IN VITRO FERMENTATION OF VITAMIN E IN VARIOUS FEEDSTUFFS IN THE RUMEN: NUTRIENT ANALYSIS OF FEEDS

THESIS

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Robin E. Hillegas-Hobdy, B.S.

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DEDICATION

This is dedicated to my Mom, who supported this goal of mine every step of the way.

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I would like to take this opportunity to thank my committee members: Dr. Pollard, Dr. Morrish, Dr. Richardson and Dr. Hustvedt. Thank you for all of your time, constructive criticism and valuable lessons that I learned throughout this process. Thank you so much for everything!

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Last but not least, Tiny Wonder. Even though you will never read this you have been my biggest motivator for me to accomplish my goals and this is no exception. Promise me you won't forget me, ever.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

ABSTRACT

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Robin E. Hillegas-Hobdy, B.S.

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SUPERVISING PROFESSOR: GREGORY POLLARD

Digestibility experiments with forages have had significant value in determining their specific nutritive content for livestock (Tilley and Terry, 1963). With the high rates of animals being produced for consumption in confinement, a negative effect of this is that the animals are not receiving the proper nutrients to have healthy body functions and growth. Research shows that supplementing animals with adequate daily amounts of fat and water soluble vitamins and various minerals help the issue (Ballet, Robert and Williams, 2000). However, there is limited research of dry matter digestibility of certain vitamins. Thus, in vitro fermentation as described in the Tilley and Terry method (Tilley and

to as the "untreated" group. Both groups were harvested and chopped in similar ways. The only difference is the addition of the bacteria. The hypothesis is that the type of silage production (treated v. untreated) will affect the vitamin E content in the forage and the level of fermentation profiles of other products. It is also predicted that the in vitro dry matter digestibility (IVTDMD) will be greater for those samples that have been treated and/or exposed to the inoculums of lactobacillus. The use of the inoculants is to ensile forage in the forty eight hour time period as compared to the typical three or four week ensiling period with traditional sources of forages and therefore, less time will be needed to degrade the levels of vitamin E and raise dry matter digestibility (DMD).

CHAPTER I

INTRODUCTION

Digestibility experiments with various feedstuffs have had significant value in determining their specific nutritive content for livestock. In studies of trace minerals and various vitamin supplements, research has shown that in order for the supplement to be absorbed by the animal, it must be made available to the animal in the small intestine and not be bound to any particulate matter in the digestive tract (Tilley and Terry, 1963). In ruminants, digestion and absorption is done by using specific microorganisms that are present in the rumen. These microorganisms have a symbiotic relationship with the animal. The animal provides the ideal host environment and the microorganisms digest, ferment, and absorb large amounts of fibrous feedstuffs that the animal ingests. There are two main factors that influence the microbial population in the rumen. The diet is very important for determining the size and type of microbes that exist in the rumen; and the pH levels in the rumen. The pH is one of the most variable factors that influence the microbial population (Ballet, Robert and Williams, 2000).

Early nutrition and digestive physiology studies have revealed the importance of rumen fermentation to the animal. Fermentation in the rumen is the combination of physical and microbial activities that convert components of the diet that can be useful such as VFAs (volatile fatty acids), microbial protein, B-vitamins or things that are harmful to the animal such as ammonia and nitrate. Ruminants can take care of the microbial population by ingesting and chewing food continuously, adding buffers to neutralize the acids that are produced, and maintaining the pH and moisture levels that are necessary for microbial growth (Owens and Goetsch, 1986).

At the basic tissue level, ruminants require all of the vitamins that are needed for normal function in other mammals. The ruminant has the ability to synthesize the B vitamins in the rumen itself, but most of the others need to come from feedstuff sources.

Vitamin E is an essential nutrient and a fat soluble vitamin that works as an antioxidant in animals and helps protect them from the harmful effects of free radicals which may ultimately cause damage to the cell membranes of vital tissues in the animals (National Research Council, 1987).

Vitamin E benefits tissues in young animals, the reproductive system, the muscle and neurological tissue integrity of the animal. Even though many animals receive their adequate daily values through lush pasture grazing or grain sources, certain animals that are kept in confinement

and fed stored feedstuffs are at risk of deficiency of the trace minerals including Vitamin E due to the fact that Vitamin E concentration in various feeds may deteriorate after prolonged storage methods (National Research Council, 1987).

According to researchers Tilley and Terry (1963) using in vitro fermentation is an efficient way to calculate the amount of dry matter digestibility in animal feedstuffs. The in vitro dry matter digestibility method has been used extensively to evaluate the nutritional value in ruminant feeds (Mabjeesh, Cohen, and Arieli 2000). Since its introduction, the Tilley and Terry method has been largely used to analyze feedstuffs and has been the most accurate and practical method available for predicting the digestibility in ruminants (Goldman et al., 1987; Stern et al., 1997).

Problem Statement

With the high rate of animals being produced for consumption in confinement, a negative effect is produced caused by confinement issues because the animals may not be receiving the proper nutrients to have healthy body functions and growth. Research shows that supplementing animals with adequate daily amounts of nutrient trace minerals such as vitamins A, E, D, K, B, C (Ballet, Roberts and Williams, 2000) and others help the issue. However, there is limited research into dry matter digestibility of these vitamins and minerals. Thus, in vitro fermentation,

as described in the Tilley and Terry method (1963), will be used to recreate the digestive tract of the animal to determine the absorption of vitamin E.

Hypothesis

The prediction for the study is that silage production will affect the level of vitamin E in forage and the levels of fermentation profiles. The other thought to suspect is that in vitro fermentation and dry matter digestibility (IVTDMD) will be greater for the samples with higher levels of vitamin E and the use of bacteria inoculums in the silage process. (referred to as treated versus untreated).

