the flavor, Firanor No. 24 (Firmenich et Cie, Geneva), was transferred through the milk at 9 ppm. Weaned pigs then had a higher intake when the flavor was added to the starter diet and had a tendency for higher average daily gains compared to pigs consuming unflavored diets.

Weaning is very stressful for most mammals, including pigs (Oostindjer et al., 2010). Although the in utero flavors may not be preferred by piglets at weaning, the familiarity of the flavors led to higher intake and resulted in a higher body weight. Of great importance was also the reduced incidence of diarrhea compared to controls when anise flavor was fed during
gestation. Fewer negative behaviors were seen in pigs that were from sows of the flavored diets, such as aggression and oral manipulation, all which can be seen in high stress environments. Human infants with prenatal exposure in breast milk or in utero exhibit fewer negative behaviors when given food with the same flavor (Mennella et al., 2001). The consumption of carrot juice either during gestation or lactation decreased facial expressions of young babies fed solid foods with carrot flavor. Palatability early and later in life may be influenced just as much by odor as well as taste during in utero exposure.

The maternal diet can alter preferences besides for taste alone (Bayol et al., 2007). Pregnant rats fed high energy, fat, sugar, and salt diets were followed through gestation and lactation and the pups monitored through growth. This type of diet was used to mimic that of high calorie, high fat, high sugar diets characteristic of many human diets. The offspring from the rats consuming the junk food diet were heavier and had a higher body mass index, while also preferring foods higher in fat, sugar, and salt at the expense of protein. The resultant hyperphagia in the female rats was associated with decreased energy expenditure and increased body fat stores, but no difference in number of offspring. Once the high fat, sugary diets were removed, the rats had a reduced intake similar to control rats.

**Nutrient content on taste**

The nutrient content of plants changes throughout the day with increasing exposure to sunlight, altering animal preference. Goats and sheep have a preference for hays harvested in the PM over hays cut in the AM; PM hays have a higher concentration in soluble carbohydrate with higher DMI (Fisher et al., 2005; Burns et al., 2007). The higher soluble
carbohydrate in the plants was at the expense of neutral detergent fiber (NDF). PM harvested gamagrass (*Tripsacum dactyloides* L.) had higher monosaccharides, polysaccharides, total nonstructural carbohydrates, and less NDF, with no change in starch or crude protein compared to AM cut hay (Sauve et al., 2009). This selection for carbohydrate may not be for energy, but rather the taste of the simple sugars.

When lambs were used to compare preference for hay from AM and PM cuttings with or without added 0.45% DM glucose and 0.35% DM sucrose, lambs preferred the PM cut hay; however, there was no preference for AM hay with sucrose and glucose over AM hay alone (Buritt et al., 2005). When lambs were offered AM hay or AM hay with 1% DM added sugar (1:1 glucose:sucrose), there was no preference for one over the other. When offered AM hay versus AM with 2% DM, AM with 3% DM, or AM with 4% DM, the sheep had preferences for the added sugar hays after the first two days of the preference test.

When glyphosate herbicide was sprayed onto annual ryegrass-silver grass pasture to delay nutrient content loss from the forage, preference in sheep was altered (Siever-Kelly et al., 1999). Herbage composition was altered where stems increased two-fold in water soluble carbohydrate content and had a smaller increase in water soluble carbohydrates in leaf and seed heads. Sheep preferred herbicide-sprayed forage over control and preferred stem over leaf in the sprayed forage. In the control, sheep preferred leaf or seed head rather than stem. Preference was for higher water soluble carbohydrate content and higher digestible plant components were chosen only when water soluble carbohydrates were the same.

With the change in fiber content of forages affecting preference (Burns et al., 2007; Sauve et al., 2009), nutritive content of forage can alter preference in many studies where
taste is not a main focus (Catanese et al., 2010). Early exposure of low quality forages (high NDF) resulted in lambs preferring more high quality forage compared to lambs that had experience with the high quality forage. Lambs with early exposure to the high quality forage (low NDF) had higher intakes of the low quality forage compared to lambs previously fed the low quality forage.

Rodents, birds, fish, insects, and some monkeys have a strong preference for high protein feeds, and when feeds are low in protein, will over-consume feeds high in energy to meet their protein requirements (Brooks et al., 2010). This overconsumption of energy may be a leading cause of obesity in some species.

Infants consuming different types of formula have different preferences for foods later in life (Mennella and Beauchamp, 2002). Sour-flavored juices were preferred by infants that consumed protein hydrolysates, while those fed soy formulas preferred bitter-flavored juice. Children fed milk based formulas demonstrated more negative behaviors later in childhood when given sour or bitter foods.

Scott and Provenza (1998) examined the effect of flavor and nutrient content of diets given to sheep at different locations. Sheep had no preference for flavor (apple, aniseed, unflavored) when the diets being compared differed in composition; however, when the basal diet was the same, lambs went to the feeding location that had a variety of flavors. Thus, nutrient content of the forage may be the primary reason for food selection with taste being of secondary importance.

When lambs were given preference tests for three feeds varying in nutrient content, the lambs chose the feed with the highest energy and protein (Provenza et al., 1996). When
LiCl was added to the hay to create an aversive stimulus, the lambs still consumed the high energy diet but in a smaller amount. This aversion did not influence the intake of the other hays most likely because the lambs were familiar with the hay. If this were a novel hay with LiCl, then lambs may have avoided the feed altogether. When given all three feeds, intake of digestible energy and digestible protein was higher. The lambs may have lost interest in the feed they were consuming and wanted variety based on post-ingestive feedback or sensory input like taste and odor.

**Post-ingestive feedback**

The degree to which an animal continues to eat a certain food is based on sensory-specific satiety and nutrient-specific satiety (Early and Provenza, 1998). After exposure to one flavor for a short amount of time, the animal preferred a different flavor; however, lambs avoided feeds that were deficient or in excess of energy when an adequate diet was available. The feedback the animal receives after eating a food helps regulate the taste with metabolic homeostasis. When heifers were given preference tests for maple-flavored or coconut-flavored hays, heifers preferred the opposite flavor in the preference test from what they had been previously fed (Atwood et al., 2001). The previous experience with the flavored hays influenced the future preference for the cattle to choose a different flavored hay of similar nutritive value.

The digestibility of feed can also affect post-ingestive feedback affecting animal preference (Early and Provenza, 1998). When lambs were fed in the morning with highly digestible feeds, their preference in the evening was for slowly digestible feeds, while
preferring different flavored feed from the morning. However, when lambs were fed in the morning with a slowly digestible feed, they continued to prefer the same in the evening, with no difference in flavor preference.

Mothers that consume alcohol during breast-feeding will give their child ethanol in the milk (Mennella and Beauchamp, 1991). The odor and the taste of the milk are altered for several hours with a peak ethanol taste around one hour after consumption of alcohol. Infants have decreased intake when breastfeeding milk after the mother consumed alcohol and the long-term effects are unknown of an infant consuming alcohol. Infants are limited in their ability to oxidize ethanol and remove the alcohol from their body.

Aversive feeds may cause nausea or vomiting and result in negative post-ingestive feedback (Launchbaugh et al., 2001). Post-ingestive feedback can alter preference based on the intensity of the feedback (Figure 1.3). Animals learn through the post-ingestive feedback to avoid feeds or continue to consume them for nutritional value (Villalba and Provenza, 2009). When lambs were given a familiar food along with a novel odor followed by LiCl to mimic toxicosis, the lambs given the LiCl consumed less food than those not given the LiCl (Provenza et al., 2000). Novel odors that may appear to be aversive to humans may not affect intake in lambs when no negative post-ingestive feedback occurs.

Adding flavors to mimic different tastes can alter intake for feeds (Grovum and Chapman, 1988). The intensity of the taste can alter the preference for the given feed. When pellets were sham-fed and preference measured with esophageal fistulas, the highest level of sucrose (120g sucrose/ kg pellet) depressed feed intake in sheep. Adding urea to the pellets for a bitter taste lowered intake at all concentrations (10-80 g urea/ kg pellet), while the
addition of HCl for a sour taste only lowered intake at the highest concentration tested (50 g HCl/ kg pellet). Monosodium glutamate for umami and NaCl for salty both increased intake when added to feed. By removing the post-ingestive feedback of feeds, preference can be altered.

When rats were given either 0.05% citric acid (sour) or 0.03% sucrose octaacetate (bitter) solutions with intragastric infusions of 16% glucose, rats preferred the solution with glucose rather than the opposing solution with a water infusion (Myers and Sclafani, 2003). When rats were given a neutral flavor with 2% sucrose and a different flavor with 2% maltodextrin, both were preferred over another non-flavored feed (Dwyer, 2005). When paired with illness, neither flavor was preferred.

When intensities of flavors were varied in combination with intragastric infusions of starch in lambs, lambs preferred the flavor intensity associated with the higher infusion of starch (Villalba and Provenza, 2000). Inexperienced lambs were then used that had no prior exposure to the starch infusion or the onion taste. Lambs preferred the weakest 5g/kg hay onion flavor over the 40g/kg onion flavor; however, they preferred the moderate 10g/kg and 20g/kg onion flavors over the weaker 5g/kg and stronger 50g/kg. Thus, strong flavor intensities may be more aversive in influencing preference, allowing grazing animals to alter their food choices to that of more moderate flavor intensities.

Eating rates

Taste is not the only factor that affects whether a food is chosen for consumption or avoided. When an animal does not have an option, certain tastes may alter the eating rate.
When rats are only given one feed that is less palatable than a normal rat chow, such as citric acid flavored rat chow, meal size is reduced and consumed at a slower rate (Johnson and Collier, 2001). Rats consumed more plain pellets when compared with 4% citric acid pellets, but had no difference in intake when plain were tested with saccharin pellets.

When dairy cows were observed with eating rates of concentrates flavored to taste sweet, salty, or bitter, the cows had faster eating rates when fed a sweet concentrate (Chiy and Phillips, 1999). No difference was seen in the first minute of eating from the prehension of feed; however, after the first minute, eating rates declined for the salty and bitter concentrates.

Horses were given 15 different flavors added to cereal grain by-products to determine preference and intake rate (Goodwin et al., 2005). Echinacea, nutmeg, and coriander were three flavors previously suggested to be added to equine diets to enhance flavor but were aversive to several horses. The flavors that had the highest preference determined by fastest intake rate were banana, cherry, carrot, rosemary, oregano, peppermint, cumin, and fenugreek. When banana and fenugreek were used to mask a mineral supplement, both flavors increased the time of consumption for the mineral.

**Total Intake**

If animals are unable to consume sufficient nutrients to meet their requirements, body energy stores are mobilized for energy depending on physiological state. During gestation, restricting intake can be beneficial to prevent excessive weight gain in the sow (Musser et al., 2004). Excess consumption of energy alters physiological states with increased plasma urea
nitrogen and IGF-1 in sows with ad libitum intake. Insulin can also be elevated during early gestation. Excessive intake results in changes in fetal composition with increased liver size and potential problems during parturition.

**Hormonal factors of intake**

Many hormones interact to signal hunger or satiation in animals. These hormones are considered to be anorexigenic or orexigenic. Anorexigenic hormones that decrease intake and increase satiety include leptin, cholecystokinin (CCK), glucagon, melanocyte stimulating hormone (MSH), and oxyntomodulin. Orexigenic hormones that stimulate intake are ghrelin and Neuropeptide Y (NPY).

Leptin is an anorexigenic hormone with many functions other than for feed intake in ruminants (Nonaka et al., 2006). Leptin is secreted from many tissues other than adipose tissue and is stimulatory towards GH secretion in sheep. Leptin secretion will also stimulate MSH secretion, while decreasing activity of NPY (Kojima et al., 2010). CCK has been shown to be one of the more potent anorexigenic hormones leading to decreased food consumption. CCK reduced the rate of feed intake in sheep when given ICV (Bueno and Riviere, 1987). CCK is a brain-gut peptide that decreases meal size and duration leading to early satiety in the rat (Moran, 2000). The hormone is most abundant in the duodenum and jejunum. CCK also works synergistically with leptin to decrease intake. Various peptide lengths for CCK occur in different species and differ in potency (Glatzle et al, 2008).

Melanocyte stimulating hormone has decreased intake in several species of animals including goldfish, chicks, and rats. Injection of MSH into the brain of chicks resulted in a
rapid decrease of food consumption (Smith et al., 2008). Glucagon has been shown to induce satiety in rats as well (Heppner et al., 2010). GLP-1 has been shown to decrease food intake in humans and rodents (Heijboer et al., 2006). GLP-2 has similar effects in rats as GLP-1; however, no effect was seen in humans. Oxyntomodulin is similar to GLP 1 and 2 and has been seen to decrease feed intake in both rats and humans. Oxyntomodulin can decrease plasma ghrelin, while stimulating insulin secretion and glucose production, along with increasing activity-related energy expenditure in humans (Heijboer et al., 2006; Wynne et al., 2006).

Ghrelin is stimulatory towards feed intake and peaks before meal periods (Reynolds et al., 2010; Sauve et al., 2010). Ghrelin concentrations decrease after feeding. Ghrelin can stimulate feed intake when administered peripherally (Heijboer et al., 2006). NPY is an orexigenic hormone leading to increased feed intake (Kojima et al., 2010). Agouti related protein has also been demonstrated to have orexigenic effects in rodents similar to NPY during fasting (Heijboer et al., 2006).

Most of the anorexigenic hormones are secreted postprandially to decrease feed intake. Each hormone may stimulate several neurons in the brain due to satiety factors. There appear to be many more satiety hormones that decrease feed intake than there are that stimulate feed intake. Feed intake may be influenced by many other factors other than hormones.

Taste and hormones
Wethers fed a high salt diet had reduced intake from the decreased palatability of a 20% salt dry matter diet (Blache et al., 2007). Plasma leptin and insulin concentrations decreased in wethers fed the high salt diet, with plasma glucose declining as well. When rats were given rat chow and water with sucrose, glucose, or fructose, rats had increased caloric consumption over those given rat chow and water, yet depressed rat chow intake (Lindqvist et al., 2008). The caloric intake from the liquid was designed to mimic soft drink consumption in humans, while finding this increased caloric intake altered normal hormonal feedback. Consuming any of the sugar solutions resulted in increased serum leptin concentrations, while decreasing serum PYY and hypothalamic NPY mRNA; however, serum ghrelin concentration increased with fructose consumption. Consuming foods that produce a hedonic response from sweet taste, intake regulation may be altered and cause increased feed consumption which may be undesirable.

When monosodium glutamate was fed to men to enhance the umami taste of a high protein diet, passage rate through the stomach was increased (Zai et al., 2009). The increased gastric emptying may be a result of taste receptors along the digestive tract that lead to increased gastric excretions altering passage rate of ingesta. When taste was evaluated in humans to compare salivary flow, taste receptors appear to have an influence (Hodson and Linden, 2006). Citric acid, monosodium glutamate, and NaCl all had higher salivary flow rates, possibly relating to the taste receptors from transfer of ions across the membrane rather than through G-coupled protein receptors.

Hormone receptors may also be found in the taste tissues of animals, at least in mice (Kawai et al., 2000). Leptin administration affected taste nerves of circumvallate papillae
affecting the response to sweet tastes. This decreased response to both sucrose and saccharin did not alter the taste sensitivity for sour, salty, or bitter food by leptin. The leptin administration altered biochemical pathways in the taste cells affecting potassium ions and changing the polarization of the cells. Intestinal absorption of galactose was inhibited by leptin in rats (Inigo et al., 2007). Leptin prevented sugar absorption by the SGLT1 transporter but did not affect intestinal permeability.

