THE EVALUATION OF A FLAVOR ENHANCER ON INTAKE AND PRODUCTION OF HIGH PRODUCING, LACTATING DAIRY COWS

by

Caitlyn Merrill

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Caitlyn Merrill

Approved: _____
Limin Kung, Jr., Ph.D.
Professor in charge of thesis on behalf of the Advisory Committee

Approved: _____
Jack Gelb, Jr., Ph.D.
Chair of the Department of Animal and Food Science

Approved: _____
Mark Rieger, Ph.D.
Dean of the College of Agriculture and Natural Resources

Approved: _____
James G. Richards, Ph.D.
Vice Provost for Graduate and Professional Education

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ABSTRACT

The objective of this study was to evaluate the effect of improving forage palatability on DM intake, milk production and composition, rumen pH, and sorting behavior of lactating cows. Twenty one multiparous and 7 primiparous Holstein cows averaging 697 kg in body weight (SD ± 81), 54 DIM (SD ± 32), and consuming 23 kg/d of DM (SD ± 8) were fed a base TMR comprised of 45% corn silage, 10% alfalfa haylage, and 45% concentrate (DM basis). After a 2-wk pretreatment period, cows were blocked by production, parity, and DIM and randomly assigned to one of two treatments for 10 wk. Each treatment had two cows with previously fitted rumen fistula. In-dwelling probes were placed in the rumens of the fistulated cows, once weekly, and rumen pH was measured every 30 min for 48 h. For one half of the cows, the forage portion of the diet was pretreated with a palatability enhancer (Luctarom ProEfficient, Lucta S.A., Spain) that was mixed in water to achieve a projected dose of 12 ml/cow/d prior to mixing into a TMR (**TRT**). The remaining half of the cows were fed a similar TMR but the forage was mixed with water only (**CTRL**). Production data were analyzed as a completely randomized and covaried on pretreatment values. Rumen pH was analyzed in a factorial design with repeated measures with treatment, week, and treatment x week as main effects. Sorting data was analyzed in a factorial design with treatment, hour, and treatment × hour as the main effects. For all animals, there were no differences between treatments for DMI and milk production and composition. However, when data from only multiparous animals were analyzed (n = 10 for TRT and 11 for CTRL) there was a tendency for greater DMI (+1.5 kg/d, $P < 0.07$) and milk production (+3.9 kg/d, *P*

< 0.10) for cows fed TRT. Compared with CTRL, cows fed TRT had higher mean rumen pH and spent less time throughout the day with pH below ≤ 5.8. There was no difference between treatments in particle size distribution of the TMR throughout the day. Improving the palatability of the forage fraction of the TMR fed to multiparous dairy cows has the potential to increase ruminal pH as well as DMI and milk production.

Keywords: flavor, feed intake, palatability, dairy cow

Chapter 1

INTRODUCTION

Healthy cows that efficiently convert DM to large quantities of milk result in greater net income on dairy farms. However, in early lactation, dairy cows are unable to consume sufficient quantities of nutrients to meet requirements for high levels of milk production. In order to compensate, cows mobilize energy and protein from body reserves (Bauman and Currie, 1980), which negatively impacts reproductive performance (Roche et al., 2000), health (Treacher et al., 1986), and body condition score (Waltner et al., 1993). In many instances the inability to consume sufficient amounts of dry matter limits potential peak milk production (Waltner et al., 1993).

Another factor affecting the health and therefore, milk production of lactating cows is rumen fermentation. Irregular rumen fermentation caused by consumption of an imbalanced diet can often result in low rumen pH (DeVries et al., 2007; 2008), which can lead to decreased fiber digestion and decreased production of microbial protein (Owens et al., 1998). To regulate rumen fermentation, cows are fed a total mixed ration (**TMR**) with the hope that each bite is homogeneous. Thus, the production of rumen acids is moderated over the day resulting in a more optimum pH for microbial metabolism. However, cows often sort TMR eating smaller, more rapidly fermentable carbohydrates first and leaving larger stemmed forages for later (Leonardi and Armentano, 2003). By sorting, cows consume a ration that is nutritionally inconsistent (Stone, 2004).

Consuming more rapidly fermentable carbohydrates and less effective fiber in the diet can lead to low rumen pH (Zebeli et al., 2006). These cows are thought to be at an increased risk for subacute ruminal acidosis (Leonardi and Armentano, 2003; DeVries et al., 2008). Sorting of TMR has been shown to cause milk fat depression (Grant et al., 1990). Methods to reduce sorting may prove to be advantageous to overall ruminal fermentation.

Altering forage palatability is one method used in an attempt to increase dry matter intake in early lactation and reduce sorting of lactating cows. Palatability encompasses the physical and chemical characteristics of the animal, the feed, and the environment and how these characteristics affect taste and appetite (Goatcher and Church, 1970; Baumont, 1996). There are many different factors affecting palatability and it is often hard to predict how all of these factors relate to one another and ultimately impact intake.

There are three main groups of factors directly affecting forage palatability: animal, plant, and environmental (Marten, 1978). Animal factors such as their senses, the species, breed, or age of the animal, genetics, previous experience, adaptation, and physiological condition along with individual variation affect forage palatability (Goatcher and Church, 1970; Marten, 1978). Forage palatability is also affected by characteristics related to the plant such as species, chemical composition, physical traits, succulence and maturity, and plant availability (Marten, 1978). Finally, environmental

factors such as plant diseases, soil quality, feed additives, climate, season, and weather also contribute to the palatability of feed (Marten, 1978). Ultimately, it is a unique combination of all of these factors that create forage palatability.

Because some animal species are able to differentiate among different tastes and smells, flavor additives have been used in livestock feed to alter forage palatability, impact food learning (Burritt and Provenza 1989; Favreau et al. 2009), and increase feed intake (Chiy and Phillips, 1999; Van Tien and Thong, 2001).

Chapter 2

LITERATURE REVIEW

Animals have the ability to differentiate among different flavors that can affect the preference of feeds consumed. Preference for one flavor over another is a result of taste and postingestive consequences (Provenza, 1995). Preference for flavors increases when there are positive postingestive consequences or when the flavor causes a hedonic response (Provenza, 1995). However, aversion for flavors occurs when toxins are present, when there is an excess or a deficit of nutrients, or when the flavor is unpalatable (Provenza, 1995).

Flavors and Feed Aversions

In general, livestock species tend to select diets that have lower toxin levels and higher nutrient levels (Provenza, 1995; Wang and Provenza, 1997). This selection mechanism has evolved to prevent consumption of toxic plants while grazing (Launchbaugh, 1995). Ruminant animals are most likely to form aversions based on flavor rather than sight or odor (Launchbaugh, 1995). The ability of animals to discern different flavors can cause them to associate preingestive cues with postingestive consequences. The association of these negative consequences with a certain flavor can ultimately cause the formation of feed aversions.

When feed is flavored and paired with a toxin, animals have rejected the flavor even when it is no longer paired with the toxin (Kyriazakis et al., 1997; Favreau et al., 2009). For example, Kyriazakis et al. (1997) fed sheep feeds flavored with orange or aniseed with or without oxalic acid. Oxalic acid has been shown to decrease the availability of calcium and cause mild hypocalcemia (James and Butcher, 1972). Aversion to the flavor paired with oxalic acid persisted up to 60 days after the final administration of oxalic acid (Kyriazakis et al., 1997). Similarly, Favreau et al. (2009) found that sheep that had previously been administered LiCl, a non-lethal poison; with a feed containing a specific flavor avoided that flavor even when it was no longer paired with LiCl.

Although many studies on flavor aversion have been executed using sheep, Zahorik et al. (1990) looked at flavor aversions in sheep as well as goats. Sheep and goats were fed then administered an intramuscular injection of apomorphine. This feed was then fed alongside several control feeds. The authors reported that both ruminant species avoided the feed originally paired with the injection. However, Duncan and Young (2002) found that goats had a difficult time maintaining an aversion for a plant species paired with LiCl when it was offered at the same time as a control plant species. The authors reported that the goats consumed less of the plant paired with LiCl when offered separately from the control feed.

Once an aversion is formed, flavor generalizations can occur and animals reject feeds that resemble those associated with negative postingestive consequences. Launchbaugh and Provenza (1994) fed lambs feed with oregano flavor, administered LiCl orally, and found that lambs generalized this feed aversion to other feeds flavored with oregano even when LiCl was not administered. These generalizations can be less specific and aversions for a large group of plants can be formed. For example, Burritt and Provenza (1989a) administered LiCl to lambs that grazed on the shrub *Cercocarpus montanus*. In a later trial, when these lambs were allowed to graze on a pasture, lambs that had been administered the non-lethal poison consumed less shrubbery compared to control lambs. The authors suggested that the lambs generalized the connection between shrubs and a poison which caused the animals to avoid shrubs altogether.

Aversion to a flavor can occur even when the postingestive consequence does not happen immediately after consumption (Burritt and Provenza, 1991). Burritt and Provenza (1991) reported that even when LiCl was administered 8 h after a novel food was offered, lambs still rejected that novel food at the following meal. However, aversion to a flavor tends to decrease when the negative postingestive consequence is delayed. Kronberg et al. (1993) fed calves a novel feed with or without LiCl. The authors reported that consumption of the novel feed was lowest when the LiCl was administered either 4 or 8 h after feeding compared to immediate administration. When LiCl was administered 12 h after feeding, consumption of the novel feed was still less than control but the aversion was not as great as when there was a more immediate consequence.

The concentration of the flavor can also contribute to the feed aversion. Launchbaugh et al. (1993) fed lambs sodium saccharin or aluminum sulfate at low and high concentrations. Initially, the lambs did not show a preference for either flavor or either concentration. However, after administration of LiCl, the lambs preferred the feed with the lower flavor concentration.

