BROWN MIDRIB SORGHUM AS WARM SEASON FORAGE FOR DAIRY CATTLES: INFLUENCE OF INTAKE AND STAGE OF MATURITY ON CALORIC CONTENT AND RUMEN FERMENTATION

by

JONATHAN M. LIM

(Under the Direction of Mark Alan Froetschel)

ABSTRACT

Energy and its utilization is important in dairy production in order to maximize voluntary intake and productive potential of the cows for increase profit. This research tested the effect of harvest maturity of brown midrib forage sorghum and level of intake on digestible energy, ruminal fractional disappearance rate and fermentation of Holstein steers. An In vitro technique was developed to assess accuracy and practical utility in predicting total tract apparent digestible energy. At high level of intake, steers consumed more of TMR containing early maturity of brown midrib sorghum (early head) than with late maturity (soft dough) because of the more physical fill provided by lignin on the latter diet. Digestibility of dry matter and energy showed tendency to be higher in early maturity than late maturity sorghum. Ammonia concentration was higher when animals were fed near maintenance but volatile fatty acid profile and pH were similar.

INDEX WORDS: Apparent Digestible Energy, Brown midrib forage sorghum, Volatile fatty acids, Fractional disappearance rate

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DEDICATION

To the love of my life, my wife Annie, and my lovely children, Julie Anne and Jay.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Assessing the Caloric Content of Warm Season Forages for Lactating Dairy Cows

Introduction

The prime function of feed is to furnish the animal with energy to support bodily and productive processes. The energy in feed available for animals is supplied by the organic nutrients in the feedstuff. The organic nutrients namely carbohydrates, fats and proteins comprise the bulk of the dry matter of the feeds. The fact that all these nutrients, particularly protein, may have specific and distinct functions as well does not alter their common utility as sources of energy (Maynard and Loosli, 1962).

The modern breed of dairy cows in the US, were genetically selected for high milk production. Proper nutrition and management are essential to maximize their genetic potential (VandeHaar and St-Pierre, 2006). It is particularly important, that effective strategies are developed to balance rations for higher producing dairy cattle that result in their increased consumption of calories. However, merely providing energy is not the only concern but its utilization is an equally important consideration. The chemical and physical form of the ration should be controlled such that DM and caloric intake are maximized without deleteriously affecting the rumen environment or the partitioning of calories towards either adipose storage or milk secretion (Coppock, 1985, VandeHaar and St-Pierre, 2006).

Forage is a major ingredient component of the ration for the dairy cow. It typically comprises more than 40% of the ration dry matter and provides effective fiber necessary to maintain health and proper function of the rumen microbial ecosystem. In addition to fiber, forages provide lesser quantities of other nutrients such as non-fiber carbohydrates, proteins and fats. The general strategy of feeding lactating dairy cows is to supplement forages with concentrates to create a total mixed ration that is energetically balanced by containing an optimal amount of fiber that allows the cow to consume enough dry matter to meet its nutritional requirements for calories and protein (Mertens, 1997). An energetically balanced ration is set to contain specified levels of fibrous and non-fibrous carbohydrates (Varga et al., 1998). Fiber is one of the most dynamic components of forages and inversely associated with their caloric concentration. According to Waldo (1986), and later qualified and quantified by Mertens (1997), fiber measured as neutral detergent fiber or NDF is one of the best single chemical predictors of intake. However, setting the proper fiber level to make an energetically balanced ration is difficult because individual forages are highly variable in fiber and caloric content. This variability is most dependent on plant species and maturity at harvest but is influenced by a multitude of other environmental factors. Thus, accurately assessing the energy value of forages is one of the most important steps to properly formulating cost-effective and energetically balanced rations (Weiss, 1993, 1998).

Forage quality is best defined in terms of animal performance such as daily weight gain, wool, reproduction and milk production (Ball et. al, 1991). Forage quality reflects how an animal responds when fed or given specific forage. It is representative of the total quantity of available nutrients in a given amount of forage and is highly related to caloric density. The quality of

forage is highly variable and is greatly influenced by several factors including specie and varietal differences, area, climate, management, maturity at harvest, post-harvest processing and method of preservation (Moe and Tyrrell, 1973). The Dairy NRC (2001) has listed maturity differences in energy content of several representative forages, such as warm and cool season legumes and grasses. Caloric values of these forages were predicted from chemical composition. It is known that cool season forages are more often superior in nutritive value than warm season forages because the latter usually contain more indigestible cell wall materials that physically restrict enzymatic digestion. Warm season forages are metabolically and anatomically structured to conserve water. They utilize a different photosynthetic pathway (C4 VS C3) and they have more lignified cell walls and stem material to protect the plant from dehydration (Jung and Varel, 1988, Reid et al., 1988). Although agronomic and environmental conditions are the prevalent reason for geographical preferences in basal forages used in dairy rations, the indigestible fiber component of warm season forage is why their use in dairy rations is restricted and generally limited to dairy production systems in subtropical and tropical regions.

In regions with temperate climates, corn silage, alfalfa and cool season grasses are the predominant forages fed to lactating dairy cattle. In general, these forages provide more available nutrient and caloric yield per hectare of crop production. Although biomass yield is higher, use of warm season forages in dairy rations is usually limited by their relatively high fiber content. Of the warm season forages available, sorghum forage ([*Sorghum bicolor* (L.) Moench.]) is one of the better alternatives to corn especially in areas where irrigation is limited (Lusk et al., 1984). Aside from being drought tolerant, sorghum offers high biomass yields and adaptability to later season planting dates and fits well into double cropping systems practiced in the Southern US

(Aydin et al., 1999). Sorghum harvested at flower maturity, ensiled properly, and fed as silage was shown to have potential for its inclusion as the basal forage in rations of lactating cows (Nichols et al., 1998). These researchers compared sorghum silage with tropical corn silage as the basal forages in total mixed rations at varying increments of NDF. At a specified level of NDF, sorghum silage based rations were equal or slightly higher in nutritional value than tropical corn silage based rations. However, in a feeding trial conducted before the development and use of NDF to formulate rations, sorghum silage did not compare well to corn silage based rations when fed at similar roughage to concentrate ratios because the resulting higher fiber rations were less digestible and resulted in lowered animal performance (Lance et al., 1964). At the same R:C ratio, corn silage is almost always better than sorghum silage because of differences in its fiber content. However, the negative nutritional characteristics of sorghum as compared to corn silage are amplified when it is harvested at later stages of maturity because of its relatively small seed head that contains more indigestible protein and fiber complexes in its peripheral endosperm that restricts its digestion and prevents utilization of a substantial portion of sorghum seed nutrients (Hart, 1990).