In obese and lean humans, Brondel et al. (2007) found no difference in flavor pleasure between the groups. Therefore, obese individuals may have lost the ability for taste sensitivity with sweet foods. Obesity does appear to have a role in preference for monosodium glutamate or umami taste in obese women, where obese women have lower taste sensitivity and consume higher concentrations of monosodium glutamate to receive the same benefit (Pepino et al., 2010).

**Measuring and estimating intake**

Markers are frequently used in animals to look at intake, digestibility, and rate of passage. When using markers, dosing time and the flow of the digesta are all important factors in evaluating the use of the marker with the respective animal and diet (Olivan et al., 2007). All markers come with potential errors and each marker should be evaluated to determine which is most appropriate. Sources of errors include delivery of marker, feed consumed, fecal excretion patterns, and analytical measurements (Ferreira et al., 2004). Common markers include chromic oxide, alkaline peroxide lignin, acid detergent lignin, and alkanes (Cochran et al., 1986; Ferreira et al., 2004; Momont et al., 1994). In vitro acid
detergent fiber and in vitro neutral detergent fiber do not make good internal markers due to high variation in fresh and immature forages due to high variability in marker ratios (Cochran et al., 1986). Concerns with chromic oxide as a marker have been the diurnal variation of passage of the marker (Momont et al., 1994). A desirable marker for intake would have a steady rate of passage and be indigestible. In grazing animals, collecting total feces may not be the best method of estimating intake due to interfering with natural grazing behaviors, so fecal grab samples may be more suitable, where alkanes could be used (Duncan et al., 1999).

Alkanes

Alkanes are mostly indigestible and excreted in the feces of animals (Duncan et al., 1999; Dove and Mayes, 1996). The alkanes are consumed and pass through the digestive tract of animals at a consistent rate in comparison to other external markers such as chromic oxide that may bind to digesta (Ferreira et al., 2004). Odd-chain alkanes naturally accumulate in plants and concentrations vary depending on species, while even-chain alkanes are rare in plants (Premaratne et al., 2005). Concentrations of alkanes are also variable in the part of plant that may be consumed (Dove et al., 1996). Although some of the shorter chain alkanes have lower fecal recovery rates, alkanes absent from the feed can be dosed to correct for recovery rates for intake calculations.

Alkanes can thus be used to estimate the intake of herbivorous species by measuring the alkanes in the plants consumed and the alkanes in the feces. For ruminants, adjacent alkanes are required to have an accurate estimate of intake (Ferreira et al., 2007), while in horses, any combination of odd and even-chain alkane can be used (Ordakowski et al., 2001).
This difference is noted because the fecal recovery of the short and long chain alkanes is similar in horses, while more variation is seen in cattle due to chain length (Smith et al., 2007).

One method of administering even-chain alkanes was with an intra-ruminal controlled-released capsule (CRC); however, use of CRC’s is decreasing due to the remnant cartridge left in the rumen of ruminants (Premaratne et al., 2005; Molina et al., 2004; Elwert et al., 2008) and lack of commercial supply. The manufacturer provides an expected release rate for the alkanes in the CRC for calculations; however, researchers should determine the release rate themselves to calculate the most accurate estimate of intake (Ferreira et al., 2004). Release rates can be calculated in fistulated cattle with calipers to determine the amount of capsule remaining. Alkane release from CRC’s may be inaccurate when trying to use manufacturer’s release rate and variation can over- or under-estimate intake (Charmley et al., 2003). When using a CRC, feeding frequency, use of multiple CRC’s, or level of feeding do not alter the accuracy of intake estimation (Dove et al., 2002).

A second method of administration is through a dietary supplement. Dosing can be done once daily by spraying even-chain alkanes on paper (Ferreira et al., 2007; Olivan et al., 2007; Hameleers and Mayes, 1998), grass (Stevens et al., 2002), gelatin capsule (Molina et al., 2004), or cottonseed meal (Dove and Charmley, 2008). In horses, due to the lack of a rumen and higher passage rate in the stomach, horses should be dosed two times a day with the even-chain alkane (Smith et al., 2007). Feeding a supplement may be the most desirable method of administering the even-chain alkanes because it is less invasive than other techniques. Providing the marker through drenching guns or with CRC are stressful to the
animal in comparison to feeding a less invasive dietary supplement. Feeding a supplement also increases the familiarity of animals to humans.

Fecal and feed samples must be collected to be analyzed for intake and output of alkanes as markers. Alkanes are provided by the CRC or supplement for several days to allow equilibration of the alkanes in the digestive tract before fecal samples are be collected for analyses. Horses and cattle should be fed even-chain alkanes for a minimum of seven days (Ferreira et al., 2007; Ferreira et al., 2004). There are no known diurnal patterns of excretion in ruminants for alkanes in the feces of cattle (Olivan et al., 2007); however, increased fecal sampling in horses can eliminate the variability in fecal alkane excretion (Smith et al., 2007). The digestive tract of the horse does not appear to have the same amount of mixing of digesta in the stomach, compared to the rumen of ruminants, allowing for an inconsistent flow of alkanes through the remainder of the gastrointestinal tract. Total fecal collection can be used to estimate intake with alkanes, but is very labor intensive and less feasible for grazing animals. Once daily fecal grab samples can provide an accurate estimate if the feces are pooled over a minimum of five days in horses (Smith et al., 2007). Fecal grab sampling can also be used for cattle using less labor and provide similar results to total fecal collection (Ferreira et al., 2004).

Fecal concentrations of alkanes can be used with known plant alkane concentrations to determine the amount of each forage consumed (Charmley and Dove, 2007). Depending on the number of species of plants the animal is eating, multivariate analysis can be used to determine the species and part of the plant the animal consumed (Dove et al., 1996). Lower concentrations of alkanes used in estimating intake may result in larger errors from the
equation, so alkane pairs with higher concentrations determined after analysis should be used (Olivan et al., 2007). Intake is usually estimated with the C32:C33 or C31:C32 pair for accuracy due to the similarity in alkane fecal recoveries (Ferreira et al., 2004).

**Alkane analysis**

The analysis of samples requires two steps: saponification and extraction. Tetratriacontane (C34) is typically used as an internal standard and may be used in conjunction with another alkane, such as docosane (C22), for the saponification and extraction procedures to avoid over- or underestimation of alkane concentrations (Ferreira et al., 2007; Olivan and Osoro, 1999).

Fecal and feed samples are freeze-dried and ground through a 1mm screen. Samples are then weighed and saponified with 1 M ethanolic KOH at 90 °C for several hours. Alkanes are then extracted with heptane or hexane, taken over a silica gel column, and the eluate collected and analyzed with gas chromatography (Olivan et al., 2007). Gas chromatography is then used to analyze peaks of alkanes of interest for all samples in comparison with known standards.

Fecal recovery, or indigestibility, of the alkanes from the extraction process needs to be similar in order for the estimation equation to be effective. Extraction must occur at hot temperatures (65 °C) or the alkanes will not be transferred in the heptane layer (Olivan and Osoro, 1999). Alkane extraction can vary greatly with chain length at different temperatures resulting in potential losses during the analytical procedure. Preparation of alkanes through
drying of feed and feces may also alter the natural alkane concentrations and oven-drying may decrease the true amount (Sandberg, 2000; Elwert et al., 2006).

**Estimation of intake**

Dry matter intake can be calculated with several equations modified from Mayes et al. (1986). The following is from Charmley et al. (2003):

\[ DMI = \frac{D_j}{(F_j/F_i) * H_i - H_j} \]

where \( H \) denotes the feed and \( F \) the feces, and \( i \) and \( j \) represent the odd and even alkane concentrations respectively. \( D_j \) denotes the release rate of the even chain alkane from the CRC.

Another modified equation from Stefanon et al. (1999) is

\[ DMI = \frac{((F_i/F_j) * ((D_j + S*S_j) - S*S_i) / (H_i - (F_i/F_j) * H_j)}{H_i - (F_i/F_j) * H_j} \]

where \( H, F \) and \( S \) denote the feed, feces, and supplement respectively, \( i \) and \( f \) represent the odd and even alkane concentrations, \( D_j \) is the even chain alkane from a CRC, if given, and \( S \) is the amount of supplement fed. \( D_j \) is removed from the equation for horses, while may be used in ruminants if the supplement is not dosed with additional alkanes.

Changes in the fecal ratios will influence DMI. Based on the previous two formulas, the fecal ratio is of most importance, because the supplement and forage compositions would remain consistent for the feedstuffs. Variations of alkanes higher in concentration have less impact on the final estimate than variations of alkanes in lower concentrations. When external marker was dosed at 224 mg per animal, there was no difference in twice or thrice daily dosing on DMI estimation (Smith et al., 2007). With a dosing of 448 mg per animal
even-chain marker, the marker had high variation in feces when given twice daily compared to thrice daily dosing.

**Dry Matter Digestibility**

Dry matter digestibility (DMD) can be calculated for animals to have an estimate on how well feed is digested in vivo. Simple equations for DMD can be $1 - \frac{\text{fecal output}}{\text{intake}}$, which is the same as $1$-indigestibility (Olivan et al., 2007). Different feeds are going to have different digestibilities based on the composition of the plant material. Higher cellulose and lignin will be less digestible in both ruminants and horses. Digestibility can be calculated using alkanes, where feeding level does not affect the outcome (Charmley et al., 2003). Digestibility is calculated based on the known intake of alkanes and the alkanes excreted, where the fecal recovery is known.

**Summary**

Alkanes have been used mainly in sheep and cattle to estimate forage intake and estimate digestibility; however, most researchers extrapolate that alkanes can be used in grazing animals consuming multiple forages, yet little research has been completed. Most researchers use fresh cut forages given to sheep individually housed and lack the realistic differences seen in groups of animals grazing. Alkanes can accurately estimate intake using the internal marker and externally dosed marker in hay fed sheep, cattle, and horses.

Although we have markers to estimate intake of animals, we do not fully understand the major influence affecting an animal’s feed selection when multiple feeds are available.
Palatability is a driving factor, but the influence of nutrient composition of feeds may also be a major influence. Taste has become a prime area of research because of the strong influence on palatability and associated post-ingestive feedback. With volatile compounds crossing through the mother’s blood and into the amniotic fluid, flavors have been added to sheep and swine diets to influence sheep and pig weanling intakes. Although most flavors have no nutritional benefit, a comparison between the nutritional and non-nutritional feed additives that influence taste would be beneficial to feed producers.
Literature Cited


Figure 1.1: Diagram of a) location of papillae on tongue; b) taste buds in each papilla; c) taste receptor cell within a taste bud.

Mombaerts, 2004
Figure 1.2: Mechanism of pathways through taste receptor cells leading to activation of neurons and taste discrimination.

Roper, 1992
Figure 1.3: Intensity of post-ingestive feedback determines the level of preference or aversion of a given food.

Launchbaugh et al., 2001
Chapter 2: Estimation of intake in cattle fed a blended switchgrass and alfalfa hay diet
ABSTRACT

Calculating dry matter intake of grazing herbivores has been an issue in determining factors such as efficiency and digestibility of forages. The objectives were to compare and contrast measured and estimated intake, and to measure digestibility using a supplement and estimate intake composition using fecal grab sample alkane composition. Twelve beef cattle were fed a blended switchgrass and alfalfa hay and fed a once-daily alkane supplement. Fecal grab samples were collected the last 5 d over the 14-d experiment. There was no difference between measured and estimated intake ($P < 0.35$). There was no day effect between fecal concentrations and estimated intake and no difference ($P < 0.65$) between fecal alkane concentrations when duplicate samples were analyzed. Alkanes can be used to estimate intake in cattle consuming mixed forage diets and intakes of individual components can be back-estimated using simultaneous equations.

Keywords: alkanes, steer, alfalfa, switchgrass

Introduction

Dry matter intake of grazing ruminants is difficult to determine because animals can select what they eat. Eating patterns can change from shifts in animal behavior and may be attributed, in part, to forage on offer. Environmental conditions can influence canopy structure and density of forage present; therefore, changing intake patterns and rate of passage. Various markers have been used to estimate intake; however, each marker has strengths and weaknesses. Alkanes have been used as markers for sheep, goats, and cattle.
(Mayes et al., 1986; Berry et al., 2000; Hendricksen et al., 2003; Dove and Mayes, 2005; Premaratne et al., 2005) by evaluating the ratio of odd-chain to even-chain alkanes in the supplemental dose, plant, and feces. Plants have higher concentrations of odd-chain alkanes, with little (but measurable) to no even-chained alkanes (Bugalho et al., 2004; Fraser et al., 2006).

Even-chain alkanes can be administered into the rumen in the form of a controlled release capsule (CRC) or they can be administered via treated material, such as cellulose or a feedstuff. In the former case the manufacturer provides a release rate for the CRC. However, with the fact that the CRC cartridge remains in the animal for life, unless the animal is fistulated, and release rate may not be consistent for all rations and hence rumen environments, a supplemental alkane on feed may be more desirable. For example, Berry et al. (2000) found the CRC to be within the manufacturer’s range, whereas discrepancies were noted with release rates by Ferreira et al. (2004).

Other methods of administering alkanes include once-daily offering of pelleted material previously treated with alkanes (Mayes et al., 1986; Olivan and Osoro, 1999; Ferreira et al., 2007) or the use of gelatin capsules filled with alkane-treated cellulose (Vulich and Hanrahan, 1995). Also, feed components can be treated with alkanes such as cottonseed meal (Elwert and Dove, 2005) or milled hay in aqueous suspension of xantham gum (Fushai, 2006), or forage can be used that has been treated or sprayed with alkanes (Giraldez et al., 2006).
The objectives of the experiment were to compare measured and estimated DMI in beef steers fed a mix of switchgrass and alfalfa hay and to estimate the proportions of switchgrass and alfalfa consumed based on alkane profiles of the hays.

**Materials and Methods**

The current protocol was approved by the North Carolina State University Animal Care and Use Committee.

Eleven beef steers and one beef heifer (BW = 283 ± 25 kg) were blocked by weight into three groups. They were fed for 3 wk a mixture of 75:25 switchgrass:alfalfa (wt:wt, as fed) ground in a hammer mill with a 1.91 cm screen to prevent sorting. The first week was an adaptation period since cattle had no previous exposure to hay diets, followed by the 14-d experiment. Cattle were housed individually under a roof on slotted metal platforms with access to six feeding stations. Cattle were weighed weekly and hay was provided in each location at 0.6% BW. Cattle were fed 0.92 kg DM of a supplement sprayed with C32 and C36 at 0830 daily so supplement would be consumed within 30 min. Hay orts were weighed at 0800 daily and new hay was provided at 0900 once all the supplement was consumed.