Flavor aversions could potentially be used to teach livestock species to avoid poisonous plants while grazing. For example, Snyman, et al. (2003) orally administered LiCl, epoxyscillrosidin, and tulp-hexane extract to cattle. The tulp-hexane extract served as a flavoring agent similar to the tulp plant (*Homeria pallid*), which is toxic to cattle. These cattle, along with control cattle were then allowed to graze in a field where tulp plants were growing. After grazing, 2 of 21 treated cattle had to be treated for tulp poisoning while 14 of 21 control cattle had to be treated. The results of this study indicate that the cattle that had been administered the tulp-hexane extract associated this flavor with a negative consequence. Therefore, an aversion was formed and when the cattle were allowed to graze, they avoided the tulp plants decreasing the incidence of tulp poisoning.

Flavors and Positive Postingestive Consequences

Livestock can also learn to associate flavors with the nutritional content of a feed. Burritt and Provenza (1992) found that lambs preferred nonnutritive flavors that were

paired with glucose. The authors hypothesized that this positive association was because the lambs associated the additional energy from glucose with the flavor of feed. Similarly, Villalba and Provenza (2000) found that when there was a positive nutritional consequence, such as a large amount of starch added to the feed, lambs would consume high concentrations of a flavor. Preference for a certain flavor concentration persisted even when there was no longer a nutritional benefit and the starch content had decreased.

In some cases, livestock can differentiate between deficient, adequate, and excessive amounts of nutrients and then form flavor preferences based on the nutrient dose. For example, Early and Provenza (1998) reported that lambs preferred a flavor that was paired with a diet containing 100% total digestible nutrients (**TDN**) rather than a flavor paired with a diet containing a deficient amount of TDN (90%) or an excess of TDN (110%). In another study, Villalba and Provenza, (1997a) studied lamb preference for feeds after administration of different sources of nitrogen (urea, casein, or gluten meal). At low doses of nitrogen lambs preferred feeds paired with any of the three nitrogen sources over plain feed. However, when the dose of urea or casein was high, lambs formed an aversion to the feed paired with that nitrogen source. It was hypothesized that the preference was due to the positive postingestive consequences associated with the addition of nitrogen and the aversion was due to an excess of nitrogen (Villalba and Provenza, 1997a).

Similarly, Villalba and Provenza (1997b) found that animals formed feed preferences and aversions based on the dose of acetate or propionate. The authors found that lambs preferred feeds paired with a lower dose of propionate or acetate and at higher doses the lambs rejected the feed. Suthoh et al. (2007) reported that wethers preferred flavored straw that was associated with an intramesenteric infusion of sodium propionate or sodium acetate. This ability to associate flavors with the nutritional content of a feed helps livestock animals select a more nutritionally balanced diet while grazing.

Flavors and Novel Feeds

The relationship between forage palatability and the ability to distinguish between different types of feeds is also important when considering novel feeds that are presented to an animal. Lambs always choose to consume familiar feeds even if the familiar feed contains a poison and is offered alongside a novel food that is safe to consume (Burritt and Provenza, 1989b). The use of familiar flavor additives may be beneficial in encouraging the consumption of novel feeds. Van Tien and Thong (2011) applied the juice of native, familiar grasses to a novel feed and found the time it took for goats to accept the novel feed and reach maximum intake was decreased with this added flavoring. Similarly, Launchbaugh et al. (1997) offered lambs a novel food (rice), with or without a familiar flavor added (onion). The authors reported that the lambs consumed more the rice when it was paired with the familiar flavor. However, there has been some controversy over whether using familiar flavors will actually increase the intake of novel foods. Provenza et al., (1995) reported that it took 12 days for lambs to increase intake of

a novel feed (lentils) even when a familiar flavor (onion) was added. However, lambs that were fed the novel feed with the familiar flavor consumed more by day 12 than lambs that were fed only the novel feed.

Flavors and Masking Unpalatable Feed

Some research has shown that using flavor additives may increase the intake of unpalatable feeds by masking flavors that may not be appealing to the animal (Marten and Donker, 1964; Chiy and Phillips, 1999). For example, Chiy and Phillips (1999) fed non-lactating cows a salty, sweet, or bitter diet with or without an added sweet flavor that acted as a flavoring mask. They reported that cows consumed more of the negative flavors, salty and bitter, when the sweet flavor was added (Chiy and Phillips, 1999).

Livestock often refuse to consume pasture or feed that has been contaminated with manure. However, using flavor additives may decrease this aversion and increase intake. Marten and Donker (1964) sprayed manure contaminated patches of grass with sugar water or molasses and allowed cows to graze in these fields. They reported that treatment with either sweet substance resulted in consumption of the manure contaminated patches of grass while untreated, contaminated patches went untouched. In another study, Plice (1952) sprayed table sugar, black-strap molasses, sorghum molasses, or corn syrup on pasture that was contaminated with manure. He found that the cows had no aversion for the pasture contaminated with manure once it was sprayed with any of the sugar substances.

Plice hypothesized that the cow's preference for sweet flavor was due to the high energy these substances provided. Therefore, saccharin or sodium cyclohexyl sulfamate, both of which are non-caloric, synthetic sweeteners were applied to manure contaminated pasture. Even without the added energy benefit, the cows still consumed contaminated pasture when it was treated with the synthetic sweeteners. Plice hypothesized that is was the improved forage palatability and the sweet taste that masked the pasture and caused increased intake.

Flavor additives may be beneficial in increasing the consumption of lower quality, unpalatable feeds. Wagnon and Goss (1961) fed cows dry, rank forage that was either untreated, sprayed with a mixture of molasses and urea, or sprayed with just molasses. They reported that cows fed the unpalatable feed that was treated with molasses or molasses and urea consumed more than cows fed untreated feed. Plice (1952) sprayed unpalatable feeds, broomsedge, perennial legumes, and three-awn grasses, with the sugar substances mentioned above. The author observed that when these feeds were sprayed with sugar the cows had no aversion to consuming them.

Sweet Flavor Additives

Even though animals can differentiate among different flavors, most species prefer sweet flavors with the exception of strict carnivores (Cheeke, 1991). Immature and mature horses have shown preference for sucrose (Randall et al., 1978). Rats prefer sweet flavors and the sugar they prefer most is sucrose (Collier and Bolles, 1968; Smith, 2000).

Pigs have shown preference for sucrose, glucose, and saccharin flavored water when offered alongside tap water (Kennedy and Baldwin, 1971). Ewes showed preference for feed that had been treated with glucose (Ralphs et al., 1995). Given a choice between sweet feed and normal feed, lactating dairy cows prefer to consume the sweetened feed (Murphy et al., 1997). When offered feeds representing different primary tastes such as sweet, salty, sour, and bitter, lactating dairy cows prefer the sweet flavor (Nombekela et al., 1994). Due to this flavor preference, sweet substances have been used by producers and feed companies for decades to enhance the palatability of feed and to encourage increased intake (Plice, 1952). For example, most manufactured feeds for cows, sheep, horses, and pigs contain molasses in order to reduce dustiness and increase palatability (Cheeke, 1991).

Several studies have hypothesized that the preference for sweeteners such as sucrose and molasses may be due to the nutritional benefits they provide to the animal and not just due to the sweet flavor. These sugars are rapidly fermentable and therefore may contribute to the synchrony of the rumen (Broderick and Radloff, 2004; Firkins et al., 2008). Broderick and Radloff (2004) found that replacing high-moisture corn with dried or liquid molasses caused an increase in dry matter intake. In their study, added molasses caused a decrease in ruminal ammonia and urinary nitrogen excretion indicating improved nitrogen utilization. They hypothesized that the added molasses stimulated the formation of microbial protein and therefore the use of nitrogen. In a follow up study, Broderick et al. (2008) found that when sucrose replaced starch in the diet; there was

once again a decrease in ruminal ammonia and urinary nitrogen excretion indicating the improved utilization of nitrogen.

Some studies have shown that increasing molasses (Martel et al., 2011) or sucrose (Broderick et al., 2008) in a dairy cow diet caused an increase in DMI and amount and percentage of milk fat. Fiber digestion has also been shown to be affected by increased sugar in the diet. For example, Vallimont et al. (2004) partially replaced dietary corn starch with sucrose in continuous-culture fermenters. The authors found that this replacement caused an increase in NDF digestion (Vallimont et al., 2004). Similarly, Broderick and Radloff (2004) found that the addition of molasses to a dairy cow diet improved fiber digestion. Based on all of these studies, it has been difficult to determine whether the altered forage palatability, other nutritional advantages, or a combination of these factors are responsible for livestock preference for feeds containing sucrose or molasses.

Saccharin as a Flavor Additive

Saccharin is an artificial, no-calorie sweetener that has been used in foods and drinks all over the world for the last century (Pena, 2010). Saccharin has also been used in flavor additives for animals in an attempt to increase intake. Rats show preference for saccharin solutions when offered alongside tap water (Young and Greene, 1949). Studies have shown that there is an optimum concentration of saccharin that rats will consume and anything above or below this concentration will not elicit preference (Collier and

Novel, 1967; Smith and Rashotte, 1978). This may be due to the fact that a low concentrations, the flavor is too weak to detect and at high concentrations saccharin has a bitter taste.

When given the choice between saccharin flavored water and tap water, pigs prefer the water with the added sweetener (Kennedy and Baldwin, 1972). Based on this preference, saccharin based flavor additives have been added to pig diets in an attempt to improve feed intake and consequently average daily gain. However, studies have shown that adding this flavor does not yield the desired results. For example, Sterk et al. (2008) fed pigs starter diets that were treated with nothing or a saccharin based additive. The authors reported no effect on intake or weight gain and they concluded that piglets need a period of time before sweeteners can have an effect.

Saccharin has also been added to cattle diets in an attempt to increase intake. Brown et al. (2004) fed 0, 88, 176, or 264 g/ton of Sucram, an additive containing 97% sodium saccharin, to male calves. Feeding 176 g/ton of Sucram caused an increase in dry matter intake and average daily gain (Brown et al., 2004). In a follow up study, McMeinman et al. (2006) found that there tended to be an increase in body weight when 200 mg of Sucram per kg of DM was fed to male beef calves. The authors concluded that since saccharin has no caloric value, the improved sweet flavor is what caused the increased intake.