Null Hypothesis

The null hypothesis for the study is that the silage process will have no effect on the levels of vitamin E. It also suspects that vitamin E will then have no effect on the fermentation profiles of the samples.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Around the 1920's, there was documented knowledge of vitamins A, B (thiamine) and C. vitamin D was the newest discovery at the time period of the early 1920s (Mason, 1977). However, when semi-purified diets were fed to rats (and positive growth and overall nutritional status was observed), there were some cases in which the rats failed to reproduce even though they appeared to be in good health (Osborne and Mendel, 1919; and Mattill and Conklin, 1920). After this incident Evans and Bishop (1922) discovered the existence of a fat soluble dietary fiber that was present in wheat germ, lettuce, and alfalfa that prevented fetal death in the rats. The substance was given the name "factor X" but later, researchers Sure and Evans (1924) gave the substance the name "vitamin E".

Vitamin E

Vitamin E was recognized as a vital nutrient necessary in the diets of animals and humans. In animals, this valuable nutrient serves as a preventative source to enable functions such as proper health or growth of the fetus - preventing encephalomacia in chicks, fetal death in rats,

and muscular dystrophy in rabbits (Mason and Horwitt, 1972; Scott 1978; Combs, 1981; and Machlin, 1984). A study done on pregnancy and maternal nutrition in rats showed that vitamin E is required for ensuring proper fetal growth in rats (Evans and Bishop, 1922). In contrast, excessive levels of vitamin E during pregnancy and lactation in rats showed prolonged birth and delayed maturity in the pups which will be discussed in more detail later (Martin and Hurley, 1977). Another study that was conducted using rats showed that having an adequate supply in the animal's diet can also prevent liver and testicular degradation and adult muscular dystrophy (Nockels and Menge 1976).

The most common form of vitamin E in the diet (known as α-tocopherol or 5,6,7-trimethyltocol) is found in nature (in the form of wheat germ, vegetable oils, and other plant oils). Other derivatives of the compound are synthetically produced in order to have more stable isomers (Scott, 1978). Some of the derivatives of α-tocopherol are unstable to oxidazing situations and thus makes vitamin E activity dependent upon several conditions.

Research by the National Research Council shows that the contents of vitamin E in feedstuffs are able to react with one another, making them variable and unable to be predicted. This is why supplementation of vitamin E in an animal's natural diet requires levels to be in the range of 10 to 30 IU/kg of body weight.

Like other fat soluble vitamins, vitamin E's point of absorption and utilization begins in the intestines of the animal. Most species of animals can effectively hydrolyze dietary forms of tocopheryl esters (commonly used in animal feed supplements) at the mucosal surface of the small intestine where it is absorbed as a free alcohol. However, studies show that the vitamin is insoluble in the intestinal lumen or interior of the intestine (Scott, 1978). This results in the functions of the liver and pancreas increasing to help to digest the vitamin. Absorption of vitamin E in the lumen region is related to fat digestion utilized by bile and pancreatic lipase. Any impairment of function in these organs will make vitamin E un-absorbable in the body regardless of what kind of supplement is used (Scott, 1978).

Of all of the vitamins, vitamin E is shown to be the least toxic. A study done by March et al. (1973) shows that in rare cases of high intake levels of vitamin E, toxicity will occur and produce several negative effects for the animal. Supplementing chicks with 2200 IU/kg of vitamin E resulted in an alteration of their growth process (March et al., 1973). Also reported at this level was a reduced level of hematocrits, reticulocytosis, and increased prothrombin (which increases normal blood clotting functions) when observed (March et al., 1973). Another finding was the result of depressed bone calcification in the chicks that were fed high levels of vitamin E. Both of these problems were evident in chicks that, in addition to high levels of vitamin E, had low levels of both

vitamin K (which is regulates the blood clotting or blood declotting), vitamin D, and calcium (vitamin D, which increases absorption of calcium, and calcium helps to increase bone density). The vitamin deficiencies were remedied after increasing the amounts of vitamins K and D as well as calcium into the chick's diet (March et al., 1973). Other studies showed that prolonged exposure to high levels of vitamin E would naturally increase the animal's nutritional requirement (and natural desire) for vitamins K and D (Murphey et al., 1981).

Other results shown from high levels of vitamin E include heptomegaly (enlargement of the liver) and reduced skin pigmentation in broiler chicks (Nockels et al., 1976). Additionally, vitamin A absorption in the liver was reduced when the animals were fed high levels of vitamin E over time, though there was no shown effect on absorption in the interaduodenal region (Sklan, 1983). The excessive levels of vitamin E during pregnancy and lactation in rats showed prolonged birth (21 days and more) and delayed maturity in the pups from those rats who were fed 2200 mg/kg of vitamin E daily (Martin and Hurley, 1977).

At this time, the actual effect of toxicity from vitamin E in animals is not known; therefore, the estimate for tolerable levels of vitamin E is tentative (National Research Council, 1997). Most of the dietary requirements of Vitamin E for most species are in a range from 5 to 50 IU/kg of diet (which is 2 or 4 IU/kg of body weight/day) and results from these studies done by the National Research Council suggest that at

least 20 times the nutritionally adequate levels should be well tolerated (National Research Council, 1987).

Selenium

Selenium is an essential trace mineral that activates an antioxidant enzyme called glutathione peroxidase that has certain cancer preventative qualities in humans (Sklan, 1983). It also provides aid in the detoxification of lipo-and hydrogen peroxides that are toxic to cell membranes (National Research Council, 1997).

The concentration in feedstuffs is around 0.05 to 0.3 ppm and these levels can be influenced by the types of selenium found in the ground (Wright and Bell, 1966). Furthermore, research that has been done to test the absorption of selenium in animals is high (around seventy seven percent) for non-ruminants (animals that do not chew cud). However, absorption of selenium in ruminants (animals that do chew cud) is only twenty nine percent (Wright and Bell, 1966).

Selenium and Vitamin E

The Food and Drug Administration has approved maximal selenium supplementation at 0.3 mg/kg of Dry matter (DM) in complete feeds for cattle, sheep, and swine (Food and Drug Administration, 1987). However, the supplementation of selenium for horses is only restricted by nutritional recommendations and industry practices (National Research Council, 1987).