Recently, several genotypes of brown midrib (*bmr*) forage sorghum with improved digestibility have been developed. Research has identified specific modifications in biochemical pathways resulting from *bmr* mutations that account for its improved nutrient availability. In sorghum, *bmr*-6 has been linked to a decrease in cinnamyl alcohol dehydrogenase (CAD) activity. The allelic *bmr*-12 and *bmr*-18 decrease caffeic acid *O*-methyl transferase (OMT) activity (Oliver et al., 2005). Apparently these changes in metabolism are responsible for these *bmr* varieties to having a cell wall and stem structure that is less impervious to cellulolytic

fermentation in the rumen. Several studies have shown that these brown midrib sorghum genotypes have performed better than the standard sorghum genotypes and are more similar in nutrient availability as corn silage (Aydin et al., 1999, Dann et al., 2008, Grant et al., 1995). Improved nutrient yield and availability of *bmr* sorghum as compared to conventional sorghum and other warm season forage varieties can be attributed to its lower concentration of lignin and its less protective involvement in cell wall structure resulting for its enhanced DM digestibility.

With these considerations, brown midrib forage sorghum silage has significant potential to serve as warm season forage for lactating dairy cattle. The purpose of this review is to justify experimentation using different techniques to measure energy density of brown midrib forage sorghum harvested at varying stages of maturity and stored as silage. The major objective of this thesis is to compare an in vivo assessment of energy density based on digestibility and bomb calorimetry with the prediction method proposed in the current dairy NRC (2001) and an in-vitro calorimetric technique. Another objective is to quantify rumen passage and utilization of structural and non-structural carbohydrates in brown mid rib sorghum silage based rations fed at different levels of intake.

Measuring the Caloric Content of Forages

The measurement of forage caloric content as described by the NRC (2001) is fundamentally based on a prediction of digestible energy (DE) and measured using a mathematical procedure that is a refined version of the total digestible nutrient (TDN) method. Although, DE and TDN are measured differently and expressed in different units (Mcal/unit weight VS percent concentration or unit weight), these measurements are interchangeable as a

function of a constant (4.409 Mcal /unit of TDN). Although, the NRC feeding standards are expressed in terms of net energy (NE_L, NE_M and NE_G), the net energy standards for forages are largely based on DE because it accounts for most of the variation in caloric content of different forages (Moe et al., 1972, Moe and Tyrrell, 1973). The estimate of DE, based on the assumed caloric value of digestible proximate and influential analyses is fundamental to the NRC (2001) prediction of NE_L. Apparent DE is the difference between the gross energy (GE) content of the feed and that of the feces. It is described as apparent because it does not account for endogenous and microbial contributions to fecal gross energy. Whereas, TDN is based on the digestible macro-nutrient concentrations (fat, carbohydrate and protein) and the constant relationship that digestible fat provides 2.25 times more calories as compared to digestible carbohydrates and proteins. The actual measure of forage NE_L content involves feeding the forage and measuring its inherent energy that can support maintenance and productive requirements as influenced by digestive and metabolic processes. This requires one to directly or indirectly measure heat production in the animal and the combustible gases formed in the gastrointestinal tract requires the use of highly specialized technical equipment, lot of time and expense, and is labor-intensive (Flatt, 1966). Accordingly, prediction equations to estimate NE_L from DE were established once mathematical relationships between DE and NE_L were validated (Moe et al., 1972, NRC, 1978, 1989) and these equations to convert DE as influenced by differences in productivity and intake are still use in the current NRC (2001) . Since forages are highly variable in DE due to maturity at harvest and many other factors and actual measurement of DE or TDN requires conducting an animal feeding trial, prediction equations were developed and are used to estimate DE and TDN from proximate analyses such as fiber. The latest version of the dairy NRC predicts DE using a

multivariate prediction equation that estimates the digestible energy in the proximate components of the forage in a manner resembling the estimate of TDN.

Variability in forage caloric content

Forages are highly variable in caloric content depending mainly on their genotype and maturity at harvest as well as many secondary pre- and post- harvest environmental factors. The dairy NRC (2001) lists the caloric density of several representative forages of warm and cool season legumes and grasses harvested at varying stages of maturity. It can noted in Tables 15-1 of dairy NRC (2001) that cool season grasses and legumes harvested from a pasture that had been managed intensively would yield forage that have a digestible energy (DE) value of 3.14 and 3.13 Mcal/kg of DM respectively. Warm season forage such as sorghum preserved as hay or silage will yield at the average 2.39 and 2.40 Mcal/kg of dry matter respectively. The marked difference in caloric content is also evident on maturity at harvest such that an immature cool season forage legume ensiled at a vegetative stage of maturity yields 2.83 Mcal/kg of DM versus 2.47 Mcal/kg of DM for one that is at advanced maturity. The effect of maturity on the caloric content of forage is more pronounced in warm season forages. Warm season forages which utilize the C4 pathway of photosynthesis are more prone to greater lignification than cool season that uses a C3 pathway of photosynthesis. Temperature, light, soil moisture and fertility all have either direct or indirect effects on lignification. The C4 plants have evolved to better withstand greater light and temperature, low soil moisture and fertility by concentrating lignin in the cell wall fiber matrix of the plant. Lignin is the indigestible portion of the cell wall of forages. It has an inverse effect on the caloric content of the forage because aside from being indigestible, it also interferes with the digestion of cell wall polysaccharides by acting as a physical barrier to

microbial enzyme (Moore and Jung, 2001). Lowered forage quality due to lignification is a major factor that must be accounted for in ration formulation and often limits dairy production in warmer climates. Improving quality of warm season forages without minimizing yield due its associations with heat and drought tolerance may be difficult to obtain. The only means that producers can minimize lignification is to harvest forage at a vegetative stage of maturity. There is a great need to determine the best methods of producing high quality warm season forages for feeding dairy cattle. World-wide production of dairy cattle in sub-tropical and tropical climates is increasing, especially in Asian countries. Also the impact of climate change due to global warming patterns may have to transition to producing warm season forages for dairy cattle in the regions of the world that are now considered temperate.