Flavor Additive and Livestock Production

Even though studies have shown that livestock prefer to consume feeds with added sweeteners, there is little data on the effects of altered palatability on intake and production. For example, even though Murphy et al. (1997) found that dairy cows preferred sweetened feed when offered alongside a control feed, when the cows were fed only sweet or only control feed, there was no difference in dry matter intake. Several studies where dietary starch was replaced by molasses or sucrose and fed to dairy cattle showed no effect on dry matter intake (Vallimont et al., 2004: Firkins et al., 2008; Martel et al., 2011). In sheep, Goatcher and Church (1970) reported that as a whole, sheep were indifferent to water treated with sucrose or saccharin even though some individual sheep exhibited preference for the sweetened water. Nombekela et al. (1994) found that dairy cows preferred sweet over other primary flavors. However, in a follow up study treatment with sucrose had no effect on dry matter intake of dairy cows (Nombekla and Murphy, 1995). Overall, research has shown that most livestock species prefer sweetened feed. However, the impact of sweet flavor additives on intake and production of livestock has lead to contradictory results.

Summary

Although studies on the impact of sweet flavor additives on intake and production of livestock species contradict the concept that these species have a “sweet tooth”, further research is needed. The idea of altering forage palatability with the use of flavor enhancers has been shown to contribute to food learning, feed aversions, intake of novel

feeds, and intake of unpalatable feeds. Altering forage palatability with sweet flavor enhancers may bring the best of both worlds and contribute to food learning while appealing to the animal's preference for sweetened feeds.

Chapter 3

OBJECTIVE

The objective of this study was to evaluate a flavoring agent applied to the forage portion of a TMR on DM intake, sorting of the TMR, milk production, milk composition, and rumen pH of Holstein cows.

Chapter 4

MATERIALS AND METHODS

A lactation trial was conducted with a protocol for animal care and handling approved by the Agricultural Animal Care and Use Committee, College of Agriculture and Natural Resources, University of Delaware ((17) 10-05-11R; Anon., 1998; FASS, 1999; Appendix E). Twenty one multiparous and 7 primiparous Holstein cows averaging 697 kg in body weight (SD ± 81), 54 DIM (SD ± 32), and consuming 23 kg/d of DM (SD ± 8) were housed in a sand-bedded, free-stall barn containing Calan gates (American Calan, Northwood, NH). The cows were fed a ration formulated to meet NRC (2001) requirements for the group. The TMR was comprised of 45% corn silage, 10% alfalfa haylage (2^{nd} cutting), and 45% concentrate (DM basis). The ingredient composition of the concentrate is shown Table 2. The chemical composition of the forages is shown in Table 3 and the chemical composition of the TMR is shown in Table 4.

At the end of a 2 wk pretreatment period, cows were blocked based on milk production, DIM, DMI, and lactation number, as seen in Table 1. Within each block the cows were randomly assigned one of two treatments. Each treatment group was blocked to contain two rumen fistulated cows. Animals fed the control treatment (**CTRL**) had 20 L of water mixed onto 424 kg forage (as fed basis) prior to mixing with the 347 kg (as fed basis) of concentrate. Cows fed the treated diet (**TRT**) had 180 mL of a flavor enhancer consisting of natural and artificial sweeteners (Luctarom ProEfficient, Lucta S.

A., Spain) mixed in 20 L of water and applied to the forage portion prior to mixing with the concentrate. The targeted application rate was based on supplying a cow consuming 55 kg of wert TMR 12 mL of the flavor enhancer per day (manufacturer's suggested dose). The water and the flavor additive were applied to the forage slowly using a watering can for 2 min under constant mixing. After application, the forage was mixed for an additional 2 min before adding the concentrate. Cows were fed the treatment for 10 wk.

Cows had access to fresh, clean water at all times and were fed once daily at 0700 h at 105% of their expected intake to ensure *ad libitum* consumption. The amount of TMR fed and refused were measured daily and used to calculate DMI. Cows were milked twice daily at 0500 h and 1600 h and production was automatically recorded at each milking. Milk samples were collected at 2 consecutive milkings per week and analyzed for fat, protein, MUN, lactose, and SCC using infrared methods (Dairy One Laboratories, University Park, PA). These data were used to determine 3.5% FCM (kg milk × (0.4255 + (16.425 × % fat / 100))), feed conversion (FCM/DMI), and ECM ((0.3246 × kg milk) + (12.86 × kg fat) + (7.04 × kg protein)). Body weights of the cows were measured on 2 consecutive d at the start and end of the trial.

Samples of TMR from each treatment, corn silage, and alfalfa silage were collected three times a wk and pooled for analysis of DM. Dry matter was determined weekly using a 60° C forced-draft oven for 48 h. The DM content of the feed

components was used to adjust the weekly TMR formulations. Nutrient analysis of the TMR samples was conducted weekly and analysis of the forages was conducted every other wk by Cumberland Valley Analytical Laboratory (Hagerstown, MD). Concentrate was sampled once a wk for DM determination and pooled every 2 wk for nutrient analysis. Dried forage and silage samples were ground with a UDY Cyclone Sample Mill (UDY Corp., Fort Collins, CO) with a 1-mm screen. Neutral detergent fiber was quantified using sulfite and amylase as described by Van Soest et al. (1991) and ADF was determined using methods described by AOAC (2000). Crude protein was calculated by multiplying total nitrogen by 6.25 after total combustion of samples in LECO CNS 528 Analyzer (LECO Corporation, St. Joseph, MI). Starch content of each sample was determined according to the method described by Hall (2009). Ash content was determined according to AOAC (2000) methods with the modification of using 0.5 g sample weights and the furnace temperature at 535°C. Dried forage and silage samples were also ground to pass through a 3-mm screen, using a UDY Cyclone Sample Mill (UDY Corp., Fort Collins, CO), and analyzed for soluble protein (Krishnamoorthy, et al., 1982).

During the last 2 d of each wk of the treatment period, in dwelling pH boluses (Kahne Limited, Auckland, NZ) were placed in the 4 rumen fistulated cows (2 per treatment) and set to record the pH every 30 min for 48 h. Data were combined into hourly increments. The collected data were then used to determine the average pH,

minimum pH, maximum pH, range of pH, and the number of minutes the rumen pH was below 5.5 or below 5.8.

Once weekly, TMR samples were selected from 12 cows (6 cows per treatment) at 0, 6, 12, and 24 h after feeding. The 12 cows sampled were selected at the start of the trial because of a high and consistent DMI. These TMR samples were analyzed for particle size using the Penn State Forage Particle Separator (Pennsylvania State University, University Park, PA) and physically effective fiber using the Z-box (Miner Institute, Chazy, NY).

Chapter 5

STATISTICAL ANALYSIS

All performance data were averaged over all wk then covaried with pre trial data and analyzed using the fit model procedure of JMP (Version 9.0; SAS Institute Inc.). Cow was used as a random variable and the main effect was treatment. Performance data were analyzed separately for all animals and for multiparous cows. Milk production and DMI data for all animals and multiparous cows were also covaried weekly with pre trial data with cow as a random variable and the main effect being treatment. Particle size data were analyzed with cow as a random variable and wk as the repeated measure. For these data, the main effects were treatment, h, and treatment × h. Each sieve level of the particle size box was analyzed independently. Effective fiber data were analyzed using the Fit model procedure of JMP with cow as the random variable and wk as the repeated measure. The main effects were treatment, h, and treatment × h. For rumen pH data h was included as a repeated measure and the subject was cow nested within treatment. Covariance was modeled using the compound symmetry covariance structure. The main effects were treatment, wk, and treatment × wk. For all data, outliers were removed using the distribution. Means were compared using the Tukey's test (Snedecor and Cochran, 1989), and significance was declared when $P \leq 0.05$. Trends ($P < 0.10$) are also noted.

Chapter 6

RESULTS AND DISCUSSION

The average nutrient compositions of TMR fed to the cows during the treatment period are shown in Table 4 and did not vary substantially from each other.

All Animals

Dry matter intake, milk production, milk composition, and body weight data of all animals are shown in Table 5. Milk production, kg/d of DMI, and DMI as a percent of BW were not different between the two treatments. There was no difference between the treatments in percent of milk fat, protein, lactose, or SCC. Somatic cell count indicated that the cows had minimal mammary infection. Milk urea nitrogen was not different between treatments but within normal range (Jonker et al., 1999). The production of fat was also not different between treatments but there was a trend for cows fed TRT to produce more ($P = 0.08$) kg of milk protein/d (1.28 kg/d) than those fed CTRL (1.22 kg/d). Average body weight, body weight gain, ECM, 3.5% FCM, and feed efficiency were not different between the treatments.

Dry matter intake of all animals covaried weekly with pretreatment data is shown in Figure 1 (Appendix A). Dry matter intake was significantly higher ($P \leq 0.05$) in cows fed the TRT in wk 4 and 9. Milk production of all animals covaried weekly with

pretreatment data is shown in Figure 2 (Appendix B). Milk production was not significantly different between the treatments in any week.

In the present study there was no effect of treatment on DMI of multiparous and primiparous cows. However, there has been some controversy over the effect of supplementation with a sugar substance, often sucrose or molasses, on DMI in early lactation cows. Some studies have shown that adding sugar to a TMR causes an increase in DMI. For example, Penner and Oba (2009) found that when multiparous and primiparous Holsteins in early lactation were fed a diet containing 4.5% or 8.8% sucrose on a DM basis, there was an increase in DMI as the percent of sucrose increased (P = 0.03). Similarly, Broderick and Radloff (2004) fed multiparous and primiparous, Holstein cows one of four TMR with 0%, 4%, 8%, or 12% molasses. The authors found that DMI increased as the percent of molasses increased ($P = 0.05$). In both studies the authors attributed the increase in DMI to the improved palatability of the feed.