Studies conducted by Stowe et al. (1968) have proven that there is a close relationship between the two nutrients; vitamin E and selenium (in the form of gluthainone peroxidase) as having high antioxidant defense functions for the animal (Stowe, 1968, and Roneus et al., 1986). selenium and vitamin E are located and metabolized in two different regions of the body. Vitamin E is found in the lipophilic parts of the cells (such as the cell membrane) whereas selenium (gluthainone) can be located in the cytosol and mitochondrial matrix of the cell. Together these important elements guard the cell from adverse affects of reactive oxygen forms and other free radical initiators of the oxidation of unsaturated phospholipids and of certain critical proteins (Roneus et al., 1986). However, to function effectively as antioxidants, vitamin E compounds must be easily oxidized themselves. Therefore, many of the natural forms of vitamin E are highly unstable (National Research Council, 1997). To remedy this issue, chemically esterified forms of the vitamin E (such as *d*(R,R,R)-or *d,l*(all-rac) α-tocopherol acetate) do not function as antioxidants (or oxidize) until the ester linkage has been split by enzymes (esterases) in the digestive tract. The result is tocopheryl esters that are highly stable (National Research Council, 1987). Thus, the vitamin E activity of feedstuffs depends on the chemical forms present and the conditions of storage. Moisture in feeds that is sufficient to permit fermentation and mold growth results in rapid decline in the natural vitamin E compounds. Grinding and storage of ground grains

versus the storage of whole kernels yields similar results. Grinding interrupts the internal structure of the seeds and exposes unsaturated lipids to the air which helps to cause peroxidation (Stowe et al., 1968).

Vitamin E in Forage

Forages are potentially a great source for animals to receive their necessary vitamin intake on a daily basis. Ballet, Robert and Williams (2000) found that the vitamin content in forages is variable and unpredictable which makes synthetic vitamin production crucial to intensive livestock production. Factors that make vitamin sources in forages highly variable are plant origin, climatic conditions, stage of maturity, conservation methods (drying, ensiling, and dehydration), and storage conditions.

Research also shows that certain vitamins the synthesis of the B group of vitamins—Thiamine, Riboflavin, Niacin, and of vitamin K occur in the degradation and fermentation process of feed ingredients. Vitamin D is synthesized by the action of the ultra violet radiation on the sterols that are in the animal's skin pigments. Vitamin C is synthesized from C6 sugars –galactose and glucose are found in nature and are easily synthesized by the animal and so the only vitamins A and E have specific dietary dependence (Ballet, Robert and Williams, 2000).

Animals that are raised indoor or fed rations (that are usually high in concentrate) tend to have a more difficult time gaining access to vitamins

D and Thiamine (B12). Furthermore, the consumer desire for higher quality animal products suggests a need for higher exogenous supply, particularly higher levels of Niacin (B6) and vitamin E (National Research Council, 1980; Hullar and Brand, 1993). The supply of vitamins to ruminants other than that from synthetic production is totally dependent on a supply from fresh or 'conserved forage' in the ration since the concentrate portion of the feed is lacking any natural sources of the vitamins (A,E, or D) or their precursors (Brown, 1953; Machlin, 1984; McDowell, 1989). In many cases when blood serum levels are high in α-tocopherol the requirements of the animal's dietary needs cannot be met. This happens for two reasons: first, the passage of the material through the rumen and second, intestinal absorption (Ballet, Robert and Williams, 2000). Even though forages can be a good source for vitamins, the levels at which they can contribute to the animal's dietary requirements are influenced by factors in the plants (and within the animal) that limit the quantity of vitamins available in the diet (Ballet, Roberts and Williams, 2000).

In Vitro Fermentation with Rumen Fluid

Digestibility experiments with feedstuffs have much worth when estimating the nutritive value of feedstuffs for ruminants. However, many of the experiments using animals can be tedious and require large amounts of feedstuffs (Tilley and Terry, 1963). This is why the use of in

vitro laboratory methods where feedstuffs are digested by preparations of microorganisms or enzymes with similar function to those that is naturally found in the digestive tract of the ruminant is a good substitute.

The in vitro dry matter digestibility method has been used extensively to evaluate the nutritional value in ruminant feeds (Mabjeesh, Cohen, and Arieli, 2000). Since the method's introduction, the Tilley and Terry method has been largely used to analyze feedstuffs and has been the most accurate and practical method available for predicting the digestibility in ruminants (Goldman et al., 1987; Stern et al., 1997).

A similar study that was done in 1979 by Menke et al., 1979 to predict the internal digestibility factors by recreating the in vitro fermentation of feedstuffs. This technique (Pell and Schofield, 1993) is found to be superior to digestibility and degradability techniques due to the fact that they account for contributions from soluble and insoluble feed components while providing researchers information on the dynamics of forage fermentation (Adesogan et al., 2000). Indeed, when nutrient content is not limiting, gas production measures microbial growth (Pell and Schofield, 1993).

The use of the gas production technique as the index of the nutritive value is decreased by the dependence of total gas production on sample size (Menke et al., 1979). The production of gas by the reaction of fermentation end products with the buffer can also complicate the

interpretation of the gas production files (especially as such indirect gas production is rarely accounted for; a requirement of this procedure is to be cautious when interpreting the gas production profiles and the validity of any interpretations that are recorded by accounting for the end products of the fermentation (Adesogan et al., 2000).

In addition to these potential problems, another issue that rises when using gas production measurements to estimate ruminal fluid fermentation is that the "profile must also be described with the "appropriate model" to allow estimation of the parameters of the curve.

Digestion of forages and feedstuffs in the rumen

With forage diets, VFAs (Volatile fatty acids which consist of acetic acid, lactic acid, propionic acid, butyric acid, and isobutyric acid) provide 50-80 percent of metabolic energy used by ruminants. Capacity for absorption of these VFAs is about six to eight times the maintenance level for the animal (Owens and Goetsch, 1986) thus absorption is not the limiting step in ruminant metabolism.