Fecal grab samples were collected once daily between 0800 and 1000 on days 10-14 of the trial period. If a steer did not defecate by 0930, manual fecal grabs were taken. Fecal samples were weighed and frozen until oven-drying to constant weight at 60°C. Feed samples were similarly dried to a constant weight at 60°C.
The supplement with C32 and C36 was prepared similarly to the procedure of Elwert and Dove (2005). Forty-eight grams of C32 and C36 were dissolved in 2.5 L of heated heptane (40°C) and sprayed from a hand-held spray bottle in 500 mL aliquots on 80 kg of soyhulls that were mixing in a horizontal feed mixer. Dispensing warm solution was critical to preventing alkanes from solidifying and accumulating on the spray nozzle. It took approximately one hour to apply the alkanes to the soyhulls. After 10 min of mixing, the treated soyhulls were removed from the mixer, spread on a clean plastic tarpaulin and allowed to aerate for 2 d at room temperature. The treated hulls were then mixed with corn grain and mineral supplement to provide each steer daily a totally mixed supplement of 475 g of corn grain, 475 g of soyhulls with added alkanes, and 50 g of mineral supplement. The supplements were readily accepted by the steers, and usually were consumed within 30 min. Supplements contained 342 mg/kg DM C32 and 332 mg/kg DM C36 daily.

Analytical procedures

The saponification and extraction procedures were adapted from Olivan and Osoro (1999) and Sandberg et al. (2000). Dried samples were ground through a 0.5 mm screen with a cyclotec mill (Rose Scientific Ltd, Edmonton, Alberta). Feed samples were weighed to 1.5 g and fecal samples were weighed to 0.5 g in 16 x 125 mm Pyrex glass screw-top tubes fitted with PTFE-lined caps. An internal standard (100 µg) consisting of n-tetratriacontane (C34) was added to each tube.

One molar ethanolic KOH was prepared by dissolving 56 g of KOH in 900 mL of
95% EtOH with mechanical stirring. Eighteen to 22 mL of deionized water was added to help dissolve the KOH. The solution was then brought to a final volume of 1L with 95% EtOH. Fourteen mL of 1M ethanolic KOH was added to each tube. Tubes were sealed, vortexed, and placed in a heating block at 90ºC. Samples were vortexed every 30 min for 5 h. The tubes remained in the heat block overnight or a minimum of 14 h.

After the samples were saponified, the heat block temperature was reduced to 65ºC. Caps were slowly removed to release pressure and 14 mL of hot heptane (65ºC) and 4.0 mL of hot deionized water were added to each tube. Twelve tubes were shaken by hand for a total duration of 6 min followed by centrifugation at 1000 x g for 3 min. The heptane layer was carefully transferred with glass pipette to a scintillation vial. Scintillation vials were then placed on a hot plate and heptane evaporated to reduce volume to 5 mL.

Silica gel (70-230 Mesh ASTM, Whatman Inc.) was dried for a minimum of 2 h at 105 ºC and stored in a desiccator. Silica gel columns were prepared by placing a small ball of glass wool in the bottom of a 10 mL plastic pipette tip. One mL of dried silica gel was poured into each pipette, and gently tapped to eliminate holes that might result during use. Five mL of hot heptane were added to each silica gel column and eluate was discarded. Scintillation vials containing the concentrated 5 mL samples from the extraction step were placed on a hot plate for 10 sec and the heptane was then transferred by Pasteur pipette to the top of the silica gel column. Eluate was collected into new scintillation vials. The original scintillation vials were rinsed with 3 additional 5 mL hot heptane rinses that were transferred to the silica gel columns. Scintillation vials containing approximately 20 mL of heptane were then placed on a hot plate to allow for evaporation to dryness. After evaporation to dryness, 900 µL of
hexane and 100 µL of hexane that contained 50 µg of n-docosane (C22) and n-octatriacontane (C38) internal standard were added, the vial was sealed, mixed, heated on a hot plate, and the contents were transferred with a glass pipette to the gas chromatograph injection vial.

Alkane concentrations in feed, orts and feces were quantitated on a Varian 5000 gas chromatograph equipped with an 8200 autosampler (Varian Medical Supply Inc., Palo Alto, CA). The column was ZB5HT inferno, 30 m long with 0.25 mm diameter (Phenomenex, Torrence, CA). The carrier gas was He and pressure was held constant at 1.7 atm. The injector was heated to 345 ºC and the detector was heated to 350 ºC. Sample injection volume was 1 µL, with a sample:split ratio of 1:10. Initial column temperature was 230 ºC for 1.5 min, then increased 35 ºC/min to a final column temperature of 290 ºC, then held constant for 11 min. Peaks were identified by retention times of prepared standards containing all n-alkanes between C27 and C36, and with internal standards for internal calibration adjusted for recovery of C22 (alkanes up to C28), C34 (saponification recovery), and C38 (alkanes between C29 and C36). Calibration factors were derived from multiple injections of standards containing 5 concentrations of each alkane between C27 and C36). Each sample’s concentration of alkanes was calculated as the mean of two injections for the sample.

Calculations

The alkane compositions of hays, supplement, and feces were expressed as mg/kg of DM. The apparent digestion coefficient of total diet (hay plus supplement) was calculated as
the difference between DM intake minus fecal output divided by DM intake. Fecal recoveries of alkanes were calculated as the proportion of the alkane consumed to that recovered in the feces. Hentriacontane (C31) was selected as the odd-chain alkane to use in intake calculations because it was present in higher concentrations than tritriacontane (C33) in alfalfa and switchgrass hays, while C32 was used as the adjacent even-chained alkane. Intake of hay was calculated with the equation from Stefanon et al. (1999):

\[
\text{Daily hay intake, kg DM} = \frac{(F_i/F_j) \times ((S \times S_j) - (S \times S_i))}{(H_i - (F_i/F_j) \times H_j)}
\]

Where \( F_i = \) fecal C31 concentration mg/kg, \( F_j = \) fecal concentration of C32, mg/kg, \( S = \) supplement kg/d, \( S_j = \) concentration of C32 in supplement, mg/kg, \( S_i = \) concentration of C31 in supplement, mg/kg, \( H_i = \) concentration of C31 in hay, mg/kg, and \( H_j = \) concentration of C32 in hay, mg/kg.

Supplement intake was constant for all steers at 0.92 kg DM/d, and hay intakes were adjusted for the differences between C31 content of hay fed and orts, if present, by multiplying orts DM by the quotient of C31 in orts divided by C31 in hay. Estimated digestion was calculated by 1) calculating fecal output by dividing daily C32, C36, or C31 supply by the average concentration of those alkanes in fecal grab samples, then 2) by subtracting fecal output from predicted intake for C32 and C36, and measured intake for C31.
**Statistical Analysis**

PROC GLM procedure of SAS was used with a model consisting of day with dependent variables for alkane concentrations, measured intake and estimated intake within animal. Paired student’s t-tests were used to compare duplicate samples for alkane concentrations based on the mean of two GC injections per sample.

**Results**

One steer was removed from the analysis due to inconsistent eating patterns from a leg injury. Alkane concentrations in the feces of the injured animal were erratic with the fecal ratio of C31:C32 greater than two standard deviations from the mean. Ten of the animals were within one standard deviation.

Cattle did not sort between the alfalfa and switchgrass hays as determined by visual observation and fecal alkane ratios. Alkane concentrations for alfalfa, switchgrass, and the mixed hay differed \((P < 0.01)\) for all alkanes measured except for C27 (Table 2.1). Estimated daily DMI \((5.96 \pm 0.70 \text{ kg})\) was not different \((P < 0.35)\) from measured intake \((5.51 \pm 0.68 \text{ kg})\) when using C31 and C32 in the estimation equation. There was no day effect \((P < 0.18)\) on estimated intake based on the fecal C31:C32 ratio among the 5 d of fecal grab samples, with fecal alkane concentrations provided (Table 2.2).

Duplicate samples analyzed during saponification and extraction were not different for any alkanes \((P < 0.22)\) between C27 and C36. In particular, there was no difference between duplicate samples for C31 and C32 \((P < 0.65)\), which demonstrated repeatability with this laboratory technique.
Discussion

Estimated intake did not differ from measured intake in steer using C31:C32 in the present experiment. Hameleers and Mayes (1998) found no difference in estimated intake of dairy cows consuming a mixed forage diet of perennial ryegrass and white clover. Estimated intake was calculated with C31:C32 over the C32:C33 pair due to the low concentration of C33 in the one forage (Hameleers and Mayes, 1998).

Alfalfa and switchgrass hays were blended to provide a mixed forage diet similar to grazing herbivores selection of various forages (Ferreira et al., 2007). Understanding mixed forage composition is essential towards application to grazing conditions in herbivores (Dove and Moore, 1995). Herbivores graze pastures of mixed forage with more than one plant species. Charmley and Dove (2007) estimated intake with up to four forages in the diet of sheep. Alkane concentrations in each forage can allow for back estimation to determine intake of each plant species. Using simultaneous equations with known C27 and C29 concentrations in the alfalfa and switchgrass hays, composition of the mixed hay was estimated from fecal grab samples. Estimated forage ratios were between 75-80% switchgrass hay and 20-25% alfalfa hay with a known ratio of 75:25 switchgrass:alfalfa hay mix. C27 and C29 were chosen because C31, 32 and 36 were present in the supplement, and C28, 30, and 33 were not present in at least one of the forages or fecal samples. Ratios of C31 in the orts:hay are an indicator that the concentration of C31 in the hay differed between alfalfa and switchgrass hays. Differences in ratios between the orts, having been predominantly stem, demonstrate a need to characterize alkane content of plant parts in
estimations of intake of animals that have the opportunity to select or sort feed on the basis of plant species (Ali et al., 2004; Bugalho et al., 2004) or plant parts (Laredo et al., 1991).

During analytical procedures, the recovery of internal standard at various steps of the procedure allowed for the conclusion that the incomplete recovery of alkanes was from an incomplete extraction and/or transfer from the saponification tube. Internal standard recovery ranged from 82-96 %. Not all of the heptane layer could be removed without pipetting waste material into the scintillation vial. This incomplete removal of heptane would account for most of the lost alkane. Temperature of extraction is very important because too cool of a temperature will lead to an incomplete extraction (Elwert et al., 2006; Mayes et al., 1986). Shorter chain length alkanes are often lost, while a higher recovery of longer chain alkanes is seen. Addition of C38 as an internal standard for gas chromatography of C30-C38 alkanes, and use of hexane rather than heptane as solvent in the gas chromatography injection vials improved repeatability of injections from standards and samples. Linearity and repeatability of standards ranging from 25 to 500 µg of alkane/mL hexane, corroboration of retention times with certified standards and absence of unidentified peaks in the chromatogram allowed us to quantify alkane concentrations of 10 mg/kg of feed or fecal DM.

Using the measured DMD for alfalfa and switchgrass hays in a prior experiment as reference, calculated DMD of the 75:25 mixture was 0.59. Calculation of DMD based on feed and fecal concentrations of C36 estimated a similar DMD (0.588 ± 0.02). Ferreira et al. (2007) found no difference in estimated DMD from in vivo DMD measurements in horses, but DMD estimations varied depending on alkane used for cattle. Ordakowski et al. (2001) reported no difference in total collection digestibility and estimated DMD with fecal alkanes.
The laboratory technique used was satisfactory with only one saponification of each sample since there was no difference between duplicate samples. Vulich and Hanrahan (1995) had no increase in precision from duplicate saponifications and reported single saponification of samples and single GC injections were sufficient; however, Berry et al. (2000) reported that duplicate saponifications should be maintained, but with single injections on the gas chromatograph.

**Conclusion**

Alkanes can be used to estimate intake of cattle consuming a mixed forage diet if a representative sample can be collected. Using fecal grab samples and individual feed samples consumed by the cattle, proportions of individual feedstuffs consumed can be estimated. Individual samples can be analyzed and duplicate samples are not needed during the saponification and extraction procedures when multiple days of fecal samples are analyzed and averaged for estimated intake calculations.

**Literature Cited**


Charmley, E., and H. Dove. 2007. Using plant wax markers to estimate diet composition and 
intakes of mixed forages in sheep by feeding a known amount of alkane-labelled supplement. 

estimate intake, digestibility and diet composition of grazing/browsing sheep and goats. 

46:1535-1544.


Elwert, C., H. Dove, and M. Rodehutscord. 2006. Effect of roughage species consumed on 
fecal alkane recovery in sheep, and effect of sample drying treatment on alkane 

Osoro. 2007. Estimation of feed intake and apparent digestibility of equines and cattle 
grazing on heathland vegetation communities using the n-alkane markers. Livestock Sci. 
110:46–56.

Ferreira, L. M. M., M. Olivan, M. A. M. Rodrigues, K. Osoro, H. Dove, and A. Dias-Da- 

Fraser, M. D. V. J. Theobald, and J. M. Moorby. 2006. Determining diet composition on 

Fushai, F.M., 2006. Estimates of intake and digestibility using n-alkanes in yearling 
Holstein-Friesian and Hereford heifers grazing on kikuyu (Pennisetum clandestinum) 


Hameleers, A., and R. W. Mayes. 1998. The use of n-alkanes to estimate herbage intake and 
diet composition by dairy cows offered a perennial ryegrass/white clover mixture. Grass 
Forage Sci. 53:164-169.


Table 2.1. Alkane composition of hays (mg/kg sample DM)

<table>
<thead>
<tr>
<th>Alkane</th>
<th>Alfalfa(^a)</th>
<th>Switchgrass(^b)</th>
<th>Mix(^c)</th>
<th>SEM</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>C27</td>
<td>17.85</td>
<td>16.06</td>
<td>19.54</td>
<td>3.32</td>
<td>0.34</td>
</tr>
<tr>
<td>C28</td>
<td>6.29</td>
<td>3.94</td>
<td>5.29</td>
<td>0.76</td>
<td>0.01</td>
</tr>
<tr>
<td>C29</td>
<td>139.38</td>
<td>37.53</td>
<td>73.37</td>
<td>9.53</td>
<td>0.001</td>
</tr>
<tr>
<td>C30</td>
<td>13.47</td>
<td>0.00</td>
<td>6.62</td>
<td>0.51</td>
<td>0.001</td>
</tr>
<tr>
<td>C31</td>
<td>330.93</td>
<td>15.95</td>
<td>118.00</td>
<td>7.46</td>
<td>0.001</td>
</tr>
<tr>
<td>C32</td>
<td>9.54</td>
<td>0.00</td>
<td>4.47</td>
<td>0.69</td>
<td>0.001</td>
</tr>
<tr>
<td>C33</td>
<td>21.90</td>
<td>4.01</td>
<td>11.00</td>
<td>0.72</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\(^a\)Average of 4 samples  
\(^b\)Average of 2 samples  
\(^c\)Average of 12 samples
Table 2.2. Alkane concentrations of fecal samples (mean ± SD)

<table>
<thead>
<tr>
<th>Alkane</th>
<th>Fecal Concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C27</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>C28</td>
<td>9.4 ± 0.9</td>
</tr>
<tr>
<td>C29</td>
<td>134 ± 12</td>
</tr>
<tr>
<td>C30</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>C31</td>
<td>217 ± 13</td>
</tr>
<tr>
<td>C32</td>
<td>107 ± 16</td>
</tr>
<tr>
<td>C33</td>
<td>21 ± 1</td>
</tr>
<tr>
<td>C36</td>
<td>101 ± 16</td>
</tr>
</tbody>
</table>
Chapter 3: Intake estimation of horses grazing tall fescue (*Festuca arundinacea*) or fed tall fescue hay
ABSTRACT

Most estimates of intake measurements and digestibility have been done in hay-fed horses, cattle, and sheep, because estimating intake of grazing animals is difficult. Estimating daily DMI of grazing horses is important for best horse management practices. Therefore, the purpose of the experiment was to use alkanes to estimate intake of horses eating non-toxic endophyte-infected tall fescue hay or pasture. Six mature geldings of light horse breed were randomly assigned to a hay or pasture treatment for a 14-d period in a random crossover design. Pasture-fed horses were housed in stalls from 0700 to 1300 daily with access to water and then grazed pasture as a group in a single 0.4 ha pasture from 1300 to 0700. Hay-fed horses were maintained individually in pens and given ad libitum hay from 1300 to 0700. All horses were individually fed 225 g oats twice daily sprayed with hexatriacontane (C36, external marker to provide 341 mg of C36 daily) and fecal samples were collected at 0700 and 1900 on d 10 through 14. Fecal grab samples were mixed, dried, subsampled, and analyzed for alkane analysis using gas chromatography. Estimated intake did not differ ($P < 0.49$) from measured intake when horses were fed hay using either tritriacontane (C33) or hentriacontane (C31) as internal alkane marker. Tritriacontane estimated pasture intake ($13.90 \pm 0.53$ kg DM/d) higher ($P < 0.001$) than C31 ($11.74 \pm 0.51$ kg DM/d). In conclusion, alkanes can be used to estimate intake of horses grazing pasture and provide a better resources for grazing management of horses.