However, some studies show that there is no effect of sugar supplementation on DMI. Nombekela and Murphy (1994) fed 24 early lactation cows which were a mix of Holstein and Jersey as well as primiparous and multiparous cows, one of two treatments for 12 wk. The control diet contained a control concentrate that did not have sucrose. The concentrate used in the sweet diet contained sucrose as 1.50% of the concentrate. The authors found that DMI was not different between treatments. The authors hypothesized that the lack of an effect of supplementation with sucrose may be due to lack of diet

variety (Nombekela and Murphy, 1994). It has been shown that several animal species consume more when they are fed a diverse diet. For example, Treit et al. (1983) conducted an experiment on rats and showed that the rats consumed more of a four-course meal when the courses were flavored differently. Similarly, Early and Provenza (1998) fed lambs either apple or maple flavored feed for a day. The next day, the lambs were offered a choice between apple or maple flavored feed. The authors found that after exposure to a flavor, the lambs preferred the alternate flavor at the next meal.

Another explanation suggested by the authors for the added sucrose having no effect on DMI is that preference and desire to consume a certain flavor may be reduced by repeated exposure to that flavor for long periods of time (Nombekela and Murphy, 1995). This phenomenon, sometimes called "sensory-specific satiety", can be caused by feeding monotonous diets to animals for a long time (Rolls, 1986). Although offering different flavors may increase intake, the present study was designed with single diets offered to reflect the industry standard. The concentration of the sugar used in the study conducted by Nombekela and Murphy (1995) was less than that used by Broderick and Radloff (2004) and Penner and Oba (2009). This difference in concentration may have lead to the contradictory results between the studies.

Researchers have consistently found that whether DMI increased (Broderick and Radloff, 2004; Penner and Oba, 2009) or was not affected (Nombekela and Murphy, 1994), supplementation with sugar had no effect on milk production. It has also been

reported that treatment with sugar has no effect on 3.5 % FCM (Nombekela and Murphy, 1994). Nombekela and Murphy (1994) reported that sugar also had no effect on feed efficiency. All of these results are similar to the current study; however these previous studies were conducted using sugars that had an added energy value. In our study, the flavor additive contained a sweetener with no caloric value and no nutritional composition. Therefore, any changes seen in our study were due to the varied taste. Feed efficiency was within a normal range throughout the trial (Hutjens, 2005). Nombekela and Murphy (1994) reported a trend for decreased body weight in cows fed a TMR supplemented with sucrose.

In the present study, there was no effect of treatment on milk fat yield. However, previous studies have shown that sugar supplementation often causes an increase in fat yield. For example, Nombekela and Murphy (1994) reported that cows fed a TMR supplemented with sucrose produced more milk fat per day. Penner and Oba (2009) also reported that fat yield was increased with added sucrose. Similarly, Broderick and Radloff (2004) reported that treatment with molasses caused an increase in fat yield. In our study there was a trend for increased production of milk protein in cows fed TRT. Previous studies have reported the opposite effect of sugar supplementation on milk protein. For example, Nombekela and Murphy (1994) reported that cows fed TMR with added sucrose produced less milk protein per day. It is unknown why in the present study there was an increase in milk protein.

Although no-calorie sweeteners have not been added to lactating cow diets, saccharin has been added to feed for male calves. Brown et al. (2004) found that when male beef calves were offered increasing amounts of a saccharin-based additive, DMI increased as well as average daily gain. In a follow up study, McMeinman et al. (2006) reported that when male calves were offered the saccharin-based additive, there was a tendency for increased body weight. The authors concluded that the improved palatability of the feed caused an increase in DMI and therefore an increase in body weight.

Multiparous Animals

Data for only multiparous animals are shown in Table 6 (n = 10 for TRT and 11 for CTRL). There was a tendency for greater DMI (+1.5 kg/d, $P = 0.07$) and milk production (+3.9 kg/d, $P = 0.10$) for cows fed TRT. Therefore, though no detectable difference in intake or milk production was observed for the entire experimental group, there was a difference when parity was taken into consideration. There was no difference between the treatments in percent of milk lactose, percent of milk protein, percent of milk fat, or kg/d of milk fat but cows fed TRT produced more milk protein (1.33 kg/d) than cows fed CTRL (1.24 kg/d; $P = 0.04$). Milk urea nitrogen was within a normal range (Jonker et al., 1999). Somatic cell count was not different between treatments and indicated minimal mammary infection. Average body weight, body weight gain, ECM, 3.5% FCM, and feed efficiency were not different between the treatments. Feed efficiency was within a normal range throughout the trial (Hutjens et al., 2005).

Dry matter intake of multiparous animals covaried weekly with pretreatment data is shown in Figure 3 (Appendix C). Dry matter intake was significantly higher ($P \leq 0.05$) in cows fed the TRT in wk 3, 4, and 9. There was a trend ($P \leq 0.10$) for increased DMI in wk 5 and 7. Milk production of multiparous animals covaried weekly with pretreatment data is shown in Figure 4 (Appendix D). Milk production was significantly higher ($P \leq 0.05$) in wk 3. There was a trend ($P \leq 0.10$) for increased milk production in wk 4, 9, and 10.

Previous work on the effect of added sugar on DMI and milk production of multiparous cows has resulted in contradictory results. For example, some studies have reported that when a sugar additive is added to a TMR and fed to multiparous cows, there is no effect on milk production or DMI. For example, Murphy et al. (1996) offered multiparous, Holstein cows in early lactation either a control TMR, a TMR sweetened with a brown sugar food product, or a choice between the control and the sweetened TMR. These authors reported that there was no difference among treatments in DMI or milk production. There was also no difference in BW, percent of milk fat, or percent of milk protein. However, the cows in this study showed a preference for the sweetened feed when offered a choice ($P < 0.02$). In another study, Martel et al. (2011) replaced corn grain in a TMR with 0%, 2.5%, or 5% molasses then fed this TMR to second-lactation Holstein cows. The authors found that treatment with molasses had no impact on DMI.

Other studies on sugar added to a TMR have reported an effect of treatment on DMI. For example Broderick et al. (2008) fed multiparous cows TMR with increasing amounts of sucrose, 0%, 2.5%, 5.0%, and 7.5%. The authors found that DMI increased as the amount of sucrose in the diet increased ($P = 0.02$). These results are similar to the current study. However, unlike the current study, the authors reported no effect of treatment on milk production. The authors attributed this increase in DMI to improved palatability of the TMR with the added sugar.

When dairy cows calve for the first time at around 24 months old they have not reached mature body weight (Coffey et al., 2006). Therefore, during their first lactation primiparous cows have to partition energy and nutrients for milk production and maintenance as well as for growth. Metabolic differences between primiparous and multiparous cows have been shown to limit the partitioning of nutrients into milk and therefore primiparous cows tend to produce less milk than multiparous cows (Wathes et al., 2007). Primiparous cows also tend to consume less feed than multiparous cows (NRC, 2001). A combination of this decreased intake and partitioning of energy into growth for primiparous cows may have impacted the effect of the flavor additive on DMI and milk production of the primiparous cows. In the current study the effect of the flavor additive on milk production was greater in multiparous cows because they were consuming more feed and putting most of this consumed energy towards milk production.

Rumen pH

Table 7 shows the rumen pH mean, minimum, maximum, and range as well as the time in min that the rumen pH was below 5.5 and below 5.8. There was an effect of wk × treatment on mean rumen pH (P =0.03). Compared with CTRL, cows fed TRT had a higher mean rumen pH although the rumen pH of both groups of cows fluctuated weekly. There was an effect of wk × treatment on time the pH was below 5.8 ($P < 0.01$). The rumen pH of cows fed TRT was below 5.8 for fewer hours throughout the day when compared to CTRL cows. The interaction is due to a greater difference between the treatments in wk 6 and 9 than other wks in the trial. There was an effect of treatment on minimum ($P < 0.01$) pH and maximum pH (P = 0.05) with CTRL cows having a lower minimum and maximum pH when compared to TRT cows. There was an effect of treatment on time the rumen pH was below 5.5 ($P < 0.01$). The rumen pH of TRT cows was below 5.5 for a shorter period of time than CTRL cows. There was no effect of week, treatment or, wk × treatment on rumen pH range.

Theoretically, it has been thought that added sugar would cause decreased rumen pH and therefore an increased risk of acidosis because the sugar is rapidly fermentable (Leonardi and Armentano, 2003; DeVries et al., 2008). Previous studies have found that adding sugar, such as sucrose and molasses, to a TMR actually causes an increase in rumen pH (Penner et al., 2009; Penner and Oba, 2009). In these studies the impact of the sugar product on rumen fermentation may be related to the increased energy brought to

the diet in the form of rapidly fermentable carbohydrates. Martel et al. (2011) fed second lactation cows TMR where corn grain was replaced with 0%, 2.5% or 5% molasses. Average rumen pH increased as the percent of molasses in the diet increased ($P = 0.02$). The authors hypothesized that increased uptake of VFA from the rumen caused the increase in rumen pH.

In another study, Penner et al. (2009) fed cows TMR with or without Fermenten (Church and Dwight, Princeton, NJ) and with added sucrose at 2.8% or 5.7% of the dietary sugar. The authors found that increased sucrose supplementation tended to cause an increase in daily minimum pH (5.61 vs. 5.42; $P = 0.09$) as well as average rumen pH (6.30 vs. 6.17; 5.5$P = 0.09$). The rumen pH of cows fed the high sugar diet tended to spend less time below 5.8 (139 vs. 283 min/d; $P = 0.08$) but the reason for this finding was unknown. Similarly, Penner and Oba (2009) fed Holstein cows starting at 1 DIM either a low sugar (4.5% DM basis) or high sugar (8.8% DM basis) diet. Average rumen pH ($P = 0.08$) and maximum rumen pH ($P = 0.07$) were higher in cows consuming the high sugar diet.

Penner and Oba (2009) provided several hypotheses for why increased sucrose in the TMR caused an increase in rumen pH. First, they thought some of the sugar may have been respired, however, they concluded that since the TMR was made each day, respiration losses would be minimal. They thought that sucrose may provide less carbon

than starch causing reduced fermentation and reduced production of microbial protein. However, cows fed the sugar gained weight indicating that they were not energy deficient due to the lack of carbon. It was hypothesized that bacteria could have converted the sugar and stored it as glycogen rather than fermenting it to acids. Finally, the sugar may have increased the passage rate out of the rumen causing a decrease in fermentation acids. Overall, all of these theories were speculation and the authors provided arguments against each theory. Ultimately, the authors concluded that the cause of the increased rumen pH with added sugar was unknown (Penner and Oba, 2009).