The development of *bmr* mutant forages may provide an opportunity for expansion of dairy production in sub-tropical and tropical regions. Brown midrib mutant have shown in trials that this genotype is low in lignin and high dry matter digestibility (Aydin et al., 1999, Dann et al., 2008, Grant et al., 1995). Although less research emphasis has been made to test the use of *bmr* sorghum in lactating dairy cow feeding experiments as compared to more conventional forages for dairy production in more temperate regions, it does have potential of becoming a more prevalently used forage for southern dairy cattle and perhaps those in more northern climates in the not too distant future. Thus it is becoming critical that production and nutritional qualities of *bmr* sorghum varieties be studied more intensively to insure successful utilization. With this in mind, *bmr* sorghum appears to be a useful experimental forage for testing the appropriateness of estimating the caloric density of warm season forages harvested at different maturities.

Predicting caloric content of forages

Predicting the caloric content of forages is an established practice. This is due to the expense and constraints posed by actual measurement of caloric content of forages that necessitates in vivo determination because energy is an animal-influenced parameter. More so, caloric content of forages are highly variable; hence, producers have to assess the caloric content of every forages produced to insure accurate ration formulation. Prediction equations are a practical method to assess the caloric value of forage and are more accurate than using tabular values. Prediction of caloric content of forages is very important in order to formulate cost-effective total mixed rations for the lactating cow herd (Weiss, 1998). Forage and feed testing laboratories utilize different prediction equations. Some laboratories use equations that are specific to certain species or agronomic classifications of forages. Most prediction equations for estimating energy content of forages are based mainly on detergent fiber analysis because of its inverse relationship between fiber and calories. The impact of maturity at harvest on fiber and predicted caloric content of warm season forages is related to forage type. Maturity at harvest has a linear impact on the NDF, ADF and lignin content of grasses; whereas, it has a quadratic impact on NDF ADF and lignin content of warm season cereal grain producing forages. In general, NDF is inversely related to caloric concentration of forages and this is the basis of most prediction equations (Donker, 1989). The different relationship between fiber and caloric content of warm- and cool-season grasses and grain producing forages has led to development of prediction equations that are specific to forage type.

The dairy NRC (2001) put forth a generic prediction equation, independent of forage type that estimates caloric content. This equation is based on an updated version of the estimation of

total digestible nutrients (TDN) using several approved analytical procedures (AOAC, 1990) that approximate nutrient content and are mathematically related to digestibility energy. The analytical values employed in the Dairy NRC (2001) TDN prediction equation include: neutral detergent fiber, neutral detergent fiber insoluble nitrogen, acid detergent insoluble nitrogen, crude protein (nitrogen X 6.25), ether extract, and lignin. The values for eight different analyses are used in separate equations to predict digestible non-fiber carbohydrate, digestible protein, digestible fatty acids and digestible fiber. The DE content of forages fed at maintenance is predicted as a summation function based on the premise that for most forage feeds contain 4.2 Mcal/kg of DE per unit of digestible carbohydrate and 5.6 Mcal/kg digestible protein and 9.4 Mcal/kg of digestible fat. The DE content of the forage fed at multiples of maintenance is estimated by discounting the DE fed at maintenance as a function of its intake and TDN content at maintenance. Prediction equations with constants used to adjust for differences in metabolic efficiencies of nutrient use are employed to convert DE at a given level of intake to metabolizable energy or net energy (net energy of lactation (NE_L), maintenance (NE_M) or gain NE_G). The NRC warns that the prediction of the caloric content of feeds is subject to overestimation depending on associative factors involving limitations in rumen fermentation of feed mixtures.

Adoption of the methods of predicting energy as put forth by the NRC is limited due to the complication and expense of a system that uses several laboratory procedures. The practice of estimating forage caloric content necessitates simple equation(s) based on fewer laboratory analyses. Furthermore, the NRC prediction equation assumes that it is feasible to predict the energy content of different forages with one equation. It is assumed that the NRC 2001 prediction equation was developed with a data set that included a representative sample of

different forages fed to dairy cattle that vary in specie and maturity at harvest. However, the data set used to establish and test the NRC prediction equation presumably is based on more digestible forages fed in more temperate climates. It is likely that high fiber warm season forages are under-represented in this data-set because their use in dairy cattle feeding is limited to the sub-tropical or southern region of the U.S. where relatively smaller proportion of the dairy cattle are produced. Testing the accuracy of prediction equations for estimating caloric content of warm season forages for dairy cattle is needed. It is the aim of this research to test the accuracy or fitness of current NRC prediction equation for estimating caloric content (DE) of warm season forages as compared to that determined using calorimetric procedures. Also we aim to establish a more practical method to assess caloric content of warm season forages using an in-vitro procedure.