Soluble carbohydrates are partially released as cell walls are destroyed during the fermentation process. The remaining cell contents are rapidly released and hydrolyzed to monomers which are fermented to VFAs in the rumen. The VFAs then reduce the overall pH of the rumen environment. With concentrate diets, ruminal fluid is to have a pH level between 5.4 and 6.5 levels. (roughage diet alone can have a pH level from

6.2 to roughly 7.0). The time after feeding in which pH is the lowest can be attributed to an increase of acid production, the input of buffers from saliva, and the presence (or release) of buffers due to the amount in the feeds. (Owens and Goetsch, 1986). Absorption of VFAs stabilizes the overall ruminal pH levels.

Digestion can be expressed in a variety of ways. For the total digestive tract, digestibility is usually expressed as total extent of digestion independent of time. However, to determine the quality of a nutrient which is indigestible, feeds can be fermented for various times with one period of time being extended to estimate the total extent of digestion. (Ballet, Roberts and Williams, 2000).

Particle size

Feed products that are either steam-flaked or ground are more fermentable than whole kernels and large pieces. Generally, particle size is determined by weighing the amount of wet or dry particles that, as a result of the siling process, are different sizes (Owens and Goetsch,1988). Particle size can be further reduced at several points. Many feeds are processed (treated/dried) prior to feeding to reduce particle size. Mastication from the animal also contributes as well as the microorganisms in the rumen that work on deteriorating the larger particles.

CHAPTER III

METHODOLOGY

The study was conducted in order to determine the dry matter digestibility of various feedstuffs of ruminants using rumen fluid and to determine a nutrient analysis of feedstuffs particularly Vitamin E, volatile fatty acids, and crude protein. Other factors that were determined as a result of the study were the effect of in vitro fermentation on pH levels in the rumen and in vitro dry matter digestibility in relation to the types of feeds stuffs. The samples that were used were collected from states from the regions of the plains, the Midwest, and the west over a period of several weeks. The two groups that were collected are referenced as "treated" and "untreated" in relation to time. The treated group, is exposed to an inoculums of lactobacillus bacterium in the ensiling process. The untreated group does not have the inoculum of bacteria.

The types of silage used were mainly corn, but other types that were used were sorghum, alfalfa, and wheat. The source of inoculums and the time at which it was collected has no effect on the in vitro dry matter digestibility of feedstuffs

The in vitro dry matter digestibility (IVDMD) method that was used in the study has been extensively used to evaluate the nutritional value of

ruminant feeds in multiple studies. The Tilley and Terry IVDMD method has been used most often in such studies due to the fact that it is the most practical and accurate method for determining data regarding nutrient digestibility of ruminants (Goldman et al., 1987; Stern et al., 1997). Many other studies have used this method due to its simplicity and reproducible results. However, despite its accuracy of predicting DMD, the method's two step process is time and labor consuming (Mabjeesh, Cohen and Arieli, 2000).

Method and Preparation

Before the samples of silage could be used for the in vitro fermentation process, each of the samples were ground with a laboratory grain mill and then dried for a period of no fewer than twenty four hours at 180 degrees Celsius.

The method has two stages. In the first stage, "a small amount of ground and dried silage was digested anaerobically with rumen microorganisms at thirty-eight degrees Celsius over a period of forty eight hours. Then a relatively large amount of a buffer solution was added to the inoculums to maintain the pH levels around the same level that would be found in the animal, and then a pepsin digestion stage to digest any remaining protein" (Tilley and Terry 1963).

However, due to time and material availability constraints, each of the samples were sent to an off-site forage analysis lab for the in vitro

fermentation process. The lab analyzed several components of the forage over the 48 hour period: Vitamin E levels of the forage, dry matter in vitro digestibility (IVDM), pH of the samples, volatile fatty acid levels (VFAs), and protein digestibility. The particle size analysis, grinding, and drying of all samples was conducted at Texas State University-San Marcos.

Limitations to the method

Some variation in digestion efficiency can be expected when using similar forages in the same experiment. Some factors that could alter the nutrient analysis of the feedstuffs could be the type of silage used. Corn, wheat, alfalfa, and sorghum all have different levels of vitamin E (as well as VFA's and protein) so variation between the samples is expected. The other factor that could be affecting the nutrient content is storage locations. Many of the samples were frozen and thawed then refrozen for a long period of time due to transporting and time restrictions which could degrade the overall nutrient quality of the silage.

CHAPTER IV

RESULTS AND DISCUSSION

The results of the in vitro fermentation process of the untreated silage samples are represented in tables 1.0-1.5. The results of the in vitro fermentation process of the treated silage samples are represented in table 2.0-2.5. The percentage of dry matter (DM) of the untreated samples remains constant throughout the period of 672 hours (four weeks) an average of 30.5 percent DM.

The Effect pH Levels of the samples

The pH levels of the untreated samples decline gradually over the zero to forty-eight hours due to the increase in acidic conditions during the fermentation process. There is a slight increase from the period of forty-eight hours to the one week mark (168 hours) but that can be attributed to the added buffer solution during the in vitro process as the levels never get higher than a pH level of 5.3 which is slightly acidic. The pH level drops even further towards the end of the in vitro process due to the fact that as more time goes past, the more acidic the conditions will be. The variation in the pH is 0.221, which is a very low amount of variation. The graphical representation shows peaks of the pH rising and dropping but never below 3.9 and never higher than 5.3 which are still slightly

acidic conditions that are present in the rumen fluid during the fermentation process.

The pH level for the treated silage remains more constant in the slightly acidic range. Even though the sample did become more acidic over time, the samples did not show a sharp increase or decrease in the pH levels. The levels of the pH averaged around 4.3 which is slightly acidic and never dropped below 4.0. There is little variation in the pH of the treated samples.