Even though several studies reported an increase in rumen pH with sugar supplementation, Broderick and Radloff (2004) reported no effect. The authors supplemented TMR with increasing levels of molasses and did not see an effect of sugar on rumen pH. However, the authors hypothesized that this lack of effect may have been due to the fact the pH data was only collected for 12 h after feeding.

In the present study, the flavor additive contained no energy and therefore, changes in rumen pH were due to the change in taste. It is thought that the increase in rumen pH seen in cows fed the TRT diet was due to the increased DMI. An increase in DMI could have lead to a greater consumption of effective fiber and studies have shown that rumen pH increases with increased consumption of effective fiber (Zebeli et al., 2006). With the increase in DMI there could have been an increase in chewing and saliva

production, even though saliva production was not measured in this study. If saliva production was increased, this would mean more sodium bicarbonate would be produced to buffer the rumen and therefore increase rumen pH.

Sorting Behavior

Particle size and physically effective fiber data are shown in Table 8. Overall, treatment with Luctcrom did not affect the distribution of particle size or physically effective fiber from the TMR over time in the feed bunk. There was an effect of sampling hour on both particle size ($P < 0.01$) and physically effective fiber ($P < 0.01$). As time went on, the proportion of fine particles decreased while the proportion of medium and short particles increased. The proportion of long particles was greatest 6 h after feeding. The amount of physically effective fiber increased over time. This change in particle size proportions and physically effective fiber throughout the day could indicate that cows were sorting.

Similar to the current study, Maulfair and Heinrichs (2010) found that the amount of small particles in a TMR decreased over a 24 h period while the amount of large and medium particles increased. In a study on the effect of sugar supplementation on sorting behavior, Penner and Oba (2009) fed cows in early lactation either a low (4.4% DM basis) or high (8.8% SM basis) sugar diet. The authors found that sorting behavior was

altered in cows fed the high sugar diet. These cows decreased their sorting for medium particles ($P = 0.03$) and increased their sorting for fine particles ($P = 0.01$). In these previous studies, the sugar additive was added to the whole ration. In the current study, the sugar additive was applied directly to the forage portion of the ration because it was believed that this would make the flavor of the forage more uniform and therefore should discourage sorting. However, this theory was not supported in the current study.

Chapter 7

CONCLUSION

Adding the flavor additive, Luctarom ProEfficient, did not have an effect on DMI, milk production, or milk composition when all the data from multiparous and primiparous cows was combined. Treatment with the flavor additive resulted in a trend for increased DMI and milk production for multiparous cows, however there was still no effect on milk composition. There was no effect of treatment on TMR sorting behavior. There was some evidence that adding this flavor additive may alter ruminal pH and metabolism as supported by the increase in average rumen pH, minimum rumen pH, and maximum rumen pH in cows fed the flavored TMR. Further studies are needed to better understand why these changes occurred.

TABLES

Table 1. Preliminary data for all animals from the 2 wk pretreatment period.

Item	DMI, kg/d	Milk, kg/d	DIM	BW, kg	Lact[1] Number
Control[2]					
Mean	22	40	54	688	2.3
Median	23	42	60	658	2.0
Minimum	17	22	9	617	1.0
Maximum	26	52	114	880	4.0
SD[3]	3	9	36	91	1.0
Treated[4]					
Mean	22	41	55	708	2.6
Median	21	38	57	689	2.0
Minimum	14	31	14	608	1.0
Maximum	28	55	103	821	5.0
SD	4	9	29	74	1.4

[1]Lactation number.
[2]The forage portion of the TMR was treated with water prior to mixing into the TMR.
[3]Standard deviation.
[4]The forage portion of the TMR was treated with Luctarom ProEfficient (Lucta S.A., Spain) prior to mixing into the TMR.

Table 2. Ingredient composition of concentrate.

Ingredient	%, DM basis
Corn, medium ground	28.25
Canola meal, solvent	16.55
Turbo meal[1]	14.12
Corn hominy	10.69
Dried citrus pulp	10.07
Soybean meal, 47.55%	2.67
Distillers grain	2.64
Palm fat	2.20
Blood meal	1.90
Molasses	1.83
Soybean hulls, ground	1.80
Sodium bicarbonate	1.57
Calcium carbonate	1.49
DCAD plus[2]	0.97
Urea	0.84
MM3 chelate[3]	0.70
Rumensin[4]	0.67
NaCl	0.59
Magnesium oxide	0.31
Smartamine® M[5]	0.14
Biotin	<0.01
Selenium premix, 0.06%	<0.01
Levucell SC[6]	<0.01

[1]Extruded and expelled soybean meal (Renaissance Nutrition Inc., Roaring Spring, PA).
[2]Potassium carbonate (Arm and Hammer Animal Nutrition, Princeton, NJ).
[3]Vitamins and trace minerals premix containing (minimum/kg) 1.75% Ca, 31.8% Mg, 7.90% S, 4.5% K, 9002 ppm Zn, 1919 ppm Fe, 1857 ppm Cu, 4670 ppm Mn, 139 ppm Co, 219 ppm I, 86 ppm Se, 640 kIU Vitamin A, 161 kIU Vitamin D, 3.2 kIU Vitamin E (Renaissance Nutrition Inc., Roaring Spring, PA).
[4]Monensin sodium (Elanco Animal Health, Greenefield, IN).
[5]Encapsulated methionine for ruminants (Bluestar Adisseo Nutrition Group, Alpharetta, GA).
[6]*Saccharomyces cerevisiae* CNCM-1077 product 20×10^9; (Lallemand Animal Nutrition, Milwaukee, WI).

Table 3. Average chemical composition of corn silage, alfalfa silage, and concentrate fed in the 10 wk lactation trial as % DM unless otherwise stated (n = 5).

Item	Corn Silage	Alfalfa Silage	Concentrate
DM, %	36.72 ± 0.95	32.03 ± 4.86	90.66 ± 0.92
CP	8.54 ± 0.35	19.22 ± 0.61	25.50 ± 0.53
SP[1], % of CP	53.68 ± 3.61	63.30 ± 4.75	25.50 ± 3.43
NE_L, Mcal/kg	1.58 ± 0.03	1.23 ± 0.12	1.77 ± 0.01
ADF	28.20 ± 1.14	40.28 ± 3.34	9.20 ± 0.46
NDF	45.34 ± 1.45	46.04 ± 3.65	15.52 ± 1.14
Ash	4.02 ± 0.25	8.26 ± 0.38	9.76 ± 0.34
Starch	30.36 ± 4.99	ND[2]	31.00 ± 3.22
NFC	39.82 ± 1.91	25.32 ± 3.28	ND
Ca	0.29 ± 0.02	1.35 ± 0.04	1.40 ± 0.08
P	0.24 ± 0.01	0.32 ± 0.04	0.51 ± 0.03
Mg	0.20 ± 0.00	0.31 ± 0.02	0.71 ± 0.12
K	1.28 ± 0.04	1.89 ± 0.18	1.58 ± 0.06
Na	0.01 ± 0.00	0.05 ± 0.01	0.81 ± 0.04
Fe, ppm	123 ± 8	356 ± 52	386 ± 30
Mn, ppm	21 ± 1	34 ± 3	82 ± 13
Zn, ppm	26 ± 1	23 ± 1	128 ± 24
Cu, ppm	8 ± 1	11 ± 1	26 ± 9

[1]Soluble protein.
[2]Not determined.

Table 4. Average chemical composition of TMR samples fed during the 10 wk lactation trial as % of DM unless stated otherwise (n = 10).

Item	Control[1]	Treated[2]
DM, %	46.99 ± 0.93	47.38 ± 1.10
CP	16.63 ± 0.84	16.75 ± 0.62
SP[3], % of CP	39.61 ± 4.19	38.17 ± 4.05
NE_L, Mcal/kg	1.67 ± 0.02	1.68 ± 0.03
ADF	22.58 ± 1.41	22.05 ± 1.47
NDF	33.45 ± 1.96	32.78 ± 1.94
Ash	6.97 ± 0.31	7.11 ± 0.20
Starch	25.62 ± 1.57	26.33 ± 1.82
NFC	39.68 ± 1.20	40.14 ± 1.30
Ca	0.85 ± 0.08	0.85 ± 0.06
P	0.35 ± 0.05	0.36 ± 0.02
Mg	0.42 ± 0.04	0.42 ± 0.05
K	1.51 ± 0.09	1.51 ± 0.07
Na	0.36 ± 0.00	0.36 ± 0.00
Fe, ppm	337 ± 31	358 ± 45
Mn, ppm	55 ± 8	54 ± 8
Zn, ppm	71 ± 12	70 ± 11
Cu, ppm	19 ± 3	19 ± 3

[1]The forage portion of the TMR was treated with water prior to mixing into the TMR.
[2]The forage portion of the TMR was treated with Luctarom ProEfficient (Lucta S.A., Spain prior to mixing into the TMR).
[3]Soluble protein.

Table 5. Effect of treating the forage portion of the TMR with a flavoring agent on covariate-adjusted least squares means of lactation performance for all cows (primi- and multiparous).

Item	Control[1]	Treated[2]	SEM	*P*-value
DMI, kg/d	25.85	26.80	0.57	0.25
DMI, % BW	3.65	3.64	0.06	0.93
Milk, kg/d	40.48	42.49	1.41	0.32
Milk fat,				
%	4.02	4.01	0.09	0.97
kg/d	1.58	1.66	0.05	0.28
Milk protein,				
%	3.02	3.04	0.04	0.79
kg/d	1.22	1.28	0.03	0.08
MUN, mg/dl	13.47	13.64	0.42	0.77
Milk lactose, %	4.73	4.77	0.03	0.31
SCC, × 1000/mL	241	186	57	0.48
3.5% FCM, kg/d	44.12	45.27	1.63	0.62
ECM, kg/d	42.61	44.53	1.40	0.34
Feed efficiency[3]	1.68	1.67	0.04	0.79
BW, kg	716	733	8	0.13
BW gain, kg	22	37	8	0.18

[1]The forage portion of the TMR was treated with water prior to mixing into the TMR (n = 14).
[2]The forage portion of the TMR was treated with Luctarom ProEfficient (Lucta S.A., Spain) prior to mixing into the TMR (n = 14).
[3]FCM/DMI.