Utilizing Sorghum as a forage for dairy cattle

Sorghum forage preserved and fed as silage has been investigated and most results indicate the potential of this warm season forage for feeding dairy cattle in sub-tropical regions (Aydin et al., 1999, Lusk et al., 1984, Nichols et al., 1998). As stated previously, there is a nonlinear relationship between maturity, NDF and caloric content of conventional grain producing forages because with maturity, starch concentration increases and diluting NDF during seed or grain development. As a result of the extent of its yield and its changing chemical composition and physical structure with advancing maturity the window of time for harvesting corn silage to maximize nutrient yield is longer than that for sorghum silage. There is generally a 30 d time period for harvesting corn for silage at an ideal stage of maturity, qualitatively measured by the distance of the starch/milk line (1/3 to 2/3) in the corn kernel for maximizing its

nutrient yield. Whereas, there is a 14 d time period for harvesting forage sorghum for silage, qualitatively measured as the development of the starch in the sorghum seed head as being described in stages of maturity known as milk to soft-dough stage (Black et al., 1980). Even though the NDF decreases in conventional sorghum forage with seed/grain development at advancing stages of maturity it does not behave like corn produced as silage because the physical and chemical nature of the mature sorghum seed is more resistant to both fermentative and hydrolytic digestion. Reduced caloric content of sorghum forage harvested at advanced stages of maturity is typically associated with decreased digestion of the non-fiber carbohydrate portion of the forage. This problem is associated with the physical and chemical composition of the seed coat and it is amplified at higher levels of intake. Brown midrib forage genotypes of sorghum contain less lignin and greater digestibility. Research is needed to accurately predict the energy content of warm season forages especially the available genotype of brown midrib sorghum forage. It is also needed to evaluate lignin content and other chemical components of brown midrib sorghum that are influenced by advancing stages of maturity at harvest and their impact on digestibility and ruminal disappearance rate of rations containing *bmr* sorghum fed at high and low levels of intake.

Objectives and Hypothesis

The objective of this research is to compare the actual measurement and predicted digestible energy (DE) content of total mixed rations that vary substantially in DE due to forage maturity at harvest and level of feeding intake. Specifically, to develop a method to measure DE based on in-vitro disappearance of gross energy, and quantify ruminal passage of non-structural carbohydrate of brown midrib sorghum silage harvested at advancing stages of maturity.

It is hypothesized that prediction of energy content of warm season forages can be improved using an in-vitro technique and bomb calorimetric procedure as compared to the NRC (2001) equations. This hypothesis will be tested using brown midrib forage sorghum harvested at different stages of maturity and fed as part of a total mixed ration at different levels of intake.

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CHAPTER 2

BROWN MIDRIB SORGHUM AS WARM SEASON FORAGE FOR DAIRY CATTLES: INFLUENCE OF INTAKE AND STAGE OF MATURITY ON CALORIC CONTENT AND RUMEN FERMENTATION

Lim, J. M., M. A. Froetschel, L. O. Ely and P. G. Smith. To be submitted to the Journal of Dairy Science.

Abstract

Four yearling steers were used in a balanced 4 x 4 Latin Square designed experiment to determine caloric content and ruminal passage of a total mixed rations (TMR) containing silage from a genotype of brown midrib (*bmr*) forage sorghum (*Sorghum bicolor* (L.) Moench.). The animals were assigned to one of four treatments with a 2 x 2 factorial arrangement with 2-week periods. Treatment include TMR's composed of 60% bmr sorghum silage harvested at two stages of maturity (early maturity (EM) at a flower stage of seed head development and late maturity (LM) at a milk to soft dough stage of development) and fed at 2 levels of intake (~1.5x maintenance and meal feeding for a 5 h interval/d). Treatments are designated as follows: **T1**= EM *bmr* sorghum silage as basal roughage in TMR fed at 1.5X maintenance; **T2**=early maturity *bmr* sorghum silage fed at approximately 3X maintenance with 5 h meal feeding; **T3**= LM *bmr* sorghum silage fed as basal roughage in TMR 1.5 X maintenance; and **T4= LM** *bmr* sorghum silage as basal roughage in TMR fed at approximately 3X maintenance with 5 h meal feeding. The TMRs or experimental diets were all formulated to contain 60% roughage as *bmr* sorghum silage and 40% concentrate, containing mainly corn and soybean meal, on dry matter basis. Chromic oxide (approximately 0.4 %) was included with the concentrate in the diets to serve as an external digestibility marker. The first seven days of each period were used for adapting animals to dietary treatments while the remaining seven days were used for data collection. Daily DMI was measured and weekly samples of silage, concentrate, and TMR were collected during the entire experiment chemical analysis of nutrients. Fecal samples were collected at 12 hour intervals from day 8 to 13 of the collection period to represent the fecal output at every 2 hours during an entire 24 hour period. On day 14 of each period, contents of rumen of the steers were evacuated 5, 11, 17 and 23 hours after feeding. Ruminal digesta contents were weighed and

sampled for nutrient analysis. Ruminal fluid was sampled for pH and stored for subsequent analysis of ammonia and volatile fatty acids (VFA). Animals were weighed weekly for purpose of adjusting intake to feed the steers on the lower intake treatment according to 1.5X maintenance. Due to seed head development, the EM *bmr* sorghum silage had more CP, NDF, and ADF but lower ADL and ash than the LM *bmr* silage sorghum. As by design, the DMI of steers were higher for steers in T2 and T4. Steers fed rations containing the EM *bmr* sorghum silage at the higher feeding level consumed more NDF but less acid detergent lignin as compared to those fed rations with LM. Apparent digestible energy, % DE and dry matter digestibility was not different among treatments while intake of DE was highest for T2, T4, T3 and T1 respectively. Digestible energy was predicted using the NRC prediction model and with an in vitro method. Predicted DE from the two methods and actual DE were compared by correlation analysis. The NRC and in vitro method were correlated with each other but not actual DE, and NRC regression analysis appeared to be the least accurate to assess DE. Fractional disappearance rate of ruminal DM , NDF, NDS, OM and ash were not different among treatments when estimated using the 5, 11, 17 and 23 h rumen emptying time observations. Including a 2 h-time value extrapolated as the sum of the 23 h observation and intake in the model did result in different fractional disappearance rates for all ruminal components except OM. Steers in T2 had the highest fractional disappearance rate for all the ruminal contents, intermediate for T4 and T3, and lowest for T1. Pool sizes of ruminal DM, NDF, NDS, OM, ash and VFA at emptying times were different. Ruminal pH was not different among treatments. Ammonia concentration was higher for those fed 1.5X maintenance (T1 and T3). Volatile fatty acids concentration was higher in steers fed at higher intakes but did not differ except for the branch chain VFA when expressed in terms of molar percentages. The ratio of acetate to propionate was not different among

treatments. It appears that nutrient content of *bmr* sorghum forage, especially with regard to DE, prepared as silage and fed as the basal forage in TMR for dairy cattle is not as negatively influenced by harvesting at later maturities as conventional sorghum varieties. Although NRC predicted DE correlates with an in vitro assessment procedure, predicted DE of the *bmr* sorghum silage using the NRC method and an in vitro method does not correlate with in vivo estimates of DE. In conclusion, the *bmr* type of sorghum forages appears to be very digestible and less influenced by stage of maturity and intake effects than its non *bmr* counterparts. Futhermore it does appear that more research is needed to accurately and practically assess the caloric content of warm season *bmr* forages.