Particle Size

Particle size was measured by separating out parts of the silage before the grinding process. The silage was separated into four categories based on size: greater than one inch, one inch to a half inch, a half inch to a quarter inch, and less than a quarter inch. Once they were all separated into groups, the groups were weighed and then totaled. The average weight of the untreated samples particle size was 11.64. The bulk of the weight of the samples fell into the categories of one inch to a half inch, and a half inch to a quarter inch which can be attributed to the way it was chopped originally. Since all of the untreated silage was chopped with the same size blade, it makes sense that the smallest category and largest category would have the smallest amount of silage in it.

For the treated silage, it was chopped similar to the way that the untreated silage was chopped. The particle size of the treated samples

shows a steady decrease overall unlike the untreated sample. This could be due to the fact that while the treated samples were chopped in a similar fashion to the untreated samples, these samples were drier and had more shape; which meant that the measuring of each individual category may have been easier to determine the exact size of the silage product. The average particle size was 11.08 which is only slightly smaller than that of the untreated sample.

In Vitro Dry Matter Digestibility

The in vitro dry matter digestibility (IVTD) of the untreated silage samples was relatively high, but increased only slightly over the period of forty-eight hours. This could be due to the condition of the untreated samples at the time of analysis (i.e. storage conditions, transport methods etc). However, the IVTD levels remained constant over the time period for incubation. This indicates that while there was not a steady increase in the IVTD levels, the condition of the samples did not have a large impact on IVTD. In the treated silage sample however, there is a steady rising of IVTD over time in comparison to the IVTD levels of the untreated silage. Like in the untreated samples, the condition of the samples was better overall than that of the untreated silage upon its arrival; etc. In contrast, the untreated samples were moist and did not have much shape or compactness before the grinding process.

The effect of IVTD on the treated silage samples showed a steady increase over the period of forty-eight hours. This makes sense with the hypothesis that the in vitro digestibility should increase over time under favorable conditions.

Volatile Fatty Acid production

The overall effect from in vitro dry matter digestibility (IVTD) on the volatile fatty acid (VFA) production tended to show an increase in VFA production over time which is to be expected. The longer the feed is being fermented the more VFAs are released for utilization for the animal. The same is shown in the treated silage sample. There is a steady increase of VFA production over time that is a result of the fermentation process.

The effect of IVTD on Vitamin E levels

The effect on Vitamin E as a result of the in vitro fermentation process show a sharp decline in the level of vitamin E over time for both treated and untreated samples which mean that the vitamin E levels that are present in silage in the beginning decrease. This is to be expected since as the animal ferments the feed and forage, vitamins should be released and utilized by the animal. The graphical representation tables 1.5 and 2.5 show that the in vitro fermentation digestibility of vitamin E decreases over time.

In the treated silage sample, the Vitamin E levels start to show an expected decline, but then there is a steep increase at the twenty four hour mark. This is not typical, and assumes this could be the result of a bad sample.

If Vitamin E is to be supplemented, the fermentation process only utilizes the vitamin E during the first forty-eight hours. Constantly feeding an excess of vitamin supplements over time would most likely not benefit the animal overall.

Overall, the results of the in vitro fermentation digestibility (IVTD) of vitamin E levels are degrading; and can be caused due to the fact that the lab only tested for the one type of vitamin E; α-tocopherol. Vitamin degradation is common with various storage situations, isomers present etc, and is well documented in other studies. "Several derivatives of Vitamin E exist in nature, whereas the most common form is α-tocopherol; other forms of this ester are proven to be more stable and thus, vitamin E in feedstuffs depends upon both the chemical form provided and the storage conditions present. In practice, vitamin E contents of practical feedstuffs are variable and not readily predictable" (Dairy NRC, 1997). Because often α-tocopherol is not found in feedstuffs alone, could be why the vitamin E levels in this particular study degraded so quickly.

Statistical Analysis

Looking at the figures 1.0, 1.1 and 2.0, these show the descriptive analysis of both groups together; as well as treated and untreated individually. Typically, the larger the number of standard deviation results in more variance between the samples. This excludes time, as time was not measured in the same way that the other samples were. Variance among the samples was low between particle size, pH levels, and of VFA production. The most variance was shown between Dry Matter (DM), In Vitro Fermentation Digestibility (IVTD) and Vitamin E level concentrations. Variance among samples is due to many factors. For one; Dry Matter (DM) levels were very large numbers (min=18 and max=46) across both treatment groups. Second, the vitamin E concentrations have high variance between the two samples due to the large range of reported content. Because of the factors contributing to Vitamin E degradation stated previously, there is the possibility for a large amount of variance between the samples.

In figures 1.2 and 2.2, the correlations are listed between the treated groups. There is a noted significance in correlation between time and Dry Matter percentages (0.083), as well as between VFA scores and Dry Matter percentages of the treated samples. This is a positive correlation (as time increases the VFA scores increase etc.). The other results show no significance between variables. Likewise, in Figure 2.2, a correlation between the untreated samples there is a positive significant correlation

between time and the VFA scores (0.006). All other results are not significant (based on the p = < 0.05).

In figure 2.3 an ANOVA test was done on both treated and untreated sets of data. There was significance shown between the treated and untreated groups for DM percentage, pH levels and IVTD. However, there is no significance with the vitamin E content levels.

In figure 2.4 a reliability test was done between both groups, treated and untreated, looking at vitamin E content, DM percentages, in vitro fermentation digestibility, as well as pH and VFA scores of the two groups. The results of the test indicated a Cronbach's alpha score of 0.486; meaning that the test that was used is not as reliable for predicting future Vitamin E analysis, VFA scores, and in vitro dry matter digestibility. This is a result of the wide variation in the samples that affect the nutrient quality overall of the feedstuffs (storage methods, siling process, transport, type of nutrients analyzed, etc).