Key words: alkanes, intake, horses, tall fescue
Introduction

Management of horses often incorporates grazing into the system to decrease amounts of hay fed and to minimize the amount of grain required to meet the daily nutrient requirements; however, determining pasture intake in horses has remained difficult. Horses selectively graze shorter grasses for the higher nutritive value in comparison to taller swards above 17 cm (Fleurance et al., 2010). Horses would also prefer taller swards of similar nutritive value over shorter swards to maximize intake (Edouard et al., 2009). Dowler (2009) estimated DMI rates for horses grazing pasture in different seasons between 0.088-0.166 kg DM/100kg BW/h over an 8-h period. This would extrapolate to 0.704-1.32 kg DM/100kg BW/d. Although these estimates were based on mature horses at maintenance, horse intake could differ based on different physiological needs, forage availability, forage species, and duration times of grazing. Duran et al. (1989) found that during a 3-h grazing period, yearling horses did not differ in forage intake when exercised for 30 min prior to grazing (865 mg/bite) or non-exercised (861 mg/bite).

Alkanes are naturally occurring waxes found in plants that can be used as intake markers; however, research in horses has been restricted to dried forage comparisons between estimated and measured intake with minimal information on intake estimation of grazing horses (O’Keefe and McMeniman, 1998; Smith et al., 2007; Stevens et al., 2002; Ordakowski et al., 2001). Each plant species has its own characteristic concentrations of
alkanes (Dove et al., 1996). The naturally occurring waxes appear to be indigestible and ratios excreted in feces can be used to estimate intake (Ordakowski et al., 2001).

The objectives of the study were to compare estimated and measured intake of hay-fed horses and to use the alkane method to estimate pasture intake by horses grazing pasture.

**Methods and Materials**

The current protocol was approved by the North Carolina State University Animal Care and Use Committee.

Six light breed geldings (553 ± 37 kg) between 5-8 yr of age were randomly assigned to either a pasture or stall-fed treatment in a crossover design during October and November, 2009. Each treatment lasted 14 d. Horses assigned to the grazing treatment were taken to pasture from 1300 to 0700 and taken to individual stalls (3.7 m x 12.2 m) from 0700 to 1300. Horses grazed in groups of three in a 0.4 ha quadrat with ad libitum intake. After the first week, horses were moved to a second pasture for the remainder of the period. Horses returned to the original pastures at the start of period 2 and were again moved for the second half of the period for rotational grazing. Pasture consisted primarily of non-toxic endophyte infected tall fescue (*Festuca arundinacea* Schreb.; Max-Q, Pennington Seed, Madison, GA; Table 3.1). Horses in the stall treatment were fed tall fescue hay ad libitum (Table 3.1) that had been harvested one year prior from the same pasture used for the grazing horses. Stalled horses remained in stalls for the 14 d except for fecal collection. Hay was removed daily at 0700, orts were weighed, and fresh hay was weighed and presented at 1300.
The alkane supplement was prepared by dissolving hentriacontane (C36) in warm heptane and then spraying the mixture onto whole oats (80 kg) while mixing in a horizontal mixer to provide approximately 750 mg C36/kg oats. Oats were placed on a tarp and air-dried for 48 h. Horses were fed 225 g of alkane treated oats twice daily at 0700 and 1900 for the duration of the experiment. Fecal samples (100g) were collected twice daily at 0700 and 1900 on d 10-14 by manual removal from the rectum unless the horse defecated within 30 min prior to or during fecal sampling. The samples collected at 0700 and 1900 on each of the five sampling days were mixed by day for each horse to give a representative fecal profile and frozen.

Pasture height measurements were taken at the beginning and end of each period with a plate drop meter. Twelve measurements were taken for calibration and three samples/ha were collected using a 0.25 m² frame randomly throughout the pasture using the clip-weigh-dry-weigh-compute method (National Academy of Science, 1962) for later analyses and 80 grass height measurements were collected with a plate drop meter. Grass and fecal samples were freeze-dried over 96 h, grass samples were ground through a 5mm screen on a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA), and both grass and fecal samples were ground through a 0.5mm screen in a Cyclotec 1093 mill (Rose Scientific Ltd, Edmonton, Alberta, Canada). Alkane concentration in forage and fecal samples were determined as described by Chavez et al. (2011). Intake estimation was calculated with tritriacontane (C33) and the C36 maker or hentriacontane (C31) and the C36 marker with the following equation from Stefanon et al. (1999):
Forage DMI= \( \frac{(F_i/F_j \times ((S_j \times S) - (S_i \times S)))}{(H_i - (F_i/F_j \times H_j))} \),

Where \( F_i \) is the concentration of odd chain alkane (mg/kg) in feces, \( F_j \) is the concentration of even-chain alkane (mg/kg) in feces, \( S \) is amount of supplement (kg), \( S_i \) is the concentration of odd-chain alkane (mg/kg) in supplement, \( S_j \) is the concentration of even-chain alkane (mg/kg) in supplement, \( H_i \) is the concentration of odd-chain alkane (mg/kg) in forage, and \( H_j \) is the concentration of even-chain alkane (mg/kg) in the forage.

Statistics were calculated using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC) to compare intake estimates across days with the model including horse, treatment and period. Actual and estimated intakes were compared using PROC ANOVA with a model including horse, treatment, period, treatment by period interaction. Estimated intakes using C31 and C33 were analyzed using paired student’s T-test to determine a difference between estimates. Significance was considered at \( P < 0.05 \).

**Results**

Horses readily consumed the alkane supplemental oats within five min after offer. Measured mean DMI (9.35 ± 0.98 kg DM/d) did not differ \((P < 0.49)\) from estimated hay mean DMI (9.18 ± 0.53 kg DM/d) using C33 and C36 alkane pairs or C31 and C36 (9.16 ± 0.51 kg DM/d). Hay intake differed \((P < 0.01)\) among horses with actual and both estimated intakes (Figure 3.1). Actual and estimated intakes with both markers differed \((P < 0.01)\) as a percentage of BW (Figure 3.2). Neither estimated nor actual hay nor pasture intake differed \((P = 0.97)\) by day. Estimated pasture DMI (11.74 ± 0.51 kg DM/d) was higher \((P < 0.001)\).
than estimated hay DMI (9.16 ± 0.51 kg DM/d) with C31 and was higher (P < 0.001) with C33 for pasture (13.90 ± 0.53 kg DM/d) over hay (9.18 ± 0.53 kg DM/d). Estimated DMI of pasture with C31 differed (P < 0.001) from C33. Estimated DMD of pasture (58.6 ± 2.9) was greater than estimated hay DMD (43.0 ± 10.7). During laboratory analysis using an internal standard of C34, alkane recovery rates for the assay ranged between 82-90%. DMD estimates ranged from as low as 40.0% for the hay and as high as 59.2% for pasture (Table 3.2). The alkane concentrations of the feeds are provided (Table 3.3) that were used in intake estimate calculations.

Discussion

Oats were an acceptable carrier for even-chain alkanes to be supplemented to horses and provided approximately 1.3 Mcal in the 405g DM consumed daily. Oats were used as a carrier for ease of dosing and for immediate consumption at the desired time of dosing, while having no C31 or C33 to influence intake estimation. The use of C36 as an external marker would not make a difference since adjacent alkanes are not used to estimate intake in horses (Ordakowski et al., 2001).

In the current study, use of C31 or C33 did not differ in estimating fescue hay intake of horses (Figure 3.1); however, the estimates differed when estimating fescue pasture intake. Alkane concentrations were higher in grass than hay (Table 3.3), with C31 concentrations higher than C33. Although both C31 and C33 estimated hay DMI the same, C31 and C33 estimated pasture intake differed. Small changes in C33 concentrations in feces would have a greater influence on the intake estimate calculation. Stefanon et al. (1999) concluded that the
estimated intake was lower than actual when feeding fescue hay and concentrate to horses, and the estimate using C31:C32 was preferable due to lower concentrations of C33 in the hay. Smith et al. (2007) also concluded that C31 as the internal marker provided a better estimate over C33 in horses fed hay. O’Keefe and McMeniman (1998) estimated within 5.4% accuracy when feeding mixed diets varying in proportions. With C31 and C33 estimates reported as the most accurate, no estimates were calculated using other odd-chain alkanes. Stevens et al. (2002) fed fresh cut grasses and hay and found the C33:C32 estimate gave a more accurate estimate when feeding Kikuyu grass or Kikuyu hay while C31:C32 had a more accurate estimate for fresh ryegrass. Kikuyu grass was higher in C33, while Ryegrass had higher concentrations of C31. Along with the current experiment, using odd-chain alkanes of higher concentrations in the forage may give better intake estimates in horses.

The problem associated with estimating intake of pasture with markers is the lack of a comparison to a known intake. Thus, using the alkane method in grazing horses only allows for an estimate and with a difference between the C31 and C33 estimates with the dosed marker can only make for a broad estimate of what the horses may have actually consumed. Horses consuming grass hays eat around 2.0-3.0% BW (Dulphy et al., 1997; Crozier et al., 1997). Horses in this experiment eating 2-3% of their body weight during grazing would be expected to consume between 11.0-16.6 kg DM/d of fresh pasture.

Hay DMD was lower than grass and ranged from 40-59.2% (Table 3.2). Ordakowski et al. (2001) found total collection DMD ranging from 54.1-60.9% in mixed grass and legume hay diets. Stevens et al. (2002) measured DMD for Ryegrass at 53.5% and for Kikuyu grass at 58.6% and Kikuyu hay at 33.8%. The current experimental DMD are similar
for the grasses. The hay is less digestible than the grass, similar to the findings by Stevens et al. (2002) with Kikuyu.

Alkanes have become a common method of estimating intake in cattle and sheep to allow for diet composition estimates (Charmley and Dove, 2007). The pasture in the current study was predominantly tall fescue with red clover (*Trifolium pratense*) and several other weed species. Dove and Moore (1995) used least-squares mathematical procedures to determine intakes of different forage species based on alkane composition; however, horses appeared to consume only the fescue during daily visual observation so no diet composition was calculated and only fescue alkane concentrations were used in estimating intake. Pasture samples were analyzed based on samples collected from visual observation near the horses to identify similar plant parts that were being consumed. Dove et al. (1996) found varying concentrations of alkanes in different plant parts of each species. With changing forage composition as forage ages, the plant composition may have affected the intake estimates slightly based on the exact forages consumed by the horses.

In conclusion, alkanes can be used to estimate intake of horses stall-fed hay. The estimated DMI of horses grazing pasture gave reasonable intakes within the expected DMI range. The use of the odd-chain alkane that is highest in forage may give better estimates for grazing horses since a difference was seen in DMI estimates for C31 and C33. Intake estimates are only estimates and can be used to improve management practices where pasture is the main source of nutrition for horses to better identify time horses should be allotted to pasture.
Literature Cited


Table 3.1. Chemical Composition of feeds$^a$

<table>
<thead>
<tr>
<th></th>
<th>Hay$^b$</th>
<th>Grass$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE (Mcal/kg)</td>
<td>1.98</td>
<td>2.27</td>
</tr>
<tr>
<td>CP (%)</td>
<td>11.92</td>
<td>15.60</td>
</tr>
<tr>
<td>NDF (%)</td>
<td>64.41</td>
<td>56.09</td>
</tr>
<tr>
<td>ADF (%)</td>
<td>35.56</td>
<td>30.84</td>
</tr>
<tr>
<td>DE (Mcal/kg)</td>
<td>1.98</td>
<td>2.26</td>
</tr>
<tr>
<td>Non-fiber carbohydrate (%)</td>
<td>13.83</td>
<td>18.12</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>1.89</td>
<td>3.38</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>0.37</td>
<td>0.33</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.22</td>
<td>0.31</td>
</tr>
</tbody>
</table>

$^a$samples were sent to the North Carolina Department of Agriculture and Consumer Services Feed and Forage Lab, Raleigh, NC, for analysis

$^b$ n=2

$^c$ n=8
Figure 3.1. Hay intake in individual horses with actual and estimated intake using C31:C36 or C33:C36 alkane pairs.
Figure 3.2. Hay intake in individual horses with actual and estimated intakes as a % body weight using C31:C36 or C33:C36 alkane pairs.
Table 3.2. Estimated intake and digestibility of horses consuming grass or hay using C31 or C33 intake markers with C36.