(Key Words: Apparent Digestible Energy, Volatile Fatty Acid, Brown Midrib Sorghum)

Introduction

Energy is a major nutrient requirement in all animals. Aside from water, energy is required in the diet in greater amount than all other nutrients. It costs far more because it contains the bulk of the diet and is the only nutrient that cannot be measured using a direct or proximate analysis (Coppock, 1985, Maynard and Loosli, 1962). Feed energy is an animal influenced parameter and its actual measurement requires an animal feeding trial. Due to the difficulty of measuring energy in feeds, prediction of the energy value of feeds had become a common practice in animal nutrition. Accurately predicting the caloric content or energy value of feedstuff and especially forages is a very important part of the ration formulation process. In dairy production, inaccuracy of prediction may either lead to under-feeding or over-feeding nutrients which can impair milk yield, feed conversion, and ultimately profitability. Furthermore,

malnutrition may make the cow more susceptible to reproductive and metabolic disorders and further negatively impacting profitability (Weiss, 1993, 1998).

The current Dairy NRC (2001) prediction equation for NE_L is based on as many as eight different chemical analyses of a given feedstuff. These analytical values are used in a series of summative equation to estimate truly digestible non-fiber carbohydrates, digestible protein, digestible fat, and digestible fiber. The estimates of digestible nutrients are in turn multiplied by their respective heat of combustion to provide an estimate of digestible energy (DE). A correction factor for metabolic fecal energy is employed to convert the estimate of true digestible energy to apparent DE. Subsequently, metabolizable energy and net energy are predicted using equations that adjust DE by its relative efficiency of use for maintenance or productive functions when consumed at varying levels of intake above maintenance. Actual use of the NRC method to predict energy value of feedstuff seems to be of limited practical value because of expense, time and labor necessary to produce the required analytical measurements. Furthermore, its accuracy is erroneous for certain ingredients. Research is needed to develop methods of predicting caloric content of forages and feeds that is more practical and dependent on fewer laboratory tests. In the Southeastern US and other warmer regions of the world, warm season forages are an integral part of the nutritional program for feeding dairy cattle. In sub-tropical and tropical climates the ability to grow corn, the crop that produces the most DE from land resources, is limited, and this necessitates the alternative utilization of warm season forages (Lusk et al., 1984). Several studies were conducted comparing corn silage to silages prepared from other warm season forage species in total mixed rations for high producing dairy cows. Among the warm season forages tested, brown midrib *(bmr)* forage sorghum ([*Sorghum bicolor*

(L.) Moench.]) and its hybrid crosses with sudan grass have shown similar feeding value compared to corn silage for lactating dairy cows (Aydin et al., 1999, Dann et al., 2008, Grant et al., 1995, Lusk et al., 1984). The satisfactory response of the dairy cows fed with total mixed rations containing the brown midrib genotypes was attributed to the improved digestibility of these genetically altered forages and their low and less anti-nutritional lignin content.

The objective of this research was to determine the effect of *bmr* forage sorghum harvested at two stages of maturity and different levels of feed intake on the energy content of total mixed rations and fractional turnover rate of ruminal components. In addition, it is the aim of this study to develop a method to measure digestible energy using an in-vitro technique.

Materials and Methods

The research was conducted at the University of Georgia Dairy Teaching Center, Athens from October 23 to December 25, 2008. Animals were handled and managed under the guidelines approved by the University of Georgia Institutional Animal Care and Use Committee.

Forage and Silage Production

The *bmr* forage sorghum (Red Top +, Production Plus, Plainview Texas) used in this study was planted in June 4 and ensiled in September 2 and 16 during the summer 2008 season. The *bmr* forage sorghum was fertilized with 135, 112 and 100 kg of nitrogen, phosphorous and potassium respectively per hectare of land planted. Brown midrib sorghum forage was harvested at two stages of maturity 1) at an early head or flowering stage and 2) at milk to soft dough stage on September 2 and September 16, 2008 respectively. Approximately 8 hectares of homogenous

and a relatively high yielding section of the forage stand were selected for harvesting at the specified maturities. The forage was harvested with a pull-type mower conditioner at approximately 1100 H. The harvested forage was allowed to wilt in a wind-row for approximately 2 hours prior to chopping at a theoretical length of 20-25 millimeters. Chopped whole plant forage *bmr* sorghum was ensiled in eight one-ton concrete stave silos with 4 silos for each level of maturity, Each silo was lined and sealed with silo bag plastic (Southern Silage Supply, Andalusia, AL). The forage was packed manually, excess air was removed by vacuum, and the plastic sealed with adhesive tape. The forages were ensiled without inoculants and packed within 4 hours of chopping. Representative samples of the wilted sorghum during filling were sampled and analyzed for dry matter and nutritional content (NDF, ADF, CP, ash, lignin and water-soluble carbohydrates).

Experimental Animals and Diets

Four yearling Holstein steers (initial mean BW ~ 501.25 kg ± 11.63) surgically fitted with ruminal fistula were restrained individually in tie stalls and fed once daily at 800 h. Orts were removed after 5 hours (1300 h) to encourage meal feeding behavior. Orts were weighed, sampled for later analysis and discarded. The steers were then released to a dirt corral for the evening and brought back into the barn at 800 h the next day for feeding. The diets were total mixed rations containing 60% of either the two different maturities of brown midrib forage sorghum silage on a dry matter basis. The concentrate portion of the ration was composed of ground corn and 48% soy bean meal with mineral and vitamin supplementation included at a rate of 32, 7.5 and 0.5% respectively. The steers were fed either at 1.5 times maintenance using the recommended dairy NRC 2001 equation (NE_M=.80*kg.^{75}) or ad libitum (~110% of the previous

days intake) for 5 hours. Rations were mixed daily few hours before feeding. Any spoilage observed on top of newly opened silos was discarded.