Table 6. Effect of treating the forage portion of the TMR with a flavoring agent on covariate-adjusted least squares means on lactation performance of multiparous cows.

Item	CTRL[1]	TRT[2]	SEM	*P*-value
DMI, kg/d	26.29	27.83	0.58	0.07
DMI, % BW	3.63	3.66	0.07	0.70
Milk, kg/d	41.26	45.12	1.60	0.10
Milk fat,				
%	4.01	3.97	0.10	0.76
kg/d	1.69	1.73	0.08	0.72
Milk protein,				
%	3.01	2.97	0.04	0.51
kg/d	1.24	1.33	0.03	0.04
MUN, mg/dl	13.72	13.77	0.46	0.95
Milk lactose, %	4.69	4.74	0.03	0.28
SCC, × 1000/mL	271	280	90	0.94
3.5% FCM, kg/d	44.83	48.33	1.95	0.21
ECM, kg/d	43.54	46.78	1.71	0.19
Feed efficiency	1.72	1.72	0.05	0.96
BW, kg	735	754	10	0.19
BW gain, kg	21	34	10	0.34

[1]The forage portion of the TMR was treated with water prior to mixing into the TMR (n=11).
[2]The forage portion of the TMR was treated with Luctarom ProEfficient (Lucta S.A., Spain) prior to mixing into the TMR (n = 10).
[3]FCM/DMI.

Table 7. Effect of treating the forage portion of the TMR with a flavoring agent and week on rumen pH.

Item	Avg[1] pH	Min[2] pH	Max[3] pH	Range	Time (min) pH ≤ 5.5	Time (min) pH ≤ 5.8
Control[4]						
Wk 2	6.26^{abc}	5.53	6.79	1.27	53	128^{bcd}
Wk 3	6.02^{c}	5.33	6.58	1.24	173	398^{ab}
Wk 4	6.19^{abc}	5.36	6.75	1.39	75	218^{abcd}
Wk 5	6.18^{abc}	5.38	6.81	1.43	60	218^{abcd}
Wk 6	6.05^{c}	5.27	6.69	1.42	143	375^{ab}
Wk 7	6.10^{bc}	5.27	6.72	1.44	75	232^{abcd}
Wk 8	6.05^{c}	5.30	6.66	1.36	98	420^{a}
Wk 9	6.06^{bc}	5.36	6.76	1.41	158	360^{abc}
Wk 10	6.05^{c}	5.35	6.77	1.42	53	263^{abcd}
Average	6.11^{B}	5.35^{B}	6.72^{B}	1.38	98^{A}	290^{A}
Treated[5]						
Wk 2	6.48^{a}	5.95	7.07	1.13	30	15^{d}
Wk 3	6.24^{abc}	5.41	7.08	1.68	0	210^{abcd}
Wk 4	6.18^{abc}	5.46	6.87	1.41	53	203^{abcd}
Wk 5	6.16^{bc}	5.44	6.80	1.36	53	263^{abcd}
Wk 6	6.33^{abc}	5.65	6.72	1.13	83	75^{cd}
Wk 7	6.31^{abc}	5.45	7.08	1.62	15	150^{abcd}
Wk 8	6.36^{abc}	5.56	6.94	1.38	23	90^{bcd}
Wk 9	6.43^{ab}	5.68	7.01	1.33	30	45^{d}
Wk 10	6.34^{abc}	5.73	6.95	1.22	0	90^{bcd}
Average	6.31^{A}	5.59^{A}	6.94^{A}	1.36	32^{B}	128^{B}
SEM	0.08	0.10	0.15	0.24	42	64
			P - value			
Wk[6]	0.02	<0.01	0.65	0.22	0.51	0.02
Trt[7]	<0.01	<0.01	0.05	0.93	<0.01	<0.01
Wk × Trt[8]	0.03	0.26	0.27	0.09	0.29	<0.01

[a,b,c,d]Least squares means within a column with unlike superscripts differ wk × trt ($P \leq 0.05$).

[A,B]Least squares means with unlike superscripts differ by treatment ($P \leq 0.05$).

[1]Average rumen pH.

[2]Minimum rumen pH.

[3]Maximum rumen pH.

[4]The forage portion of the TMR was treated with water prior to mixing into the TMR.

[5]The forage portion of the TMR was treated with Luctarom ProEfficient (Lucta S.A., Spain) prior to mixing into the TMR.

[6]Effect of week.

[7]Effect of treatment.

[8]Effect of treatment by week.

Table 8. Effect of treating the forage portion of the TMR with a flavoring agent and time on sorting, expressed as % retained on an as-fed basis, of long, medium, short, and fine particles and on physically effective fiber at different times after feed delivery.

	Long[1] Particles	Medium Particles	Short Particles	Fine Particles	Pef[2]
Control[3]					
0	5	38	38	19	0.63
6	6	39	38	17	0.64
12	5	39	39	17	0.65
24	5	40	40	15	0.65
Treated[4]					
0	6	37	38	19	0.62
6	7	37	38	18	0.64
12	6	38	39	17	0.64
24	6	39	39	16	0.64
SEM	0.41	0.72	0.43	0.62	0.01
			P - value		
Trt[5]	0.06	0.16	0.55	0.67	0.48
Hr[6]	0.01	0.01	0.01	0.01	0.01
Trt × Hr[7]	0.65	0.74	0.06	0.67	0.85

[1]As measured using Penn State Forage Particle Separator (Pennsylvania State University, University Park, PA).
[2]Physically effective fiber, as measured using the Z-Box (Miner Institute, Chazy, NY).
[3]The forage portion of the TMR was treated with water prior to mixing into the TMR.
[4]The forage portion of the TMR was treated with Luctarom ProEfficient (Lucta S.A., Spain) prior to mixing into the TMR.
[5]Effect of treatment.
[6]Effect of sampling hour.
[7]Effect of treatment by hour.

FIGURES

Figure 1. Effect of treating the forage portion of the TMR with a flavoring agent on DMI (kg/d) of all cows (primi- and multiparous), covariate-adjusted (on pretreatment) least squares means by week. Control (the forage portion of the TMR was treated with water prior to mixing into the TMR; - - -) and treated (The forage portion of the TMR was treated with Luctarom ProEfficient (Lucta S.A., Spain) prior to mixing into the TMR; --). [a,b]Least squares means on the graph with unlike superscripts differ ($P \leq 0.05$).

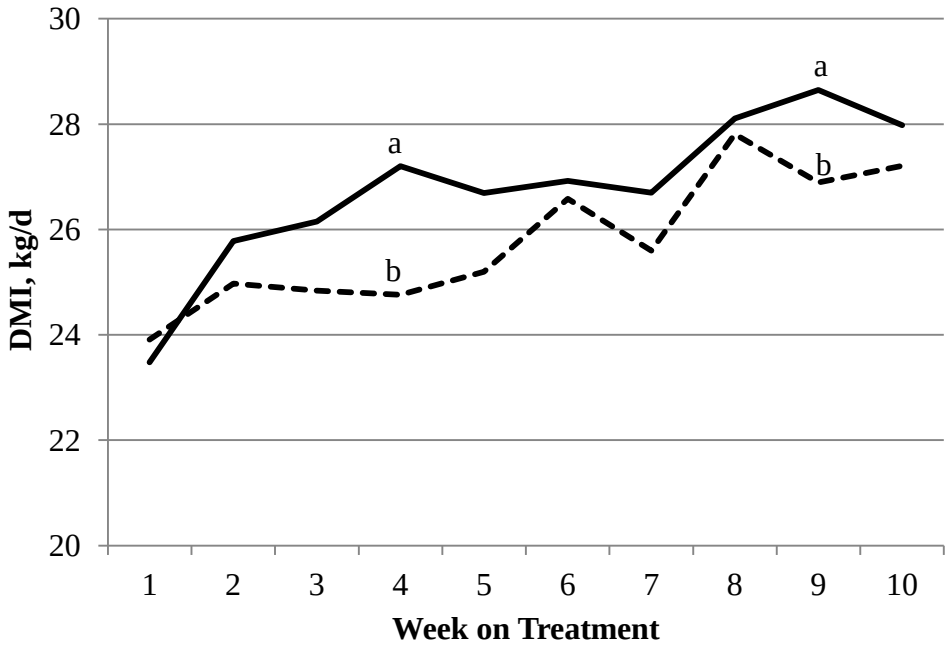


Figure 2. Effect of treating the forage portion of the TMR with a flavoring agent on milk production (kg/d) of all cows (primi- and multiparous), covariate-adjusted (on pretreatment) least squares means by week. Control (the forage portion of the TMR was treated with water prior to mixing into the TMR; - - -) and treated (The forage portion of the TMR was treated with Luctarom ProEfficient (Lucta S.A., Spain) prior to mixing into the TMR; --).