<table>
<thead>
<tr>
<th>Period</th>
<th>Hay 1</th>
<th>Hay 2</th>
<th>Grass 1</th>
<th>Grass 2</th>
<th>SE</th>
<th>Pd</th>
<th>Trt</th>
<th>PdxTrt</th>
</tr>
</thead>
<tbody>
<tr>
<td>C31 est. DMI (kg/d)</td>
<td>11.0</td>
<td>7.6</td>
<td>11.7</td>
<td>13.6</td>
<td>1.2</td>
<td>0.51</td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>C33 est. DMI (kg/d)</td>
<td>10.8</td>
<td>7.9</td>
<td>14.3</td>
<td>12.4</td>
<td>1.2</td>
<td>0.09</td>
<td>0.01</td>
<td>0.68</td>
</tr>
<tr>
<td>Actual DMI (kg/d)</td>
<td>9.7</td>
<td>9.0</td>
<td>14.3</td>
<td>12.4</td>
<td>0.2</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Est. fecal DM output (kg/d)</td>
<td>6.7</td>
<td>4.1</td>
<td>6.4</td>
<td>5.7</td>
<td>0.7</td>
<td>0.06</td>
<td>0.39</td>
<td>0.23</td>
</tr>
<tr>
<td>Fecal grab C36 (mg/kg)</td>
<td>48.7</td>
<td>67.2</td>
<td>52.3</td>
<td>48.1</td>
<td>7.2</td>
<td>0.35</td>
<td>0.32</td>
<td>0.16</td>
</tr>
<tr>
<td>DMD C31 estimate</td>
<td>41.6</td>
<td>48.3</td>
<td>47.1</td>
<td>59.2</td>
<td>2.3</td>
<td>0.01</td>
<td>0.01</td>
<td>0.28</td>
</tr>
<tr>
<td>DMD C33 estimate</td>
<td>40.5</td>
<td>50.6</td>
<td>56.6</td>
<td>55.4</td>
<td>1.8</td>
<td>0.04</td>
<td>0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Table 3.3. n-alkane concentrations (mg/kg DM) of feeds consumed in treatments

<table>
<thead>
<tr>
<th></th>
<th>Oats\textsuperscript{a}</th>
<th>Grass\textsuperscript{b}</th>
<th>Hay\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>C27</td>
<td>10.9 ± 1.0</td>
<td>13.2 ± 1.6</td>
<td>14.5 ± 2.6</td>
</tr>
<tr>
<td>C28</td>
<td>ND\textsuperscript{d}</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C29</td>
<td>14.9 ± 0.2</td>
<td>84.6 ± 8.4</td>
<td>98.2 ± 0.2</td>
</tr>
<tr>
<td>C30</td>
<td>ND</td>
<td>20.4 ± 2.1</td>
<td>ND</td>
</tr>
<tr>
<td>C31</td>
<td>ND</td>
<td>311 ± 28</td>
<td>246 ± 2</td>
</tr>
<tr>
<td>C32</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C33</td>
<td>ND</td>
<td>84.6 ± 7.9</td>
<td>72.2 ± 1.9</td>
</tr>
<tr>
<td>C35</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C36</td>
<td>757 ± 12</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Oats sprayed with C36, n=2  
\textsuperscript{b} n=9  
\textsuperscript{c} n=3  
\textsuperscript{d} ND: alkane not detected during analysis
Chapter 4: Intake estimation of horses grazing tall fescue (*Festuca arundinacea*) during morning or evening
ABSTRACT

Throughout the day, photosynthesis changes the composition of grasses by increasing sugar concentrations. This change in grass composition can influence animal acceptance of the forage, either increasing intake over another grass or total intake. The objective of the study was to use alkanes as markers to estimate DMI of horses grazing in the morning or evening. Six light breed horses (588 ± 39 kg) with maintenance only requirements were randomly assigned to either an AM or PM grazing treatment for 14 d and then switched to the other treatment for an additional 14 d. The AM grazing horses were allowed to graze non-toxic endophyte infected tall fescue pasture from 0700-1300, while PM horses grazed from 1230-2030. Horses were given access to approximately 0.40 ha to graze and were housed in individual pens (3.7m x 12.2m) when not grazing. Horses were given 200g of oats twice daily at 0830 and 2030 that were sprayed with dotriacontane (C32) dissolved in heptane to provide approximately 430 mg C32/horse/d. Oats were not fed on d 12 and 13. Rectal fecal grab samples (50g) were collected twice daily at 0830 and 2030 on d 8-12 of each period and mixed per day and frozen. Grass samples were collected at the beginning and end of each period. Grass and fecal samples were freeze-dried and ground through a 0.5mm screen for alkane analysis. Grass and fecal samples were saponified in ethanolic KOH and alkanes were extracted for gas chromatographic analysis. Intake estimates were calculated using C32 with either hentriacontane (C31) or tritriacontane (C33) as the internal marker. Estimated DMI were higher in the PM than AM (6.76 ± 0.25 kg/d vs. 5.74 kg/d) for with C33. When estimating intake with C31, estimated DMI was higher (P < 0.03) in period 2 (10.52 ± 0.93 kg/d vs. 6.88 ± 0.93 kg/d) and tended (P = 0.10) to be higher in the PM (9.92 ±
0.93 kg/d vs. 7.48 ± 0.93 kg/d). There was a tendency for an interaction where horses had higher estimated DMI ($P < 0.07$) in period 2 compared to period 1 (7.12 ± 0.25 kg/d vs. 5.36 ± 0.25 kg/d). The change in forage composition of the tall fescue may have prevented the horses from selecting higher intakes of fescue in the PM of period 2. NSC content of fescue during the second period ranged from 11.84% to 14.81%, where it was lower at all times during the day compared to period 1, ranging from 15.19% to 20.98%. Horses likely changed eating rates to adjust for eating the seed heads of fescue rather than leaves. Depending on the stage of growth of tall fescue, horses may increase their intake during PM grazing due to preference for higher sugar content of the grass.

**Keywords:** horse, grazing, alkanes, intake

**Introduction**

Throughout the day, plant composition changes as photosynthesis occurs and increases the content of nonstructural carbohydrates (NSC) in the plant (Burns et al., 2007). Dry matter intake increased throughout the day as NSC increased and fiber fractions decreased, with an increase in DMD in sheep (Burns et al., 2007). The increase in total NSC increases glucose, fructose, sucrose, fructan, and insoluble starch, which can influence animal preference (Mayland et al., 2000). Cattle preferred grazing tall fescue higher in total NSC. Sheep preferred PM cut hays compared to AM cut hay, but preferred AM hay with sugar added at 2, 3, or 4% over AM hay alone (Burritt et al., 2005). Taste and post-ingestive feedback from the high sugar concentrations may increase preference for the higher NSC in
forages; however, high intakes of NSC by horses grazing pasture may cause laminitis by altering the hindgut microbial populations and their subsequent fermentation products (Longland and Byrd, 2006). When horses were dosed with the NSC oligofructose, clinical laminitis developed (van Eps and Pollitt, 2006).

Estimation of intake by grazing horses has remained difficult; however, alkanes have been used as internal markers to estimate intake of hay-fed horses with no difference in estimated and measured intakes (Smith et al., 2007; Chapter 3). Alkanes have been used to estimate intake of grazing sheep and cattle (Charmley and Dove, 2007); however, no research has been done in horses grazing pasture. Alkanes have been used to estimate intake in horses fed hay or fresh cut forage (Ordakowski et al., 2001; Smith et al., 2007; O’Keefe and McMeniman, 1998; Ferreira et al., 2007).

If increased consumption of NSC can lead to laminitis, equine management practices should be evaluated to determine if horses have higher intakes of NSC during PM grazing as compared to AM. The objective of the study was to estimate DMI of horses grazing tall fescue during the AM or PM using alkanes as a marker to determine pasture DMI.

**Materials and Methods**

Protocols were approved by the North Carolina State University Animal Care and Use Committee.

Six light horse breed geldings (588 ± 39 kg) were randomly assigned to an AM or PM grazing treatment in a crossover design consisting of two 14-d periods at the university research farm in Raleigh, NC from April-May 2010. The AM treatment grazed from 0700-
1500 and the PM group from 1230-2030 to account for similar turnout times to most horse owners. When horses were not grazing non-toxic endophyte infected tall fescue pasture (MaxQ, Pennington Seed, Madison, GA), the horses were housed in stalls (3.7 m x 12.2 m) with access to water. Pasture was divided into 4 quadrants of approximately 0.40 ha of predominantly tall fescue (Table 4.1). Grass heights were collected using a plate drop meter at the beginning and end of each period. Forage samples were collected using a 0.25 m² frame randomly throughout the pasture using the clip-weigh-dry-weigh-compute method for forage availability estimates (National Academy of Science, 1962). Additional grass samples were collected to represent actual horse intake during each period by visual observation of what portion of the forage was being consumed. Grass samples were freeze-dried and ground through a 5mm screen on a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA), followed by a 0.5 mm screen on a Cyclotec 1093 mill (Rose Scientific Ltd, Edmonton, Alberta, Canada) for alkane analysis. Total nonstructural carbohydrate concentrations were determined using near-infrared spectrophotometry, NIRS Model 5000 (FOSS NIRSystems Inc., Laurel, MD, USA).

The external alkane supplement was prepared by dissolving dotriacontane (C32) in warm heptane. The dissolved C32 was then sprayed onto oats at approximately 1000 mg C32/kg oats in a horizontal mixer (Table 4.2). The sprayed oats were mixed for an additional ten min and then spread on a tarpaulin for 48 h to allow for evaporation of the heptane.

Horses were fed 200g of supplemental oats twice daily, at 0830 and 2030. Oats were not fed on d 12-13 of each period. Rectal fecal grab samples (50g) were collected twice daily at 0830 and 2030 on days 8-12 of each 14-d period and mixed for the daily sample.
samples were then freeze-dried and ground through a 0.5mm screen on a Cyclotech. Grass and fecal samples were then saponified in ethanolic KOH, followed by subsequent extraction of alkanes and later analyzed using gas chromatography as described by Chavez et al. (2011). Dry matter intake estimations were calculated with C32 and tritriacontane (C33) or C32 and hentriacontane (C31) using the following equation by Stefanon et al. (1999):

Forage DMI = \((F_i/F_j \times ((S_j \times S) - (S_i \times S)) / (H_i - (F_i/F_j \times H_j)))\),

Where \(F_i\) is the concentration of odd chain alkane (mg/kg) in feces, \(F_j\) is the concentration of even-chain alkane (mg/kg) in feces, \(S\) is amount of supplement (kg), \(S_i\) is the concentration of odd-chain alkane (mg/kg) in supplement, \(S_j\) is the concentration of even-chain alkane (mg/kg) in supplement, \(H_i\) is the concentration of odd-chain alkane (mg/kg) in forage, and \(H_j\) is the concentration of even-chain alkane (mg/kg) in the forage.

Apparent Dry Matter Digestibility (DMD) was calculated as:

\[ DMD = ((I - F) / I) \times 100, \]

where \(I\) is total estimated DMI and \(F\) is estimated total fecal DM output using the even-chain marker.

Statistics were calculated using the PROC ANOVA procedures of SAS (SAS Inst. Inc., Cary, NC). Means were calculated treatment, period, and treatment by period. The model included AM or PM treatment, period, and period x treatment interaction tested by horse within treatment and period. Estimated intakes were compared with linear regression and estimated intakes using C31 and C33 were analyzed using paired student’s T-test. NSC content was calculated using PROC MIXED procedures of SAS with the model period, time and period x time interaction, where time was a repeated measure.
Results

Alkane concentrations of feeds that were used for intake estimation are provided in Table 4.2. Estimated DMI was calculated with C31 paired with C32, and with C33 paired with C32. When estimating intake with C31, estimated DMI was higher ($P = 0.03$) in period 2 than period 1 (10.52 ± 0.93 kg/d vs. 6.88 ± 0.93 kg/d) and tended ($P = 0.11$) to be higher in the PM as compared to AM (9.92 ± 0.93 kg/d vs. 7.48 ± 0.93 kg/d). There was a tendency for an interaction (Table 4.3) where horses had higher estimated intakes ($P < 0.07$) in period 2 (7.12 ± 0.25 kg/d vs. 5.36 ± 0.25 kg/d), while intakes were higher in the PM than AM (6.76 ± 0.25 kg/d vs. 5.74 kg/d) for estimates with C33. There was no difference in estimated DMI with either C31 ($P = 0.99$) or C33 ($P = 0.65$) across days. Horses lost an average of 20 kg BW (588 ± 39 kg vs. 568 ± 39 kg) at the end of period 1 with no change at the end of period 2 (568 ± 39 kg). NSC content was lower ($P < 0.01$) at 8 AM compared to 2 PM and 8 PM (Table 4.4). NSC content was higher ($P < 0.01$) in period 1 than period 2 with a time x period interaction ($P < 0.02$). Significance was determined at $P < 0.05$.

Discussion

Horses had higher intakes during PM grazing during period 1 (Table 4.3). Fisher et al. (1999) found cattle, sheep, and goats had increased preference for fescue hays cut in the PM compared to the following morning. The preference was associated with increased NSC and decreased fiber components. Orr et al. (1997) found sheep grazing in the PM had higher bite masses associated with increased grass DM. This increase in bite mass may also influence
the DMI in horses grazing in the PM. With grazing time limited to 8 h, horses were eating continuously during their allotted times. Consumption rates were not measured to determine differences between horses, but changes in grass composition may have influenced changes in DMI. Orr et al. (1997) found that water soluble carbohydrates (WSC) increased in ryegrass from 15.6% to 18.3% from 0730 to 1930, where the NSC in the current experiment changed approximately 4% for period 1 (Table 4.4). In period 2, the fescue was more mature and horses consumed predominantly stem and seed heads which may not have changed in composition as much as period 1 for fiber components, resulting in no difference in AM and PM intake estimates. The NSC were also lower in the PM for period 2 than in the AM for period 1. If the forage composition changed enough that the DE of the forage declined, the horses would have consumed more grass to meet their energy requirements. More mature forages have lower DE and higher ADF, with lower NSC content (NRC, 2007).

As the experiment progressed over the one month of the experiment, the developmental stage of the tall fescue changed from the elongation stage to reproductive stage with the formation of seed heads in period 2. The change in plant maturity may have caused horses to increase consumption to meet energy needs based on changes in forage composition, such as NSC (Table 4.4). Period 2 had lower NSC content throughout all times measured during the day compared to period 1. Lechtenburg et al. (1972) reported decreased sugar concentrations over 30 d when forages were in different growth stages; however, fructosan concentration was constant throughout the day.

Stevens et al. (2002) concluded that alkanes can be used for grazing horses after feeding freshly cut ryegrass or Kikuyu, where both C31:C32 and C33:C32 estimates were
approximately 0.5 to 1.0 kg less than measured intake, but C33:C32 was closer. Stefanon et al. (1999) had similar conclusions with fescue hay. In sheep, estimated intake from C33:C32 did not differ from measured, while C31:C32 estimates overpredicted intake between 50-100g OM/d (Charmley and Dove, 2007). Without having a measured intake in the present experiment from horses grazing pasture, both C31 and C33 estimates have been used to give a range for possible daily intakes (Table 4.3). Alkane pairs used for intake estimates in horses have been shown to affect the estimated DMI (Ferreira et al., 2007). Intake estimates with alkanes were within 5.5% of actual intakes when horses were fed diets with varying proportions of feedstuffs (O’Keefe and McMeniman, 1998). In the current experiment, C31 concentrations were higher in the grass consumed than C33 (Table 4.2), where Stevens et al. (2002) had better estimated DMI in horses fed different forages using the odd-chain marker of higher concentration in the forage. Therefore, C31 may be the better estimate in this experiment.

Horses also were losing weight and may have increased intake rates due to limited grazing time of 8 h for period 2. Horses lost an average of 20 kg in body weight over the 14-d experiment, which may have been a combination of gut fill, water loss, and/or fat mobilization from decreased grazing times. The change of maturity of the forages would have increased the NDF and ADF and decreased digestible fractions of the grass. Horses did not lose or gain body weight during the second period of the experiment. The first period losses may have resulted from a lack of adjustment to the limited grazing period. Smith et al. (2007) found that frequent dosing and fecal collections would minimize the estimated intake error based on minimizing the variation in fecal marker excretion from the
diurnal variation of passage. In grazing horses, frequent fecal grab samples can be difficult and moving the horses to the barn for rectal grab samples may not be feasible. Twice daily sampling was used to reduce the amount of moving horses during grazing times and sample when horses were housed in stalls. Fecal samples were collected beginning on d 8 of each period to allow for several days for the dosed marker to reach a steady state. Ferreira et al. (2007) determined that dosed markers reach a steady state in horses after 4 d of dosing.

Estimates for DMD appear higher in period 2, compared to period 1 using C31 (Table 4.3) in digestibility is unlikely given the change in forage composition to lower NSC. Stevens et al. (2002) calculated DMD between 0.445 to 0.597 in fresh Ryegrass and Kikuyu with C31 and C33. The current estimates may have been influenced by the forage composition with higher amounts of seed heads on the fescue, where horses consumed more digestible seeds and less fibrous stem. If an incomplete recovery of the marker occurs, the DMD estimates would not be reliable unless adjustments are made for incomplete recoveries (Stevens et al., 2002).