Experimental Design and Sampling Protocol

The experimental design used was a balanced 4 X 4 Latin Square Design with a 2 X 2 factorial arrangement of treatment. The steers were assigned to one of the following treatments during one period of each experimental period:

T1 = TMR with 60% of DM as early maturity *bmr* sorghum silage fed at 1.5X maintenance

T2 = TMR with 60% of DM as early maturity sorghum silage fed free choice for 5 hours

T3 = TMR with 60% of DM as late maturity sorghum silage fed at 1.5X maintenance

T4 = TMR with 60% of DM as late maturity sorghum silage fed free choice for 5 hours

Experimental periods were set at 14 d with the first 7 d of each period for adjustment and 7 d for sample collection. Steers were weighed at weekly intervals during the experiment. Individual DMI was measured daily by weighing the feed offered and weighing the orts after the feed had been removed for each animal. Digestibility was measured using chromic oxide as an external digestibility marker which was mixed to the concentrate part of the ration. Fecal samples were collected from steers either upon defecation or by rectal palpation over a 6 d period (d 8-13) at approximately 12 h intervals to represent every 2 h sampling interval of a 24 h period. Daily fecal samples were collected on d 8 at 800 and 2000 h, d 9 at 1000 and 2200 h, d 10 at 1200 and 2400 h, d 11 at 1400 and 200 h, d 12 at 1600 and 400 h, d 13 at 1800 and 600 h.

Immediately after fecal samples were collected they were placed on drying trays for each animal by treatment by period, placed into a forced air oven set at 60^{o}C, and dried to a constant weight. The dried weekly composite sample of feces representing all sampling times for each animal and period were weighed, then air-equilibrated at ambient temperature and humidity for 24 hours, and ground via Wiley Mill (Arthur H. Thomas, Philadelphia, PA) to pass through a 1 mm screen. On d 14 of each period, ruminal contents were completely evacuated manually at 5-6, 11-12, 17-18 and 23-24 h after feeding through the rumen fistula. The total evacuated rumen contents were weighed, sampled and immediately returned to the rumen. Samples of rumen digesta were collected into tared aluminum pans and dried to constant weight to obtain estimates of % DM. The rumen digesta samples were then processed by the same procedure as fecal samples in preparation for subsequent analysis of ash, organic matter, and NDF. The mass and composition of rumen evacuation measurements were used to estimate the fractional rate of rumen disappearance of the DM, NDF, neutral detergent soluble (NDS), ash, and organic matter. These measurements were taken with the goal that rumen fractional turnover rate of fiber and non-fiber fractions could be used to determine if *bmr* sorghum acts like conventional forage sorghum that has decreased DE due to ruminal passage of its non-fiber fraction with advancing stages of maturity. Rumen fluid was also collected during rumen evacuation and immediately analyzed for pH, placed in nalgene plastic bottles and stored frozen until subsequent analysis of volatile fatty acids (VFA) and ammonia. Daily samples of silage, concentrate and TMR were collected and combined into a weekly composite sample and placed in frozen storage (-40^{o}C). Silage, concentrate and TMR samples were thawed after the experiment and dried at 60^{o}C. Dried feed samples were weighed, air-equilibrated, and ground via Wiley mill to pass through a 1 mm screen prior to nutrient analysis. Composite samples of silage, concentrate, TMR and feces were

analyzed for DM, NDF, ADF, neutral detergent fiber insoluble nitrogen, acid detergent insoluble nitrogen, CP, lignin, GE and chromic oxide (Cr_2O_3) using standard analyses to be described. In addition, an adaption of the Tilley and Terry in-vitro method was conducted to predict DE content of TMR samples and was compared to the other methods of DE determination. These comparisons were made to gain preliminary data to develop an in vitro DE method that may have greater utility and practicality than the current NRC method.

Analytical Techniques

The digestible energy (DE) content of the *bmr* sorghum silage based diet samples was estimated using three methods. Digestible energy was determined as apparent in vivo total tract DE based on the difference between feed gross energy input and fecal gross energy output and the ratio of chromic oxide in the feed and feces in steers. The DE content of the diets was predicted using the equation as specified by the dairy NRC (2001). It was also estimated using an adapted version of the Tilley and Terry in vitro procedure that accounted for in vitro digestion of gross energy.

The GE measurement for all feed ingredients, fecal, and residue for the in-vitro technique were determined by calorimetric procedure using a PARR 1261 adiabatic bomb calorimeter (PARR Instrument Company, Moline, Illinois). Compressed pellets of samples weighing approximately 1 g were prepared in duplicate and placed in a metal cup supported by the screw top of the bomb. A 10 cm piece of ignition wire fuse was securely connected to the bomb terminals and was set to touch the pelleted sample. The top was then placed on the bomb and screwed tightly. The bomb was charged with oxygen and placed in the calorimeter jacket containing 2 liter of distilled water. The stirrer was started until a constant temperature reached

and the charge was ignited electrically and the sample burned. Readings were taken on the thermometer of the instrument and the number of calories produced by the burned sample was estimated after accounting for the fuse that was combusted.