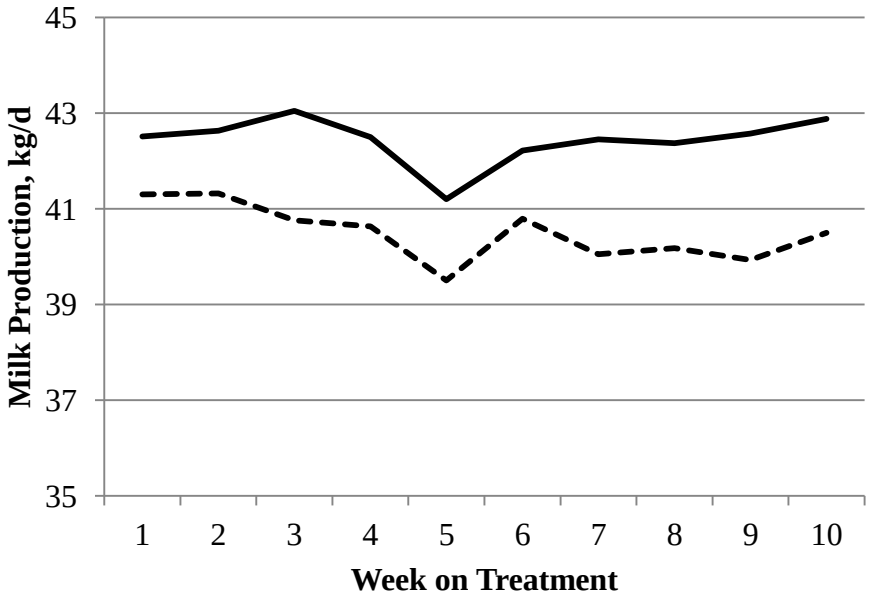


Figure 3. Effect of treating the forage portion of the TMR with a flavoring agent on DMI (kg/d) of multiparous cows, covariate-adjusted (on pretreatment) least squares means by week. Control (the forage portion of the TMR was treated with water prior to mixing into the TMR; - - -) and treated (The forage portion of the TMR was treated with Luctarom ProEfficient (Lucta S.A., Spain) prior to mixing into the TMR; --). [a,b]Least squares means on the graph with unlike superscripts differ ($P \leq 0.05$). [C,D]Least squares means on the graph with unlike superscrips differ ($P \leq 0.10$).

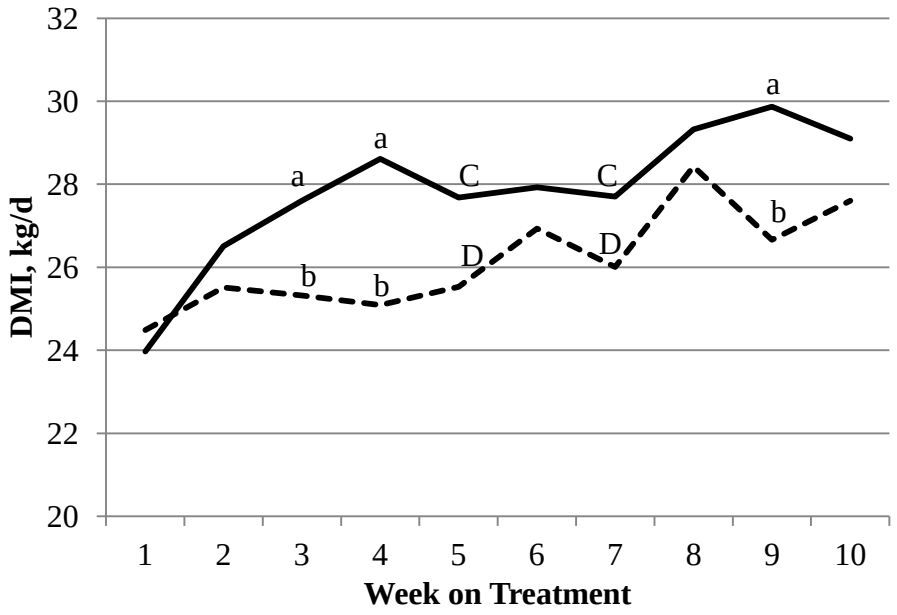
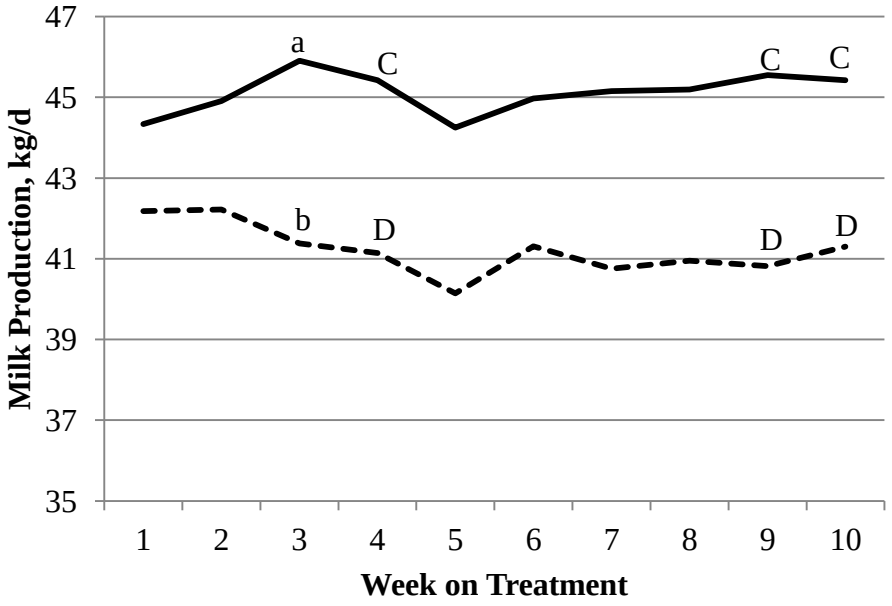


Figure 4. Effect of treating the forage portion of the TMR with a flavoring agent on milk production (kg/d) of multiparous cows, covariate-adjusted (on pretreatment) least squares means by week. Control (the forage portion of the TMR was treated with water prior to mixing into the TMR; - - -) and treated (The forage portion of the TMR was treated with Luctarom ProEfficient (Lucta S.A., Spain) prior to mixing into the TMR; --). [a,b]Least squares means on the graph with unlike superscripts differ ($P \leq 0.05$). [C,D]Least squares means on the graph with unlike superscrips differ ($P \leq 0.10$).



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Appendix A

EFFECT OF TREATING THE FORAGE PORTION OF THE TMR WITH A FLAVORING AGENT ON DMI (KG/D) OF ALL COWS (PRIMI- AND MULTIPAROUS), COVARIATE-ADJUSTRED (ON PRETREATMENT) LEAST SQUARES MEANS BY WEEK

Week	Control[1]	Treated[2]	SEM	*P* - value
1	23.91	23.48	0.32	0.35
2	24.97	25.78	0.41	0.17
3	24.84	26.15	0.56	0.11
4	24.76[b]	27.20[a]	0.66	0.01
5	25.20	26.69	0.67	0.13
6	26.58	26.92	0.70	0.74
7	25.60	26.70	0.64	0.24
8	27.81	28.11	0.78	0.79
9	26.29[b]	28.65[a]	0.76	0.04
10	27.21	27.98	0.73	0.46

[1]The forage portion of the TMR was treated with water prior to mixing into the TMR (n = 14).

[2]The forage portion of the TMR was treated with Luctarom ProEfficient (Lucta, S.A., Spain) prior to mixing into the TMR (n = 14).

[a,b]Least squares means on the graph with unlike superscripts differ ($P \leq 0.05$).

Appendix B

EFFECT OF TREATING THE FORAGE PORTION OF THE TMR WITH A FLAVORING AGENT ON MILK PRODUCTION (KG/D) OF ALL COWS (PRIMI- AND MULTIPAROUS), COVARIATE-ADJUSTRED (ON PRETREATMENT) LEAST SQUARES MEANS BY WEEK

Week	Control[1]	Treated[2]	SEM	*P* - value
1	41.29	42.51	0.81	0.30
2	41.32	42.63	1.12	0.42
3	40.76	43.05	1.30	0.22
4	40.63	42.50	1.44	0.37
5	39.50	41.20	1.64	0.47
6	40.79	42.22	1.66	0.55
7	40.05	42.45	1.62	0.31
8	40.18	42.37	1.63	0.35
9	39.93	42.57	1.65	0.27
10	40.50	42.88	1.52	0.28

[1]The forage portion of the TMR was treated with water prior to mixing into the TMR (n = 14).
[2]The forage portion of the TMR was treated with Luctarom ProEfficient (Lucta, S.A., Spain) prior to mixing into the TMR (n = 14).

Appendix C

EFFECT OF TREATING THE FORAGE PORTION OF THE TMR WITH A FLAVORING AGENT ON DMI (KG/D) OF ONLY MULTIPAROUS COWS, COVARIATE-ADJUSTRED (ON PRETREATMENT) LEAST SQUARES MEANS BY WEEK

Week	Control[1]	Treated[2]	SEM	*P* - value
1	24.49	23.97	0.36	0.31
2	25.51	26.51	0.45	0.13
3	25.32[b]	27.60[a]	0.55	0.01
4	25.09[b]	28.61[a]	0.66	0.01
5	25.53[D]	27.68[C]	0.76	0.06
6	26.93	27.93	0.77	0.36
7	26.01[D]	27.70[C]	0.70	0.10
8	28.43	29.32	0.75	0.40
9	26.66[b]	29.87[a]	0.75	0.01
10	27.60	29.10	0.75	0.17

[1]The forage portion of the TMR was treated with water prior to mixing into the TMR (n = 11).
[2]The forage portion of the TMR was treated with Luctarom ProEfficient (Lucta, S.A., Spain) prior to mixing into the TMR (n = 10).
[a,b]Least squares means on the graph with unlike superscripts differ ($P \leq 0.05$).
[C,D]Least squares means on the graph with unlike superscrips differ ($P \leq 0.10$).

Appendix D

EFFECT OF TREATING THE FORAGE PORTION OF THE TMR WITH A FLAVORING AGENT ON MILK PRODUCTION (KG/D) OF ONLY MULTIPAROUS, COVARIATE-ADJUSTRED (ON PRETREATMENT) LEAST SQUARES MEANS BY WEEK

Week	Control[1]	Treated[2]	SEM	*P* - value
1	42.18	44.34	1.02	0.14
2	42.22	44.91	1.30	0.15
3	41.38[b]	45.91[a]	1.42	0.03
4	41.14[D]	45.42[C]	1.71	0.09
5	40.14	44.25	1.89	0.14
6	41.31	44.97	2.00	0.21
7	40.75	45.15	1.93	0.12
8	40.95	45.19	1.86	0.12
9	40.82[D]	45.55[C]	1.82	0.08
10	41.30[D]	45.42[C]	1.73	0.10

[1]The forage portion of the TMR was treated with water prior to mixing into the TMR (n = 11).
[2]The forage portion of the TMR was treated with Luctarom ProEfficient (Lucta, S.A., Spain) prior to mixing into the TMR (n = 10).
[a,b]Least squares means on the graph with unlike superscripts differ ($P \leq 0.05$).
[C,D]Least squares means on the graph with unlike superscrips differ ($P \leq 0.10$).