Table 1.0 Statistical Analyses of Treated Groups

	Std. Dev.	Variance	Mean	Minimum	Maximum
Dry Matter	7.37	54.30	37.60	23.50	46.00
Particle Size	2.05	4.24	11.08	9.09	15.05
VFA Score	1.59	2.53	6.32	4.13	8.44
IVFTDMD	3.24	10.50	85.00	79.00	88.00
pH	0.25	0.06	4.30	3.90	4.80

Table 2.0 Statistical Analyses of Untreated Groups

	Std. Dev.	Variance	Mean	Minimum	Maximum
Dry Matter	4.18	17.50	23.50	18.10	32.90
Particle Size	0.92	0.86	11.64	10.49	13.88
VFA Score	2.22	4.96	3.84	0.99	7.80
IVFTDMD	4.80	24.00	78.67	68.00	85.00
pH	0.54	0.29	4.82	3.90	5.40

Table 3 Correlations between both groups (* indicates statistical significance)

	Dry Matter	pH	IVFTDMD	VFA	Time
Dry Matter					
Pearson Correlation	1	0.382	0.113	0.626	0.607
Significance (2-tailed)		0.31	0.772	0.071	0.083
N	9	9	9	9	9
pH					
Pearson Correlation	0.382	1	0.471	-0.288	-0.319
Significance (2-tailed)	0.31		0.201	0.452	0.403
N	9	9	9	9	9
IVFTDMD					
Pearson Correlation	0.113	0.471	1	-0.328	-0.504
Significance (2-tailed)	0.772	0.201		0.389	0.166
N	9	9	9	9	9
VFA Score					
Pearson Correlation	0.626	-0.288	-0.328	1	0.922*
Significance (2-tailed)	0.071	0.452	0.452		0.000*
N	9	9	9	9	9
Time					
Pearson Correlation	0.607	.-319	-0.504	.922*	1
Significance (2-tailed)	0.083	0.403	0.166	.000*	
N	9	9	9	9	9

Table 4 Paired Sample Correlation of the treated samples

	N	Correlation	Significance
Pair 1 IVFTDMD and TIME	9	-0.50	0.17
Pair 2 pH and TIME	9	-0.32	0.40
Pair 3 VFA Score and TIME	9	0.92	0.00
Pair 4 DM and TIME	9	0.61	0.08

Table 5 Paired Sample Correlation of the untreated samples (* indicates statistical significance)

	N	Correlation	Significance	
Pair 1 IVFTDMD and TIME	9	0.08	0.83	
Pair 2 pH and TIME	9	-0.65	0.59	
Pair 3 VFA Score and TIME	9	-0.17	0.66	
Pair 4 DM and TIME	9	0.83	0.01	*

Table 6 ANOVA Between both groups, treated and untreated (* indicated statistical significance)

	Sum of Squares	DF	Mean Square	F	Significance	
Vitamin E						
Between Groups	11.842	1	11.842	0.265	0.614	
Within Groups	714.098	16	44.631			
Total	725.94	17				
Dry Matter						
Between Groups	894.645	1	894.645	24.892	0.000	*
Within Groups	575.051	16	35.941			
Total	1469.96	17				
pH						
Between Groups	0.889	1	0.889	4.950	0.041	
Within Groups	2.871	16	0.179			
Total	3.76	17				
IVFTDMD						
Between Groups	180.5	1	180.500	10.460	0.005	*
Within Groups	276	16	17.250			
Total	456	17				