The chromic oxide analysis was determined on the TMR, concentrate and fecal samples using the procedure adapted from Fenton and Fenton (1979). In a 2 L Erlenmeyer flask placed in an ice bath, molybdate reagent was prepared by adding 20 g of sodium molybdate to 300 ml of distilled water. Slowly, 300 ml of concentrated sulfuric acid was added to the first mixture. After the solution had cooled, 400 ml of 70% Perchloric acid was slowly added. The solution was allowed to cool to room temperature and the stored in a reagent bottle until used for heated acid digestion of the feed and fecal samples. Chromic oxide (0.1 g) for standard and samples of feed or fecal (1 g) were placed in individual etched 50 ml Erlenmeyer flasks in duplicate. The samples were dried ashed initially in a muffle ashing oven for 3 hours at 500 – 600^{o}C and then allowed to cool to room temperature. The samples were removed from ash oven and weighed after which 15 ml of molybdate reagent was added to each Erlenmeyer flask including one containing no sample. The samples were then digested on a hot plate in a perchloric fume hood until the mineral matter in the sample was dissolved and a yellow-red color appears. Digestion was continued for 10 – 15 minutes after color change and then samples were removed from the hot plate block. After cooling, the samples were transferred quantitatively into individual 50 ml volumetric flasks. The chromic oxide sample intended to generate the standard curve was transferred to 100 ml volumetric flask. Each flask was rinsed twice with a small quantity of water and then diluted to 50 ml volume. From the full volumetric flasks containing the ashed and acid dissolved sample, 5 ml were taken and placed into test tubes and centrifuged for 15 minutes at 2000 rpm. After centrifugation, the supernatants were decanted and transferred to cuvettes for

reading in a spectrophotometer (Milton Roy Spectronic 401, Milton Roy Company, USA) set at 440 nm wavelength absorbency. The concentration of chromic oxide was determined from the absorbance of the sample solutions as compared to the standard curve made from the serial dilution of the standard solution of chromic oxide. During the study, re-run of the chromic oxide (Cr_2O_3) analysis was conducted on the samples collected on the fourth period. This was done because concentrate in the first three periods contained 0.42% chromic oxide while that used in the fourth period had 0.19%. When the result of the chromic oxide analysis was used to measure apparent digestible energy of the diets at different intake levels, results from the first three periods were consistent with a relatively low SE. In contrast, the measurements obtained from fourth period were highly variable, especially with the steers fed at 1.5X maintenance. Due to the low concentration of the chromic oxide in the fourth period diets, it is suspected that the spectrophotometer response may have been inaccurate because the levels of absorbance of the 4^{th} period samples were below the range of the standard curve. For the re-run samples, it was decided that the amount of substrate to be digested be tripled from a gram to 3 grams in order to increase the amount of Cr_2O_3 to a level detectable to the machine. In addition, composite TMR sample with and without Cr_2O_3 were analyzed. This was done to determine whether or not the feed samples affected the measurement of the absorbency in the analysis, and the result of this will be used to establish correction factors in the analysis. The composite samples with Cr_2O_3 were spiked with the same amount of Cr_2O_3 (0.1 gram) with that used in making the stock reagent for the standard curve. Results obtained in the re-run were used for the measurement of apparent digestible energy for the fourth period.

Crude protein measurements for the silage, concentrate, TMRs, and feces were determined by combustion analysis (LECO FP-528 Nitrogen Analyzer, LECO Company, St.

Joseph, MI). Readings or measurement were automatically logged into a computer and nitrogen values were multiplied by 6.25 to derive the crude protein content of the sample. Neutral detergent insoluble CP and acid detergent insoluble CP was also determined through this method by analyzing NDF and ADF residues.

The method for determining the ash content of samples for the study was performed in muffle oven. Samples weighing around one gram were placed in etched crucibles and then heated at 550 – 600^{o}C for three hours. The samples were then cooled at room temperature for about 30 minutes and then transferred to a force - air oven set at 110^{o}C for 1 hour. The samples were taken out and placed in a desiccator for 20 minutes before weights were determined and recorded. The difference between the ashed sample residue and the crucible as compared to the initial sample were used to estimate % ash.

Analysis of the fiber contents of the samples were determined by using the Ankom 200 filter bag techniques (Ankom Technology Corp., Macedon, NY, Van Soest et. al, 1991) using a sequential version of the method. The neutral detergent fiber analysis, reagent was prepared by adding and dissolving 180 gm of sodium lauryl sulfate in an Erlenmeyer Flask with 2000 ml of distilled water. In a separate one liter beaker filled with 500 ml of distilled water, 27.42 gm of anhydrous sodium phosphate dibasic was added and thoroughly mixed. The beaker was filled to the 700 ml volume mark with distilled water and 111.72 gm of ethylenediaminetetraacetic acid (EDTA) and 40.92 gm of sodium borate were added and stirred thoroughly. The mixture was then added to the sodium lauryl sulfate earlier prepared and with constant stirring the Erlenmeyer flask was filled to 6 liters by adding distilled water and 60 ml of ethylene glycol monoethyl ether alternately. When solution was thoroughly mixed, it was left to sit overnight and pH was

checked next day. If pH was higher than 7.0, 1.0 – 1.5 ml of HCl was added to make the pH of the solution neutral. Solution was then transferred to carboy until used. Samples for NDF analysis approximately 0.5 gm were placed in filter bags previously weighed and labeled with acetone resistant pen and were sealed with heat sealer machine. The bags were placed in a suspender and agitated under heat in an Ankom Fiber Analyzer containing 2 liters of the NDF solution, 20 gm of Sodium Sulfite, and 4 ml of heat stable α – amylase for 60 minutes. After agitation, samples were rinse twice for 3 minutes with 2 liters of hot water and 4 ml of α – amylase. A third rinse was done using only 2 liters of hot water. After rinsing the samples, the bags were removed from the agitation vessel, squeezed out of excess water, and soak in acetone for 3 minutes. The bags were then squeezed out lightly of excess acetone, left to sit for 30 minutes to let the remaining acetone to evaporate, and placed for drying in a force – air oven set at 105^{o}C for at least 2 hours or until constant weight. After drying, the bags were placed in a desiccator for 15 minutes and then weighed and recorded.

The acid detergent fiber analysis followed the same procedure with that of NDF except that the agitation and rinsing process does not involved the use of sodium sulfite and α – amylase but only hot water. The ADF solution was a mixture of 304 gm of sulfuric acid and 6 liters of distilled water. Normality of the solution was checked by adding 3 drops of bromocresol green into 10 ml of ADF into a small beaker. The solution was titrated with tham until the solution changes from yellow to blue. The normality was then calculated by dividing the amount of tham necessary for color change by 10 (ml of ADF). When a 1.00 N was achieved, 118.2 gm of cetyltrimethylammonium bromide (CTAB) was added to the ADF solution, mixed thoroughly and transferred to carboy.