Dove and Moore (1995) used least-squares mathematical procedures to determine intakes of different forage species based on alkane composition; however, the alkane compositions were very similar in species analyzed and did not provide realistic estimates for diet composition. During period 2 of the experiment, the growth of the tall fescue prevented horses from grazing plant species low to the ground. With odd-chain alkanes as the predominant alkanes in plants, most variation occurs in those alkanes by plant species (Dove et al., 1996). The part of the plant and age also affect the alkane composition. Depending on
species and age, the stem, leaf, and seed heads may have dramatically different concentrations of each odd-chain alkane.

In conclusion, preference determined by higher estimated PM DMI in horses could not be determined due to the low concentration of NSC throughout the day in period 2. However, during period 1, PM horses tended to have higher estimated DMI. The estimated intakes using C31:C32 appear to correspond with the daily nutrient needs of the horses and the C33:C32 estimate may have underpredicted DMI. C31 would be a better estimate for intake in horses consuming fescue from higher C31 concentrations. The preference for PM grazing during higher NSC content should be examined in more detail due to the change of maturity and therefore change in NSC content of the forages in the current experiment.

**Literature Cited**


Table 4.1. Nutrient Composition of tall fescue (*Festuca arundinacea*)

<table>
<thead>
<tr>
<th></th>
<th>Tall fescue&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE (Mcal/kg DM)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>CP (%)</td>
<td>10.8 ± 2.6</td>
</tr>
<tr>
<td>NDF (%)</td>
<td>56.7 ± 3.9</td>
</tr>
<tr>
<td>ADF (%)</td>
<td>30.1 ± 3.0</td>
</tr>
</tbody>
</table>

<sup>a</sup>n=18 samples  
<sup>b</sup>DE calculated (NRC, 2007)
**Table 4.2.** Mean alkane concentrations ± SD (mg/kg DM) of feeds used for intake estimation of horses

<table>
<thead>
<tr>
<th></th>
<th>Period 1 Fescue&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Period 2 Fescue&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Oats&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>C27</td>
<td>12 ± 1</td>
<td>23 ± 3</td>
<td>ND</td>
</tr>
<tr>
<td>C28</td>
<td>ND&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C29</td>
<td>78 ± 27</td>
<td>78 ± 22</td>
<td>12 ± 0.3</td>
</tr>
<tr>
<td>C30</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C31</td>
<td>276 ± 23</td>
<td>228 ± 81</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>C32</td>
<td>ND</td>
<td>ND</td>
<td>1034 ± 42</td>
</tr>
<tr>
<td>C33</td>
<td>77 ± 10</td>
<td>82 ± 13</td>
<td>ND</td>
</tr>
<tr>
<td>C35</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C36</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> n = 4
<sup>b</sup> n = 2
<sup>c</sup> n = 4
<sup>d</sup> ND: the alkane was not detected during analysis.
Table 4.3. Intake estimates of horses grazing AM and PM in either period 1 or 2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1</th>
<th>2</th>
<th>SE</th>
<th>Pd</th>
<th>Trt</th>
<th>PdxTrt</th>
</tr>
</thead>
<tbody>
<tr>
<td>C31 est. DMI (kg/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>5.2</td>
<td>8.5</td>
<td></td>
<td></td>
<td>0.03</td>
<td>0.11</td>
</tr>
<tr>
<td>PM</td>
<td>9.8</td>
<td>11.3</td>
<td>1.3</td>
<td>0.03</td>
<td>0.11</td>
<td>0.52</td>
</tr>
<tr>
<td>C33 est. DMI (kg/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>4.48</td>
<td>6.26</td>
<td>7.00</td>
<td>7.26</td>
<td>0.35</td>
<td>0.11</td>
</tr>
<tr>
<td>PM</td>
<td>7.00</td>
<td>7.26</td>
<td>0.35</td>
<td>0.01</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>Fecal grab C32 (mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Est. feces DM (kg/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal grab C31 (mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal grab C33 (mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMD C31 estimate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMD C33 estimate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P - value
### Table 4.4. Nonstructural Carbohydrate (% DM ± SD)

<table>
<thead>
<tr>
<th>Time</th>
<th>Period 1</th>
<th>Period 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>8AM</td>
<td>15.19 ± 0.92</td>
<td>11.84 ± 0.41</td>
</tr>
<tr>
<td>2PM</td>
<td>20.98 ± 0.73</td>
<td>12.64 ± 0.40</td>
</tr>
<tr>
<td>8PM</td>
<td>19.38 ± 2.50</td>
<td>14.81 ± 1.12</td>
</tr>
</tbody>
</table>

\[ ^a \text{n=3 for each time} \]
\[ ^b \text{n=3 for each time} \]
Chapter 5: Preference of a ground switchgrass and alfalfa hay blend flavored with sucrose or citric acid in cattle
ABSTRACT

Palatability of feed is an influential variable affecting intake for animals. The objectives were to evaluate preference by bovine for sucrose or citric acid addition to a ground switchgrass and alfalfa hay blend. Flavors were dissolved in 50 mL deionized water for blending. In experiment 1, sucrose hay (S100) had 100 g sucrose added per kg hay. Citric acid hay (CA50) had 50 g citric acid added per kg hay. Control hay (CON) had 50 mL deionized water added per kg hay. In experiment 2, treatments were CON, sucrose added at 25 g per kg hay (S25), S100, 150 g per kg hay (S150). Citric acid added at 25 g per kg hay (CA25), and CA50. Hays were mixed in a horizontal mixer 3 d prior to each experimental period. Twelve beef cattle (initial BW = 283 ± 25 kg) were housed under a roof on expanded metal flooring with access to six feed slots designated I through VI, west to east. Cattle consumed 1 kg of supplement (corn, soybean hulls, and trace mineralized salt) that was fed at 0800 daily. Hay orts were removed and weighed at 0800. Cattle were offered treatment hay equal to 0.6% BW at each feed slot and treatments were randomly assigned to two of the six locations at 0830. Cattle were given a 14-d adaptation to CON followed by a 7-d preference trial. In Experiment 1, cattle preferred S100 (3.42 ± 0.04 kg/d) over CON (2.8 ± 0.04 kg/d, P < 0.01) and CA50 (0.32 ± 0.04 kg/d, P < 0.01) and preferred CON over CA50 (P < 0.01). In Experiment 2, cattle preferred CA10 over CON (P < 0.02) and CA25 (P < 0.02). S100 was preferred over S150 (P < 0.02) and S25 (P < 0.02). Cattle consumed sucrose treatments over CON and citric acid treatments independent of location. Cattle may search for food that stimulates taste receptor cells for sweetness over other flavors.
Keywords: cattle, preference, hay, sucrose, citric acid

Introduction

Palatability of feedstuffs has a dramatic effect on how much feed is consumed from post-ingestive feedback (Villalba and Provenza, 2000). Several factors influence palatability, which include texture, smell, and taste. Taste can be divided into the five basic flavors: sweet, sour, salty, bitter, and umami. (Sola-Oriol et al., 2009). Sweet flavors are associated with sugars and carbohydrates, while sour feeds are associated with acids. Palatability can be separated from post-ingestive effects through esophageal fistulas, where preference is influenced after the feed is digested (Grovum and Chapman, 1988). Preference is not differentiated from palatability and post-ingestive effects in natural settings.

Sheep selectively graze for nutrient content; however, if nutrient content is constant, flavor affects the feed consumed (Scott and Provenza, 1998). Lambs preferred alfalfa hay with sugar added at 2–4% of DM (1:1 glucose:sucrose) compared to the no sugar added hay from the same cutting (Burritt et al., 2005). Spray-topping grasses with glyphosate (180 g a.i./ha) prolonged the nutritive value of the forage and increased sheep preference for the grass (Siever-Kelly et al., 1999). Higher concentrations of water soluble carbohydrates appear to increase animal preference. Steers fed polyethylene glycol had higher intakes of legumes with high concentrations of tannins (Mantz et al., 2009).

Chiy and Phillips (1999) found that dairy cows will consume sweet concentrates (250g sugar beet pulp and 75g African locust bean / kg DM) faster, while bitter (250g rape meal, 80g shea nut extract, 100g cherko, 50g cocoa / kg DM) and salty (5g NaCl/kg DM)
concentrates are eaten slower. Nombekela et al. (1994) found that dairy cows preferred sweet diets with 1.5% dietary DM as sucrose over sour diets with 1.25% dietary DM as HCl, bitter diets with 1% dietary DM as urea, and salty with 4% dietary DM as NaCl. Sheep and goats altered their preference for juniper leaves that have several negative secondary plant metabolites depending on the amount of protein in the diet (Utsumi et al., 2009). Consumption of a variety of feeds when given more than one option may enhance feed intake in sheep (Provenza et al., 1996); however, early exposure to lower quality feeds did not change the preference by sheep for more nutritious feeds in adulthood (Catanese et al., 2010). Sheep may change feeds they consume after flavor or taste exhaustion from sensory input and post-ingestive feedback when feeds are similar or different in taste or nutrients. Thus, the objective was to evaluate a preference for sucrose or citric acid addition to a ground switchgrass and alfalfa blended hay, where nutrient content of the hay is unaltered.

**Methods and Materials**

The following experiments were approved by the North Carolina State University Animal Care and Use Committee.

*Experiment 1*

Two flavor additives were prepared prior to the preference trial. A control (CON) was formulated using 50 mL of deionized water added per kg of hay. The sucrose (S100) additive (Domino sugar) was formulated with 100 g of sucrose dissolved in 50 mL of deionized water per kg of hay, while the citric acid (CA50) solution (Citric Acid Monohydrate) consisted of 50 g of citric acid dissolved in 50 mL of deionized water.
Switchgrass and alfalfa hay were each ground in a hammer mill with a 1.9 cm screen. The hays were then mixed in a 3:1 switchgrass:alfalfa ratio (wt:wt, as fed) in a horizontal mixer. Flavor solutions were then slowly poured onto the mixed hay (86.8% DM) in the horizontal mixer and mixed for an additional 10 min. The flavored hay was then placed in a color coded cart.

Eleven beef steers and 1 heifer (283 ± 25 kg) were blocked into 3 groups of 4 by body weight. Cattle were housed individually in 13 m² pens under one roof on expanded metal flooring with access to 6 feeding stations per animal. Cattle were given a 14 d adaptation period to the unaltered 3:1 switchgrass:alfalfa hay since the cattle had no known previous exposure to a hay diet. This period also allowed time for the cattle to learn they had access to 6 feeding stations rather than one. Water was provided ad libitum. Cattle consumed 0.92 kg DM of supplement (47.5% each of corn and soybean hulls, and 5% trace mineralized salt) that was fed once daily at 0800. At 0800, orts were removed and weighed and fresh feed was provided at 0830. Hay was fed at 0.7% BW at each of the 6 feeding stations so that cattle would have ad libitum intake of hay, but would consume hay from more than two locations.

After the adaptation period, cattle were given a 7 d preference trial where each of the three treatments (CON, S100, CA50) was randomly assigned to 2 of each animal’s 6 feeding stations. Hays were randomly assigned each day in the same order for all cattle. Feeding stations were numbered I to VI from west to east.

**Experiment 2**

Four steers from experiment 1 (324 ± 18 kg) were returned to pasture for a minimum of three weeks were then used to test the level of citric acid added to the diet to determine if
lower levels of citric acid were more palatable than the control. The control diet was formulated the same as Exp 1, while citric acid solutions were formulated with 25 g of citric acid (CA25) in 50 mL of deionized water or 10 g citric acid (CA10) in 50 mL of deionized water.

Four different steers from experiment 1 (324 ± 15 kg) were returned to pasture for a minimum of three weeks were then used to test the level of sucrose added to the diet as well. The concentrations of sucrose added to the hay were 100 g (S100), 150 g (S150), or 25 g (S25) in 50 mL of deionized water.

Statistics were calculated using Proc GLM of SAS, where significance was defined as $P < 0.05$. Daily DM intake was the dependent variable, with flavor treatments and location as fixed effects and individual animal as the experimental unit.

**Results**

In both experiments, animal preference was defined as disappearance of feed, where greater disappearance meant preference for the hay. In Experiment 1, Cattle preferred S100 over both CON and CA50 ($P < 0.01$) and CON over CA50 ($P < 0.01$; Figure 1). Feeding location I was preferred over feeding location III ($P < 0.03$), while there was no difference between any other feeding locations (Figure 2). Cattle gained an average of 0.77 kg/d.

In Experiment 2, CA10 was preferred over both CON and CA25 ($P < 0.02$) and CON was preferred over CA25 ($P < 0.02$; Figure 3). S100 was preferred over both S150 and S25 ($P < 0.02$) and S150 was preferred over S25 ($P < 0.02$; Figure 4). There was no preference
for feeding location in either parts of experiment 2. Cattle gained an average of 0.73 kg/d during the citric acid phase and 0.82 kg/d during the sucrose phase.

**Discussion**

Cattle had a strong preference for the sucrose treatments during all 7 d of the preference test. After a few days, the cattle began searching for sucrose treatment over the other two treatments upon offering and usually consumed the hay at one location before stopping to ruminate. The cattle then consumed the remainder of sucrose treatment before consuming hay from either of the remaining treatments. Burritt et al. (2005) found in sheep, where animals did not initially have a preference until the second day of the trial. The preference of SUC over the other treatments was most likely due to the hedonic response seen with sweet foods (Silveira et al., 2010).

The lack of preference for the citric acid hay in the Experiment 1 (Figure 1) may be from too high a concentration of citric acid. At high concentrations, flavors may become overbearing and result in a decreased intake (Grovum and Chapman, 1988). The smell of the citric acid hay at 50 g/kg hay was very potent to humans and could have deterred the cattle from consuming the hay. Lambs were more aversive to feeds that had both a strong odor and flavor resulting in decreased intake (Provenza et al., 2000). However, citric acid treatment was not completely avoided and cattle did consume some of the hay. On one occasion in Experiment 1, one of the CON locations was accidentally blocked for several hours and the animal in that pen ate CA50 to meet the daily energy intake. Had the cattle been given previous exposure to the strong flavor, intake may have been similar to the CON. Lambs
with previous exposure to strong flavors would associate the nutrient reward with the feed and would have higher intakes of less palatable feeds (Villalba and Provenza, 2000).

The preference for location seen in experiment 1 (Figure 2) was due to the randomization of treatments where CA50 appeared by chance 4 out of 7 d in feeding location III. Scott and Provenza (1998) found that lambs ate more food and spent more time at the northern area than at the southern area during a preference test. No preference was seen for any other location, while some animals have been noted to spend more time where more preferred feed sources had been (White and Carr, 1985).

In Experiment 2, cattle preferred S100, over other sugar treatments (Figure 4). The cattle could have become habituated to that flavor and level in Experiment 1, and therefore consumed the S100 treatment over the other treatments.

Providing flavored diets may be beneficial to animals by decreasing the monotony of feeding the same forage daily (Villalba et al., 2010). Adding diversity through flavors can increase intake allowing for faster growth in younger animals. Prenatal exposure of flavors in pigs increased growth after weaning and decreased stress behaviors when the same flavors were provided at weaning (Oostindjer et al., 2010). Villalba et al., (2009) found that sociality may affect a sheep’s willingness to consume novel feeds. For this reason, feeding stations were numbered in order to prevent preferences for feeding location at adjacent feeding stations where taste preference was of primary interest. Thus, low concentrations of familiar flavoring could be used to decrease the fear of consuming new feeds or simply adding sugar to the feed could increase intake (Burritt et al., 2005).
**Conclusion**

Flavored feeds that are nutritionally adequate can be used to provide variety in the diet of cattle. Flavoring can increase the acceptance of less palatable feeds and may minimize sorting behaviors of less preferred ingredients mixed in the diet. Adding sugar to less palatable feeds or weeds in pasture may increase intake of unwanted plants in cattle since the current diet of alfalfa and switchgrass had no aversive palatability characteristics, as the feed was readily accepted by all steer during the adaptation period.