Appendix E. Animal care and use committee project application and proposal.

UNIVERSITY OF DELAWARE

COLLEGE OF AGRICULTURE AND NATURAL RESOURCES

AGRICULTURAL ANIMAL CARE AND USE COMMITTEE

Application for Use of Agricultural Animals

In Teaching or Research

AACUC Protocol Number: (11) 10-05-11 R

TITLE OF PROJECT: Flavor Enhancer Lactation Study (Revised) 10/13/2011

INSTRUCTOR/PRINCIPAL INVESTIGATOR

Limin Kung, Jr. _____ 10/5/11

Printed Name Signature Date

(This section for Committee use only)

Application Approved (date) 10-12-2011

Application Rejected (date) _____

Reason for Rejection _____

_____ 10-12-2011

Signature, Animal Care and Use Committee Date

APPLICATION INFORMATION:

Title: Flavor Enhancer Lactation Study

Instructor/Principal Investigator: L. Kung, Jr.

Address: TNS 33

Telephone: 2524 Email: lksilage@udel.edu

People involved in animal care (not listed above) for this protocol:

Name	Email	Office Phone #	Home/Cell Phone #	Received Animal Care Training	
				Yes	No
Co-investigator(s)					
Caitlyn Merrill	nerrill@udel.ec	2269	9082688901	x	
	emerrill@udel.edu				

Name the person(s) responsible for conducting the training.
R. Morris and L. Kung

If after hours participation is required by students, please describe how this is being handled. (e.g. supervisors, assistants, etc.) Please include the times and days that students may be on site.

Has everyone listed above read the application and is familiar with the proposed work?

YES ☒ NO ☐

If no, identify those needing to read application.

New or Three Year Review (mark one)

NEW ☒ THREE YEAR ☐

If this is a 3 year renewal, what is the assigned existing protocol number?

Teaching or Research Application (mark one)

TEACHING ☐ RESEARCH ☒

If TEACHING box was checked, select from the following:

Demonstration ☐ Laboratory ☐ Student Project ☐

Proposed start date: 10/~~11~~12/11 **End date:** 12/31/11

Are all proposed animal care management procedures 1) defined as "pre-approved" by the Animal Care and Use Committee, or 2) part of the Standard Operating Procedures developed by the Animal Care and Use Committee for that particular species?

YES ☒ NO ☐ to be determined by AACUC ☐

Has everyone been trained in all procedures that are listed int this protocol? Yes ☒ No ☐

Who has not been trained?

ANIMAL INFORMATION:

Common Name of the Animal Requested: **Holstein**

Amount Being Requested: **30**

Source of Animals: **UD Dairy**

Where are the animals being housed: **UD Dairy**

Briefly Describe the Goals or Objectives of this Application (use additional space as needed).
Evaluate the effect of a flavor enhancer on lactating cows

Please state or attach your animal protocol.
attached

How did you determine the number of experimental animals you are requesting? If you have a table showing treatment groups and animal numbers please insert here or include as an attachment.
"Historic, meaning 30 Calin gates were installed at the Dairy to meet the minimal statistical requirements for theres types of studies and that is the basis for the 30 animals for this study."

Please verify that the research involved in this protocol is new and is not a duplication of work already performed.
No data published on this additive

Does this procedure involve surgery? YES ☐ NO X

If yes, explain in detail the surgery.

Will the animals experience pain? YES ☐ NO ☐

If so, what is your pain management protocol? Please insert here or include as an attachment (euthanasia is an acceptable means of pain management): _____

Are drugs, vaccines and/or medications being used? YES ☐
NO ☒

If yes, describe what is being used. Include dosages and sites.

How often are animals monitored and how are sick or injured animals being handled?
Daily

What is the method of euthanasia?
Standard Farm Protocol

List the veterinarian who is on-call.
New Bolton Field Service — Name
610 925 6310 — Telephone

Does this application need approval from EHS? YES ☐ NO ☐

If yes, what form(s) are attached?_____

NOTE: EHS approval is required for experiments involving the administration of hazardous or biological materials such as pathogens, carcinogens, highly toxic, or radioactive materials.

A Proposal on

Evaluation of a Flavor Enhancer on Intake and Production of High Producing Lactating Cows

Submitted to:

Chad Risley, Ph.D.
Lucta
950 Technology Way, Ste 110
Libertyville, IL 60048
Tel: +1 847 996 3400
Fax: +1 847 996 3401
E-Mail: Chad.Risley@lucta.net
http://www.lucta.com

By:

Limin Kung, Jr., Ph.D.
Department of Animal and Food Sciences
University of Delaware
Newark, DE 19717-1303
Phone: 302-831-2522
Fax: 302-832-3651
Email: LKSILAGE@UDEL.EDU

Proposed Start Date: September 15, 2011
Proposed End Date: September 14, 2012

Introduction

Cows in early lactation are unable to consume sufficient quantities of nutrients to meet requirements for milk production. This phenomena is not well understood but is simplistically caused by high production coupled with the animal's inability to consume high levels of dry matter. Thus, methods to improve dry matter intake have great potential to enhance animal performance.

Another factor affecting the performance of lactating cows is the interaction between ruminal fermentation and its ability to safely supply nutrients to the host. Unregulated and uneven ruminal fermentations caused by excess consumption of rapidly fermentable carbohydrates (e.g. concentrates) often result in acidosis. Low ruminal pH caused by acidosis leads to lower fiber digestion and production of microbial protein and in severe cases, systemic acidosis. To moderate ruminal fermentations, cows are fed total mixed rations (TMR) with the hypothesis that every bite of TMR yields a homogenous mix of forages and concentrates. In reality, cows often sort TMR, eating concentrates first and leaving large stemmed particles for last. Methods to reduce sorting may prove to be advantageous to overall ruminal function.

Objectives

The objective of this study will be to evaluate the effect of feeding a flavor enhancer on DM intake, milk production and composition, ruminal metabolism and TMR sorting of high producing dairy cows.

Materials and Methods

Thirty Holstein cows (26 multiparous and 4 primiparous) will be used in this study. Cows typically average approximately 78-80 DIM with an average production of about 95 to 100 lb/d. Cows will be housed in a barn with Calan gates for individual intake and will be trained for a 2-wk period prior to the start of the study. Corn silages will represent a minimum of 45% of the TMR dry matter. The remainder of the TMR will consist of alfalfa hay, alfalfa haylage and concentrate. Concentrates will be adjusted based on silage compositions prior to feeding.

Rations will be formulated based on NRC (2001) requirements for the average production values of the group of cows (to be determined).

After adaption to the Calan gates, animals (15/treatment) will be randomly assigned based on DIM, pre treatment milk production and lactation number to a control treatment or treatment where Luctarom SBS-R will be incorporated into the diet. (Rough estimate of 6-7 heifers/group and 8-9 multiparous cows/group.) Each treatment group will also have 2 rumen-fistulated animals. Luctacrom (a liquid product) will be mixed into the total forage portion of the TMR prior to blending with the concentrate. The Luctacrom TMR will always be prepared last to prevent carry over. Cows will remain on treatment for 10 weeks.

Cows will be offered their TMR once daily at 105% of their expected intake to ensure ad libitum consumption and will have access to fresh water at all times. Throughout the study, daily milk production will be recorded twice daily. Milk will be sampled from two consecutive milkings each week during the study. Milk will be analyzed for somatic cells, protein, fat, MUN, and lactose by infrared analyses. Body weights will be recorded on two consecutive days at the start and end of the treatment period and bi-weekly during the treatment period. Samples of forages will be collected three times weekly and pooled each week for determination of DM content. Concentrate and hay will be sampled once weekly for determination of DM content. Dry matter of the samples will be determined in a forced-draft oven set at 60^{o}C for 48 h. The DM content of feeds will be used to adjust weekly TMR formulations. A sample of the TMR will be collected three times per week and composited weekly for analysis of nutrient components (DM, CP, soluble N, starch, ADF, NDF, NEL, macro and micro minerals) using standard methodology.

Once weekly, TMR from 6 cows (determined at the start of the study)/treatment will be analyzed for particle size via the Penn State Particle separator (Nasco, Ft. Atkinson, WI)

and physically effective fiber via the Z-box (Miner Institute, Chazy, NY) at 0, 6, 12 and 24 h after feeding.

Twice weekly, in dwelling pH probes (Kahne Limited, Auckland, NZ) will be placed in the rumen of the fistulated cows and pH will be recorded every 10 min for 24 h. Data will be analyzed for max and min ruminal pH, ruminal pH range, and time (h) less than pH 5.5 and 5.8.

Data will be analyzed as a completely randomized design with the data from the preliminary period as a covariate using JMP (SAS Institute, Cary, NC) with week of treatment as repeated measures using the first-order autoregressive covariance structure that provided the best fit according to Sawa's Bayesian information criterion. A covariate will not be used for analysis of BW change. The model will include treatment, week, and treatment by week interaction as fixed effects, and cow within treatment as a random effect. Means were determined using the least squares means statement, treatment means were compared using the PDIFF option after a significant overall treatment F-test, and interaction effects were partitioned using the SLICE option (SAS Institute, 2004). Statistical significance and trends will be considered at $P \leq 0.05$ and $P \geq 0.06$ to $P < 0.10$, respectively.

Reference

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

Estimated Time Frame (subject to change)

September 21	Begin moving cows into Calan gates for training and start pretreatment period

October 10	Allocate cows to treatment and start study period
December 31	End of 10 week treatment period