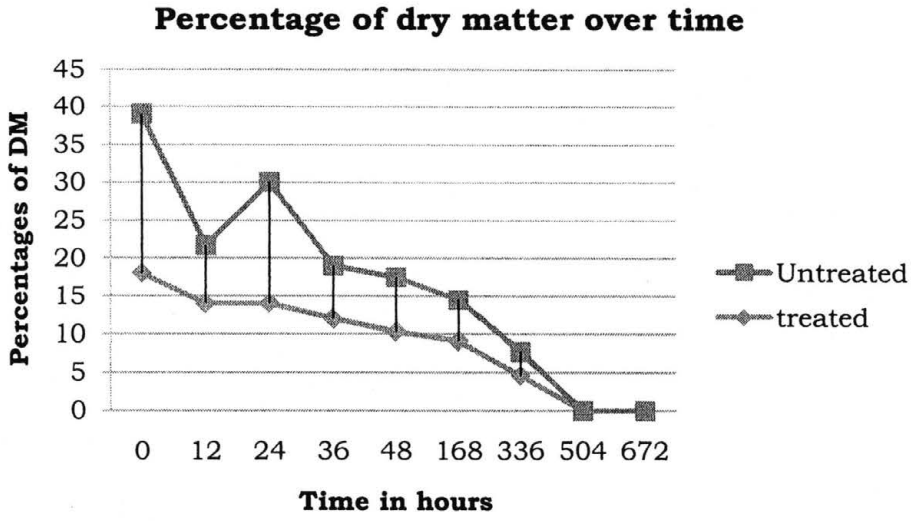

Figure 1 Percentage of dry matter over time

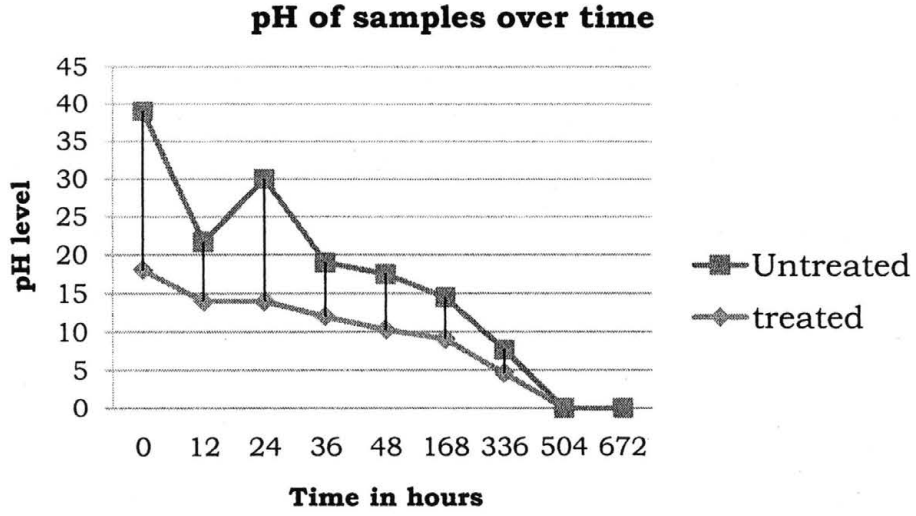

Figure 2 pH of both samples over time

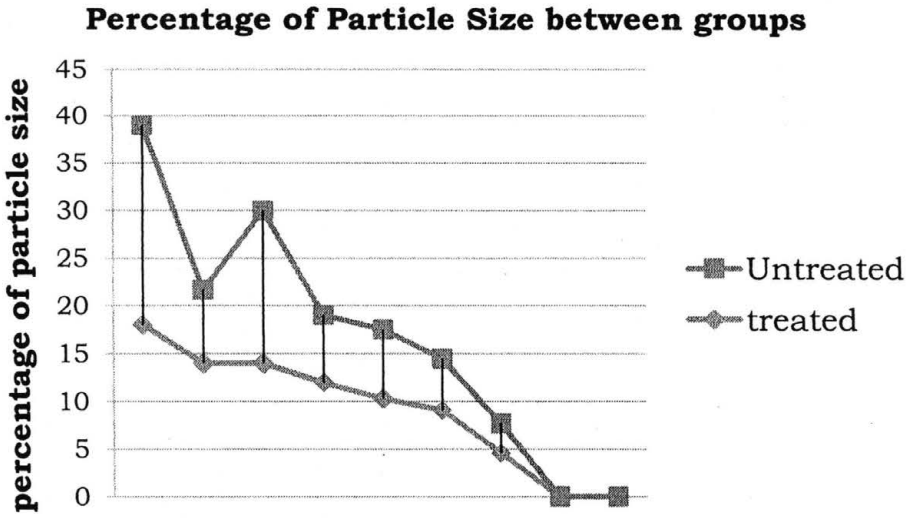

Figure 3 Percentages of particle size between groups

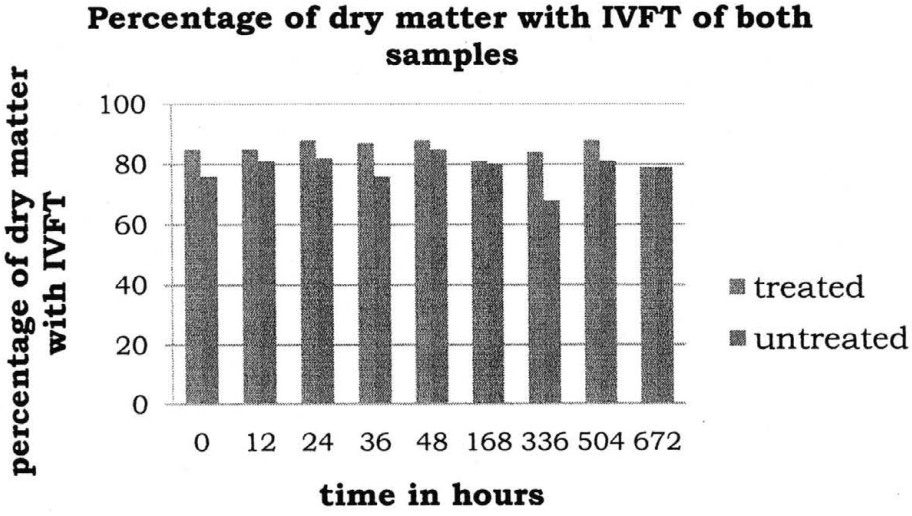

Figure 4 Percentage of dry matter from IVFT of both samples

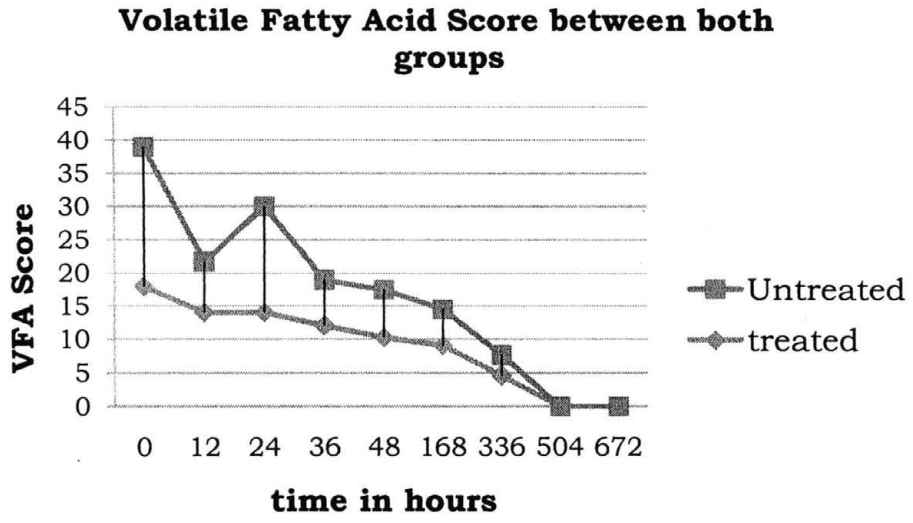

Figure 5 Volatile Fatty Acid Score of both groups

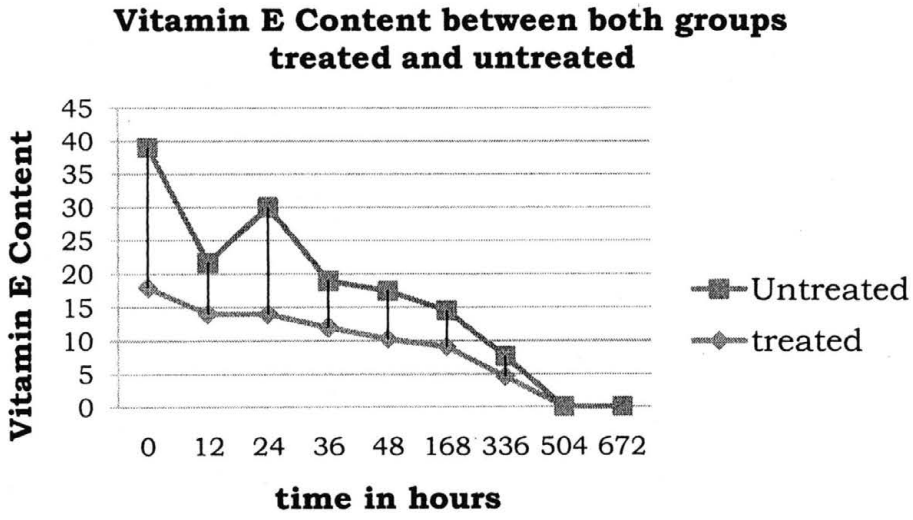

Figure 6 Vitamin E Content of both groups

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENFATIONS

The in vitro method of evaluating digestibility of ruminant feeds is used worldwide (Mabjeesh, Cohen, and Arieli, 2000). This method is easier than previous *in vivo* methods and is less invasive than surgically analyzing several portions of the animal's digestive tract. The Tilley and Terry method has also been shown to be more accurate than digestibility predictions based on the chemical compositions of feeds (Van Soest, 1994). This method was first introduced and modified according to types of feedstuffs that are being analyzed (starches, etc).

Forages, grains can be analyzed in an in vitro fermentation process that simulates the actual act of fermentation in the animal. However, certain factors affect nutrient content and utilization that must be taken into consideration. First, the types of feeds are critical for looking at the overall composition of feeds that are being analyzed. Next, the matter of how the grains and forages are processed affects the overall nutrient quality, and finally, the matter of storage can affect the nutrient content in feedstuffs.

Vitamin levels in forages can be highly variable due to how they are processed, fed, and combined with other feedstuffs. There is no doubt that vitamins and minerals are found in the natural food sources and can be utilized by the animal for certain functions. This study was conducted to determine nutrient quality and vitamin levels of various feedstuffs after the siling process, which most feedstuffs go through before it gets to its end point destination. Other things that affected the nutrient content for this study was the time between first harvest and digestion and how it was stored.

For the purpose of this study, vitamin E content was originally at a higher concentration in the treated samples than in the untreated samples. However, an obvious degradation is shown in both groups regardless of treatment. The largest impact on these vitamin levels was due to the decreasing integrity of the vitamin over time. The other factor of why the vitamin E content was found in low amounts was due to the fact that the Dairy One Forage lab that was used only tested for the most common isomer of the Vitamin E (α-tocopherol) while up to eight other isomers exist in natural states (National Research Council, 1997). Looking at the other attributes to the feedstuffs, the pH of each of the samples remained moderately similar, as well as the VFA scores of each of the feedstuff treatment groups which is primarily because the pH and the production of VFAs is dependent on the conditions and microorganism population inside the rumen. The effects on the feedstuffs

from storage and the siling process had an insignificant affect on the pH and the VFA production. There was not a correlation to these attributes and time. This is most likely due to the fact that the process of fermentation over time will result in a diminished availability of nutrients for the production of VFAs and the pH will fluctuate during the fermentation process.