**Literature Cited**


**Table 5.1.** Nutrient composition of hays and concentrate.

<table>
<thead>
<tr>
<th></th>
<th>Alfalfa hay&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Switchgrass hay&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Concentrate&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP (% DM)</td>
<td>17.6 ± 0.42</td>
<td>4.24 ± 0.14</td>
<td>11.5</td>
</tr>
<tr>
<td>NDF (% DM)</td>
<td>47.25 ± 1.34</td>
<td>79.7 ± 0.4</td>
<td>34.9</td>
</tr>
<tr>
<td>ADF (% DM)</td>
<td>35.9 ± 0.85</td>
<td>45.6 ± 0.3</td>
<td>24</td>
</tr>
</tbody>
</table>

<sup>a</sup> n=2; samples analyzed by NIRS reference databases of Dr. Joe Burns, USDA, ARS.

<sup>b</sup> n=7 (hay analyses from previous experiment, unpublished data)

<sup>c</sup> calculated using feed composition tables (NRC, 1996)
Figure 5.1: Cattle DMI (kg) ± MSE of CON, S100, and CA50 in Experiment 1

a-c Columns without a common superscript differ ($P < 0.01$).
Figure 5.2: Hay DMI (kg) ± MSE at each feeding station, Experiment 1

* Means without a common superscript differ from feeding location 1 ($P < 0.05$).
Figure 5.3: Cattle DMI (kg) ± MSE of CON, CA10, and CA25, Experiment 2

\textsuperscript{a-c} Means without a common superscript differ ($P < 0.01$).
**Figure 5.4:** Cattle DMI (kg) ± MSE of S100, S150, and S25, Experiment 2

\[ \text{Means without a common superscript differ } (P < 0.01). \]
Chapter 6: Preference in weanling pigs for sweet or umami taste after in utero exposure
ABSTRACT

The objectives of the experiment were 1) to investigate the interaction of taste perception with increased nutrient supply in determining preference after weaning and 2) to determine if the taste of gestation or lactation diets influences weanling preference. Twenty sows in second or greater parity were randomly assigned to 1 of 5 diets and blocked into a sweet or umami taste group with a control (CON) in each group. The sweet group consisted of CON, sucrose (SU), and non-caloric sweetener (SW), while the umami group consisted of CON, monosodium glutamate (MSG), and glutamate-free umami additive (UM). The SU was added at 5.0% of the diet, SW at 0.1%, MSG at 1.5%, and UM at 0.05%. Sows were fed 2.27 kg/d of their respective treatment diet beginning on d 10 of gestation. At farrowing, sows were given a lactation diet in the same treatment, while pigs were cross-fostered across treatments within sweet or umami groups. One hundred and forty pigs were weaned at 21 d and allotted to pens (1.73 m x 0.83 m) of 2-4 pigs/pen with 2 feeders/pen based on gestation and lactation treatments. Pens were given 3-d double-choice preference tests to measure feed intake of test diets. Feed intake was analyzed using the student’s paired t-test in SAS with significance at \( P < 0.05 \). In the sweet group, when SU was provided post-weaning, pigs chose SU >75% of the time for all preference tests that included SU. In the UM group, when MSG was provided, pigs chose MSG 80% of the time for all preference tests with MSG. Pigs in the UM group preferred \( P < 0.02 \) MSG over CON, preferred \( P < 0.03 \) MSG over UM, but there was no preference \( P > 0.16 \) for UM over CON. The SU and SW pigs preferred \( P < 0.02 \) SU over CON, control pigs preferred \( P < 0.01 \) SW over CON, and SU and control
pigs preferred ($P < 0.02$) SU over SW. In conclusion, pigs had a stronger preference for sweet and umami tastes associated with increased nutrient supply, which likely resulted from the expected interplay between sensorial perception and post-ingestive effects.

**Keywords:** pig, taste, sweet, umami, preference

**Introduction**

Weaning is a very stressful stage of life for pigs when they transition from a diet consisting of milk to a solid feed (Langendijk et al., 2007). Pigs given flavored feeds after weaning grew faster and ate more feed when they were weaned from sows consuming the same flavor (King, 1979). Feeding flavored diets to gestating sows reduced incidence of diarrhea and increased weaning weight in piglets exposed to flavors in utero (Oostindjer et al., 2010).

Odors can be just as influential as flavors in diet acceptability by piglets (Morrow-Tesch and McGlone, 1990). Baby pigs prefer odors from their own mother within hours after birth and avoided novel odors. Odors can be transmitted through amniotic fluid (Mennella et al., 1995) and milk in humans (Mennella and Beauchamp, 1991). However, little is known about the transmission of nutrients and how they can influence preference of pigs in utero. When choosing foods to eat, sucrose and sucralose (a non-caloric artificial sweetener), activate different taste responses in humans (Frank et al., 2008), thus, nutrients and non-nutrient feed additives may have different effects in utero and after parturition.
The first objective of the experiment was to investigate the interaction of taste perception with increased nutrient supply in determining preference after weaning. The second objective was to determine if the taste of gestation or lactation diets influences weanling preference.

Materials and Methods

Animals and design

Twenty sows in second or greater parity were randomly assigned to one of five treatments with four sows per treatment. The sweet group consisted of sucrose (SU) and non-caloric sweetener (SW), while the umami group consisted of monosodium glutamate (MSG) and glutamate-free umami additive (UM) and control (CON) was included in the design. Sucrose was added at 5.0% of the diet, SW at 0.1%, MSG at 1.5%, and UM at 0.05% (Tables 1, 2, 3) based on previous research with the non-nutritive additives in pigs (personal communication). Ten days after breeding, sows began treatment diets for the duration of gestation while housed in gestation crates. Sows were fed 2.27 kg/d of their respective diet once daily at 0600.

Blood samples were collected by jugular venipuncture 3 d prior to breeding, 1 h prior to feeding and 2 h post-feeding. The sampling protocol was repeated on d 30 and d 60 of gestation. Five mL of plasma and 7.5 mL of serum were collected and placed on ice. Plasma samples were then placed in a centrifuge, spun at 2,500 x g at 4°C for 15 min, and frozen until later analysis. Serum samples were placed in a refrigerator for 14 h at 2.8°C, placed in a centrifuge, spun at 2,500 x g at 4°C for 15 min, and frozen for later analysis. Plasma samples
were analyzed for glucose concentrations using a YSI 2700 Select Biochemistry Analyzer (YSI Inc., Yellow Spring, OH), while serum was analyzed for insulin and leptin by RIA (Millipore Corp., Billerica, MA).

Sows were moved to farrowing crates 2 wk prior to expected farrowing dates and fed a lactation diet beginning on day 110 of gestation until piglets were weaned. Piglets had ears notched, tails docked, and given penicillin and iron dextran injections within 24 h after farrowing. Piglets were weighed and then cross-fostered within sweet or umami treatment groups (Figure 6.1). Males were castrated at 7 d of age and weaned at 21 d.

Preference Testing

At weaning, 140 pigs were moved to nursery barns where they were grouped by treatment in pens (1.73m x 0.83m) of 2 to 4 pigs/pen with 2 feeders/pen. Pigs were given 3-d double-choice preference tests based on their exposure to treatments during gestation and lactation. Serum samples were collected from weanling pigs on d 13 after weaning. Weanling preference was analyzed by pen where preference was determined as:

\[ \% \text{ preference} = \left( \frac{\text{test feed intake}}{\text{total intake}} \right) \times 100 \]

where preference was indicated as test feed intake > 50% of total intake over the 3-d period. Pens per treatment ranged from 1-8, depending on survival to weaning. Some pigs were removed from the experiment prior to weaning in order to receive milk replacer.

Statistical analysis
Leptin and insulin concentrations were analyzed using the PROC GLM procedures of SAS (SAS Inst. Inc, Cary, NC) with date and within dietary treatment (sweet or umami group) as class variables and pig as the individual unit. Student’s t-test was used for comparisons of preference for each double-choice preference test.

Results and Discussion

There was no difference in litter weights ($P = 0.48$) or number born alive ($P = 0.71$) across treatments. Litters ranged from 5-13 pigs. One sow died two d prior to weaning and piglets were moved across the sows of the same lactation treatment.

In the sweet taste group, pigs preferred SU over 62% of the time when SU was one of the two choices (Table 6.4). Pigs that were exposed to the SU or SW treatments in utero or receiving the tastes through lactation preferred ($P < 0.02$) SU over CON, CON pigs preferred ($P < 0.01$) SW over CON, and SU and CON pigs preferred ($P < 0.02$) SU over SW. Taste sensory perception on the tongue is activated through various pathways. Sweet taste can be activated through sucrose stimulated adenylate cyclase through the circumvallate papillae causing cAMP release (Naim et al., 1991). Frank et al. (2008) found that sucrose and artificial sweeteners activate different taste pathways. The lack of dopamine response from the same areas of the brain may influence the post-ingestive feedback for sucrose addition to nursery diets. In the umami taste group, pigs chose MSG 66% of the time for all preference tests that included MSG except for one group (Table 6.5). Pigs in the UM group preferred ($P < 0.02$) MSG over CON, preferred ($P < 0.03$) MSG over UM, but there was no preference ($P > 0.16$) of UM over CON.
Nutrient and flavor interactions have been found in pre-weanling rats demonstrating a post-ingestive feedback mechanism (Myers et al., 2005). Rats preferred a flavor given in association with 20% glucose over a flavor with 0.05% saccharin. Rats were able to make a flavor or nutrient association within a short time frame during a conditioned stimulus test (Ackroff et al., 2009). Flavor associations appear to be learned prior to weaning and are influenced by flavor transmission through the mother’s milk (Myers et al., 2005). Mennella and Beauchamp (2002) found that human infants given different types of formulas emphasizing various tastes had preferences for foods later in life associated with those tastes. Sour tasting formula from a protein hydrosylate resulted in preference for sour-flavored juices, while soy formulas resulted in preference for bitter-flavored apple juice. Not all volatile compounds associated with flavors are transmitted in the milk (Hausner et al., 2008). Transfer of flavor compounds is selective and varies in concentration.

The nutrient and flavor interaction appears to have a strong association with animal preference; however, in mice lacking taste receptor signaling, a preference was still seen for D-glucose (Ren et al., 2010). The metabolic use of glucose affects the dopamine release associated with glucose utilization influencing the hedonic response associated with carbohydrate intake.

When paired with an olfactory stimulus, weanling rats oriented toward the odor of a sweet, low calorie stimulus in preference to an unsweetened, high calorie stimulus; however, adult rats oriented towards both (Myers and Hall, 2000). Rats consumed more of the sweet, low calorie and unsweetened, high calorie when paired with unsweetened, low calorie. Rats preferred both the taste and nutrient component of the stimuli. Although the rats were
conditioned to a stimulus, the pigs preferred the nutrient at the concentrations presented. Altering the concentrations of the non-caloric feed additives may alter the weanling preference.

When sows were fed a control diet or anise flavored diet during gestation and then similar diets to weaned pigs, pigs that were exposed to the anise flavor in utero had fewer incidences of diarrhea after weaning and higher body weights on d 2 and 5 post-weaning (Oostindjer et al., 2010). The familiarity of the flavored diet minimized the effect of stress from novel feeds. Langendijk et al. (2007) reported a higher feed intake post-weaning associated with higher intakes during lactation when pigs were given aniseed and garlic flavor. Post-weaning diet had an effect on feed intake, so early exposure to flavors can improve post-weaning acceptance of feeds that would be novel to young growing pigs. Human infants born to mothers consuming anise consumed the mother’s milk without hesitation, while infants with no experience to anise found the milk aversive or neutral (Schaal et al., 2000). Although the anise flavor is also orosensorial, pigs in the current experiment preferred the nutrient over the non-caloric additive with orosensorial properties.

Adding flavors to the diets of humans and animals may be influenced by uterine exposure and decrease stress upon re-exposure (Mennella et al., 2001; Oostindjer et al., 2009). Calves fed a milk replacer and starter diet with flavor additives did not have an increased intake at weaning with added flavors; however, calves with low solid feed intake before weaning tended to have a higher ADG post weaning when given the flavored starter diet (Montoro et al., 2011). With no exposure in utero, the lack of preference may be from the calves’ first exposure to the novel flavor in milk. Nursery pigs did not prefer added flavor
to diets containing varying levels of distillers grains with solubles; however, when no choice was offered, phase 1 starter diets with added flavor resulted in increased daily feed intake (Seabolt et al., 2010). Human newborn infants found the smell of amniotic fluid to be more soothing than breast odor or no smell (Varendi et al., 1998). The uterine environment may have a large role in acceptance of novel odors.

Not all cereal grains are found to be equally palatable by pigs (Sola-Oriol et al., 2009a). High fiber grains, such as oats, were found to be preferred less than naked oats, which had lower crude fiber. Corn and naked oats had a higher preference when they were extruded. In nursery diet formulation, diets were formulated with similar ingredients used in industry while minimizing the ingredients with strong flavor associations. Diets were kept as similar as possible with only corn removed for addition of taste additives in nursery diets (Table 3). Fish meal and blood plasma were formulated into the diet of nursery pigs to maintain highly digestible sources of protein; however, the inclusion levels were low to minimize influencing the taste of the diet. Texture of feed also affects preference depending on hardness and difficulty chewing (Sola-Oriol et al., 2009b). Diets were all prepared as a mash to avoid any heat damage from pelleting without altering the nutrient composition of each diet.

There was no effect \( P = 0.39 \) of treatment diet on insulin in weanling pigs during preference testing (Figure 6.2). In sows, there was no treatment effect due to diet on insulin post-feeding \( P = 0.38 \); however, sows fed CON had higher insulin pre-feeding compared to SW and UM sows (Figure 6.3). Musser et al. (2004) found no difference in plasma insulin or glucose of sows fed restricted or ad libitum diets during gestation. Sows consumed most of
their entire meal within minutes after feeding and, therefore, relied on the one meal to meet all nutritional requirements. The insulin spike normally seen after consuming carbohydrates would have declined over the 2 h post-feeding, and plasma insulin would have returned to baseline concentrations. There was no difference between sow diets on plasma glucose (Figure 6.4). Thus, no effect was seen feeding a diet higher in simple sugars.

Weanling pigs from sows that were on SU for both gestation and lactation had higher \((P < 0.05)\) serum leptin 2 wk after weaning than weanling pigs in any other gestation/lactation treatment (Figure 6.5). This is in contrast to Kawai et al. (2000) who found leptin as a suppressor to sweet taste in mice. The preference testing in the present experiment used weanling pigs that were growing, in comparison to adult rats. The nutrient and taste interaction may have influenced the preference for the SU over the SW and CON. No effect was seen \((P < 0.64)\) in sow leptin concentrations pre- or post-feeding (Figure 6.5). Whitley et al. (2009) found no relationship between pig leptin with growth or dam’s milk and pig growth. There was no difference \((P = 0.48)\) of treatment diet on litter weight.