Microbial fermentation in the rumen must be considered to function independently of the needs of the host animal. The animal may regulate the daily intake, saliva input, etc. The animal has limited control over the fermentation process in the rumen. Because of ruminal fermentation, the animal can survive on diets that are not efficient on energy, protein, or other nutrients. Manipulation of the rate of intake and availability of certain feedstuffs regulate the type of microbial conditions that are present in the rumen that are required to ferment such products.

Most natural feeds for ruminants (grains, forages, etc) contain adequate amounts of vitamin E so supplementation of the vitamins is not always necessary. Depending on certain conditions in which the feedstuffs are exposed to prior to consumption, this can have a negative effect on the content of the vitamin in its natural feed source. Silages or hays that have undergone excessive heating or a lengthy storage period may be partially or completely void of significant vitamin E sources (Huber, 1988).

More research is needed in regards to the effects that vitamins have on the fermentation process. This study demonstrated that that the validity of vitamin E in various feedstuffs breaks down over time. These is partially due to internal factors (fermentation in the rumen) as well as due to extraneous factors such as the treatment process, drying, siling storing, and heating all have a negative effect on the content of vitamin E, or α-tocopherol.

For another potential study it would be beneficial to look at the combination of vitamin E in the rumen with added selenium. Much research is documented (Stowe et al., 1968, Food and Drug Administration, 1987, and National Research Council 1997) about the effects that these two elements produce that are beneficial for the animal. Another potential study could be to explore the effect of the water soluble vitamins have in the rumen. Ruminants have the ability to produce certain B vitamins (niacin, thiamine, etc) when they are lacking from forages or feeds (Machlin, 1984).

The in vitro fermentation of vitamin E showed that even though certain levels of vitamins and nutrients can be estimated or predicted prior to analysis, the resulting effects are not that simple. The extraneous factors are far too critical when examining content in vitamins. Both aspects internally (fermentation process) and externally (storage, location, age, exposure to bacterium, etc) must be accommodated for when doing further study with vitamins and various feedstuffs.

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VITA

Robin E. Hillegas-Hobdy was born in Akron, Ohio, on July 17, 1981, the daughter of Pamala Buttler Hillegas and Lynn Frances Hillegas. After graduating from Lake High School in 2000, she entered Kent State University working towards her Bachelor of Science degree in Nutrition and Dietetics. She graduated with her Bachelor of Science degree from Kent State University in May, 2006. During the time after graduation, she and her husband moved from Ohio to Texas to follow her husband's job. She and her husband moved to San Marcos, Texas in December of 2006. In July of 2007, she entered the Graduate College of Texas State University-San Marcos.

Permanent Address: 11286 Mogadore Avenue N.W.

Uniontown, Ohio 44685

This thesis was typed by Robin E. Hillegas-Hobdy.