In conclusion, pigs had a stronger preference for sweet and umami tastes associated with increased nutrient supply, which likely resulted from interplay between chemosensory perception and post-ingestive effects. With palatability factors controlled except for taste, the pigs preferred the nutrient association with taste rather than taste alone, demonstrating a strong influence of dietary selection based on the hedonic response of sweet and umami flavors. Diets had no influence on insulin, glucose, or leptin concentrations during gestation.
**Literature Cited**


**Table 6.1.** Sow gestation diet composition and nutrient composition for crude protein, water soluble carbohydrate, and Na.

<table>
<thead>
<tr>
<th>g/100 g diet</th>
<th>CON&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SU</th>
<th>SW</th>
<th>MSG</th>
<th>UM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>83.16</td>
<td>77.76</td>
<td>83.12</td>
<td>81.55</td>
<td>83.05</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>12.19</td>
<td>12.56</td>
<td>12.16</td>
<td>12.31</td>
<td>12.20</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.85</td>
<td>1.85</td>
<td>1.85</td>
<td>1.85</td>
<td>1.85</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.03</td>
<td>1.02</td>
<td>1.03</td>
<td>1.03</td>
<td>1.03</td>
</tr>
<tr>
<td>Salt</td>
<td>0.58</td>
<td>0.58</td>
<td>0.58</td>
<td>0.57</td>
<td>0.58</td>
</tr>
<tr>
<td>Trace mineral&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Poultry fat</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>CON&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SU</th>
<th>SW</th>
<th>MSG</th>
<th>UM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Non-caloric</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>sweetener</td>
<td>Monosodium</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td>glutamate</td>
<td>Umami additive</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>Crude Protein (%</td>
<td>15.4</td>
<td>14.6</td>
<td>14.9</td>
<td>15.9</td>
<td>15.5</td>
</tr>
<tr>
<td>DM)</td>
<td>WSC (% DM)</td>
<td>5.1</td>
<td>11.7</td>
<td>5.6</td>
<td>5.3</td>
</tr>
<tr>
<td>Na (% DM)</td>
<td>0.303</td>
<td>0.266</td>
<td>0.256</td>
<td>0.474</td>
<td>0.286</td>
</tr>
</tbody>
</table>

<sup>a</sup> Treatments (CON=control diet, SU=sucrose addition, SW=non-caloric sweetener addition, MSG=monosodium glutamate addition, UM=non-caloric umami additive)

<sup>b</sup> Trace mineral premix composition: 10,692 ppm CU, 198 ppm I, 110,230 ppm Fe, 19,841 ppm Mn, 198 ppm Se, 110,230 ppm Zn.
Table 6.2. Sow lactation diet composition and crude protein, water soluble carbohydrate, and Na.

<table>
<thead>
<tr>
<th>g/100 g diet</th>
<th>CON(^a)</th>
<th>SU</th>
<th>SW</th>
<th>MSG</th>
<th>UM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>68.57</td>
<td>62.91</td>
<td>68.46</td>
<td>66.51</td>
<td>68.36</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>27.4</td>
<td>28.36</td>
<td>27.42</td>
<td>27.75</td>
<td>27.44</td>
</tr>
<tr>
<td>Dicalcium</td>
<td>1.75</td>
<td>1.79</td>
<td>1.75</td>
<td>1.76</td>
<td>1.75</td>
</tr>
<tr>
<td>phosphosphate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limestone</td>
<td>1.15</td>
<td>1.13</td>
<td>1.15</td>
<td>1.15</td>
<td>1.15</td>
</tr>
<tr>
<td>Salt</td>
<td>0.57</td>
<td>0.58</td>
<td>0.57</td>
<td>0.06</td>
<td>0.57</td>
</tr>
<tr>
<td>Trace mineral(^b)</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Poultry fat</td>
<td>0.32</td>
<td>0.01</td>
<td>0.36</td>
<td>1.04</td>
<td>0.4</td>
</tr>
<tr>
<td>L-lys HCl</td>
<td>0.05</td>
<td>0.03</td>
<td>0.05</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Non-caloric sweetener</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Monosodium glutamate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td>Umami additive</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>CON(^a)</th>
<th>SU</th>
<th>SW</th>
<th>MSG</th>
<th>UM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein (% DM)</td>
<td>21.3</td>
<td>22.3</td>
<td>21.4</td>
<td>21.9</td>
<td>21.2</td>
</tr>
<tr>
<td>WSC (% DM)</td>
<td>7.4</td>
<td>15.6</td>
<td>9.3</td>
<td>5.7</td>
<td>5.6</td>
</tr>
<tr>
<td>Na (% DM)</td>
<td>0.279</td>
<td>0.256</td>
<td>0.266</td>
<td>0.297</td>
<td>0.268</td>
</tr>
</tbody>
</table>

\(^a\) Treatments (CON=control diet, SU=sucrose addition, SW=non-caloric sweetener addition, MSG=monosodium glutamate addition, UM=non-caloric umami additive)

\(^b\) Trace mineral premix composition: 10,692 ppm CU, 198 ppm I, 110,230 ppm Fe, 19,841 ppm Mn, 198 ppm Se, 110,230 ppm Zn.
Table 6.3. Nursery diet composition and nutrient content for crude protein, water soluble carbohydrate, and Na.

<table>
<thead>
<tr>
<th>g/100 g diet</th>
<th>CON(^a)</th>
<th>SU</th>
<th>SW</th>
<th>MSG</th>
<th>UM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>35.83</td>
<td>30.83</td>
<td>35.83</td>
<td>34.33</td>
<td>35.67</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>27.5</td>
<td>27.5</td>
<td>27.5</td>
<td>27.5</td>
<td>27.5</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>0.92</td>
<td>0.92</td>
<td>0.92</td>
<td>0.92</td>
<td>0.92</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.77</td>
<td>0.77</td>
<td>0.77</td>
<td>0.77</td>
<td>0.77</td>
</tr>
<tr>
<td>Salt</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Trace mineral premix(^b)</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>L-lys HCl</td>
<td>0.26</td>
<td>0.26</td>
<td>0.26</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td>L-threonine</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Dried whey</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Plasma spray dried</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Fish meal</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Poultry fat</td>
<td>4.07</td>
<td>4.07</td>
<td>4.07</td>
<td>4.07</td>
<td>4.07</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Non-caloric sweetener</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Monosodium glutamate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td>Umami additive</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>Crude Protein (% DM)</td>
<td>26.0</td>
<td>25.7</td>
<td>26.1</td>
<td>26.8</td>
<td>26.9</td>
</tr>
<tr>
<td>WSC (% DM)</td>
<td>22.6</td>
<td>31.4</td>
<td>23.2</td>
<td>24.1</td>
<td>25.8</td>
</tr>
<tr>
<td>Na (% DM)</td>
<td>0.338</td>
<td>0.323</td>
<td>0.315</td>
<td>0.493</td>
<td>0.372</td>
</tr>
</tbody>
</table>

\(^a\) Treatments (CON=control diet, SU=sucrose addition, SW=non-caloric sweetener addition, MSG=monosodium glutamate addition, UM=non-caloric umami additive)

\(^b\) Trace mineral premix composition: 10,692 ppm Cu, 198 ppm I, 110,230 ppm Fe, 19,841 ppm Mn, 198 ppm Se, 110,230 ppm Zn.
Figure 6.1. Diagram of cross-fostering piglets with the number of pens represented during preference testing for each gestation/lactation combination.
Table 6.4. Preference % for type of sweet additive on weanling pigs from different sweet gestation and lactation treatments (SU/SU=sucrose gestation and lactation, SU/CON=sucrose gestation and control lactation, CON/SU=control gestation and sucrose lactation, CON/CON=control gestation and control lactation, CON/SW=control gestation and sweetener lactation, SW/SU=sweetener gestation and sucrose lactation, SW/SW=sweetener gestation and lactation).

<table>
<thead>
<tr>
<th></th>
<th>Sucrose(^a) vs. Control</th>
<th>Sweetener(^b) vs. Control</th>
<th>Sucrose(^c) vs. Sweetener</th>
</tr>
</thead>
<tbody>
<tr>
<td>SU/SU</td>
<td>79 ± 9</td>
<td>54 ± 9</td>
<td>75 ± 7</td>
</tr>
<tr>
<td>SU/CON</td>
<td>62 ± 13</td>
<td>77 ± 14</td>
<td>85 ± 10</td>
</tr>
<tr>
<td>CON/SU</td>
<td>77 ± 23</td>
<td>79 ± 24</td>
<td>90 ± 18</td>
</tr>
<tr>
<td>CON/CON</td>
<td>66 ± 16</td>
<td>71 ± 17</td>
<td>74 ± 13</td>
</tr>
<tr>
<td>CON/SW</td>
<td>88 ± 23</td>
<td>93 ± 24</td>
<td>24 ± 18</td>
</tr>
<tr>
<td>SW/SU</td>
<td>67 ± 13</td>
<td>45 ± 14</td>
<td>60 ± 10</td>
</tr>
<tr>
<td>SW/SW</td>
<td>75 ± 8</td>
<td>42 ± 9</td>
<td>73 ± 6</td>
</tr>
</tbody>
</table>

\(^a\)Preference for sucrose over control \(P < 0.01\)

\(^b\)No preference for sweetener over control \(P > 0.14\)

\(^c\)Preference for sucrose over sweetener \(P < 0.01\)
Table 6.5. Preference % for type of umami additive on weanling pigs from different umami gestation and lactation treatments (MSG/MSG=gestation and lactation treatment for MSG, MSG/CON=MSG gestation and CON lactation, UM/MSG=umami gestation and msg lactation, UM/UM=umami gestation and lactation, UM/CON=umami gestation and CON lactation, CON/UM=control gestation and umami lactation, CON/CON=control gestation and lactation).

<table>
<thead>
<tr>
<th></th>
<th>Monosodium glutamate&lt;sup&gt;a&lt;/sup&gt; vs. Control</th>
<th>Umami&lt;sup&gt;b&lt;/sup&gt; vs. Control</th>
<th>Monosodium glutamate&lt;sup&gt;c&lt;/sup&gt; vs. Umami</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSG/MSG</td>
<td>86 ± 5</td>
<td>62 ± 9</td>
<td>78 ± 6</td>
</tr>
<tr>
<td>MSG/CON</td>
<td>87 ± 11</td>
<td>52 ± 18</td>
<td>70 ± 12</td>
</tr>
<tr>
<td>UM/MSG</td>
<td>84 ± 11</td>
<td>51 ± 18</td>
<td>78 ± 12</td>
</tr>
<tr>
<td>UM/UM</td>
<td>78 ± 6</td>
<td>40 ± 11</td>
<td>77 ± 7</td>
</tr>
<tr>
<td>UM/CON</td>
<td>92 ± 15</td>
<td>8 ± 26</td>
<td>100 ± 17</td>
</tr>
<tr>
<td>CON/UM</td>
<td>76 ± 15</td>
<td>18 ± 26</td>
<td>28 ± 17</td>
</tr>
<tr>
<td>CON/CON</td>
<td>66 ± 7</td>
<td>30 ± 13</td>
<td>77 ± 8</td>
</tr>
</tbody>
</table>

<sup>a</sup>Preference for monosodium glutamate over control $P < 0.01$

<sup>b</sup>No preference for umami additive over control $P = 0.63$

<sup>c</sup>Preference for monosodium glutamate over umami additive $P < 0.01$
Figure 6.2. Serum insulin in weanling pigs across dietary treatments (MSG/MSG=gestation and lactation treatment for MSG, UM/MSG=umami gestation and msg lactation, UM/UM=umami gestation and lactation, SU/SU=sucrose gestation and lactation, SU/CON=sucrose gestation and control lactation, CON/UM=control gestation and umami lactation, CON/CON=control gestation and lactation, CON/SW=control gestation and sweetener lactation, SW/SU=sweetener gestation and sucrose lactation, SW/SW=sweetener gestation and lactation). SE = 6.0 μU/mL.
Figure 6.3. Sow serum insulin concentrations (mean ± SE) before and after feeding across dietary treatments (CON=control diet, SU=sucrose addition, SW=non-caloric sweetener addition, MSG=monosodium glutamate addition, UM=non-caloric umami additive). Pre-feeding SE = 2.7 µU/mL and post-feeding SE = 7.5 µU/mL.
Figure 6.4. Sow plasma glucose before and after feeding across dietary treatments (CON=control diet, SU=sucrose addition, SW=non-caloric sweetener addition, MSG=monosodium glutamate addition, UM=non-caloric umami additive). Pre-feeding SE = 29.0 mg/L and post-feeding SE = 38.0 mg/L.
Figure 6.5. Weanling pig serum leptin (mean ± SE) across gestation and lactation treatments (MSG/MSG=gestation and lactation treatment for MSG, UM/MSG=umami gestation and msg lactation, UM/UM=umami gestation and lactation, SU/SU=sucrose gestation and lactation, SU/CON=sucrose gestation and control lactation, CON/UM=control gestation and umami lactation, CON/CON=control gestation and lactation, CON/SW=control gestation and sweetener lactation, SW/SU=sweetener gestation and sucrose lactation, SW/SW=sweetener gestation and lactation). SE = 0.2 ng/mL.
Figure 6.6. Sow serum leptin (mean ± SE) pre- and post-feeding across dietary treatments (CON=control diet, SU=sucrose addition, SW=non-caloric sweetener addition, MSG=monosodium glutamate addition, UM=non-caloric umami additive). Pre-feeding SE = 0.3 ng/mL and post-feeding SE = 0.35 ng/mL.
Chapter 7: Conclusion
Alkanes can be used to estimate intake of cattle and horses consuming mixed forage diets. Without any precise measurement and the variability between markers, alkanes allow for intake estimates, digestibility calculations, and diet composition from feeding an external marker compared to other markers that are limited in their function. From the lack of actual grazing trials in the scientific literature, the two horse experiments provide an estimate of intake in herbivores on pasture fed a dietary supplement rather than a controlled-release capsule. Although there was a period and treatment interaction, more horses should be used on pasture of similar stage of growth. Horses may prefer higher intakes of nonstructural carbohydrates similar to goats, sheep and cattle and the higher intakes may be detrimental to their health.

Many factors influence what and how much animals eat. One of the influential factors affecting animal intake and preference is palatability, most notably from taste. Cattle and pigs appear to have a natural selection for sweet taste, while horses may as well. By adding flavors or feeds that can influence the taste, animals may find the feed more preferable and select the feedstuff or find it aversive and avoid it, unless there are no more palatable feeds. In grazing animals, most have the option to choose between multiple forages, while animals in the feedlot or industry may only have one choice. Making the food more palatable can influence the post-ingestive feedback and may influence the animal in how much feed is consumed.

Pigs preferred consuming sucrose and monosodium glutamate readily over control or non-nutritive feed additives for sweet or umami tastes. Cattle preferred sucrose addition to a blended alfalfa and switchgrass hay. Lower quality forages may be less palatable and the
addition of sugar may increase intake by ruminants. Addition of sucrose may also influence cattle to eat less palatable forage species. Feeding weanling pigs diets with monosodium glutamate and sucrose may increase intake during the first several days post-weaning because of the post-ingestive dopamine response rather than feeding a non-nutritive feed additives.

Although each animal within a species is different in their selection of feeds when given a choice, taste is very influential from the associative effects and the post-ingestive feedback associated with each feed. Adding flavors to stimulate the taste buds of animals may increase intake and elicit a hedonic response to continue eating.