The sulfuric acid method was used for acid detergent lignin analysis of feed samples. After performing ADF on samples, filter bags were placed into 3L beaker and along with a sufficient quantity (approximately 250 ml) of 72% sulfuric acid to cover bags. A 2 L beaker was placed inside the 3 L beaker to keep the bags submerged in the sulfuric acid. The bags were then agitated by pushing and lifting the 2L beaker up and down approximately 30 times. The agitation was repeated every 30 minutes. After 3 hours, the sulfuric acid was poured off and the samples were rinse with hot water to remove all acid. The samples were thoroughly rinsed until pH is neutral. The samples were then soaked in acetone for 3 minutes to removed water, squeezed out of acetone and left to sit at ambient temperature to let excess acetone to evaporate, and placed in a force – air oven at 105^{o}C for 4 hours or until constant weight has been achieved. After drying, samples were placed in a desiccator for 15 minutes, weighed and recorded.

Water soluble carbohydrates analysis was performed on green chop forage sorghum following the methods described by Dubois et. al (1956). Green chop forage sorghums were thawed out and 25 gm of samples were blended in 100 ml of distilled water and filtered through four layers of cheesecloth. One milliliter of the blended extracts was diluted with 100 ml of distilled water. From the aliquots, 2 ml were placed to a reaction tube and were added 1 ml phenol reagent and 5 ml of concentrated sulfuric acid in sequence. The solutions were vortex and left to stand for 10 minutes and then vortex again and left to stand for 20 minutes. The sample solutions were transferred to cuvettes and then read on spectrophotometer at 490 nanometers. Dextrose weighing 100 mg mixed with 1000 ml of distilled water was used as stock standard for this analysis.

Ruminal fluid samples collected during rumen evacuation were analyzed for VFA using a Varian 3400 Gas Chromatograph (Varian Inc., Palo Alto, CA). Samples were thawed out and strained through 4 layers of cheesecloth. In a test tube, 5 ml of the strained rumen fluid were added to 1 ml of 25% meta-phosphoric acid. The test tubes were covered with a rubber stopper and were thoroughly mixed. The samples were then placed in a freezer overnight. After freezing, samples were thawed and centrifuged for 20 minutes at 2000 rpm. The supernatant were decanted into a clean 5 to 10 ml test tube and placed into septum covered vials and loaded to the gas chromatograph rack for VFA determination. .

Free Ammonia-N analysis was also conducted on the ruminal fluid samples using a specific ion electrode method (Model 95 -12, Orion Research Inc., Beverly, MA). A standard curve was established from ammonium chloride. This was accomplished by adding 25 ml of standard in a 50 ml beaker with stir bar. Initial pH reading was taken and then a 50% solution NaOH was added by drops until pH was greater than 11.0. The NH_3 electrode was then placed in the beaker for ammonia reading and recorded. The ammonia concentration of the samples were fit and predicted based on a standard curve of standards.

The in-vitro technique employed to predict DE in the silage and TMR were adapted from Tilley and Terry (1963). Initially, a trial run was conducted using composite samples from the TMRs used in the study. The samples were inoculated with buffered rumen fluid and incubated for 12, 24, 48, 72, 96, and 120 hours and the residue obtained after two stage centrifugation and washing were subjected to gross energy determination by bomb calorimetry. The in-vitro measurements of gross energy before and after the in vitro digestion were then used to derive the percentage in vitro DE in the different times of inoculation and the results were compared to the

in vivo measurements determined through the chromic oxide method. The samples that were inoculated at 48 hours were the ones that most nearly compared to the in vivo method. Thus, it was decided that in-vitro digestible energy (IVDE) should utilize a 48 hour digestion of silage and TMR samples. Approximately 0.60 gm of samples was weighed into 50 ml centrifuge tubes and each sample was prepared in six tubes to obtain a residue that will be enough for bomb calorimetric procedure in duplicate. To the samples, 20 ml of McDougall buffer and 10 ml of rumen fluid taken from one of the fistulated steers used in the study which was fed with dairy TMR was added . The rumen fluid was taken from below the rumen mat, pH evaluated and was strained with two layers of cheesecloth. Tubes were gassed with CO_2 and capped tightly with one-way valve rubber stopper. The tubes were then placed in a water bath heated to a constant 39^{o}C and were inoculated for 48 hours. After inoculation, tubes were placed in a freezer overnight. The samples were thawed out next day, vortex, and centrifuged. The supernatants were discarded and the samples in the tubes were rinse by adding 20 ml of tap water, vortexed, and centrifuge. Rinsing of the samples was done twice and all supernatants were discarded. After rinsing, 20 ml of pepsin solution was added to each tube. Samples were inoculated with the pepsin solution for 24 hours in a water bath and immediately frozen after. After an overnight freezing, samples were thawed out and were rinsed following the procedure described earlier. The tubes were then placed in a force – air oven set at 60^{o}C for drying until constant weight. After drying, samples were placed in a desiccator for 15 minutes then were weighed and recorded. Residues from each sample were ground manually, pooled and were stored in plastic bottles until gross energy determination by bomb calorimetry.

Ether extract content of the feed ingredients was not measured and estimated from tabular values of the dairy NRC (2001).

Statistical Analysis

All data were analyzed by the General Linear Model (GLM) procedure of SAS (version 9.1, SAS Institute Inc., Cary, NC) for a Latin Square Design. Model effects include period, animal, maturity, intake and the interaction of maturity and intake. Repeated measurements were analyzed on a split-plot where observations over time constitute the subplot and error term was nested within period, animal and treatment. Regression and correlation of data on digestible energy estimated through in vivo, NRC and in vitro method were analyzed using the PROC REG and PROC CORR procedures of SAS. Data on rumen turnover rate were analyzed by the Non-Linear Model procedures of SAS and logarithmic transformation and measurements obtained were analyzed by GLM procedure. Level of significance was declared at $P < 0.05$ unless otherwise stated. The PDIFF procedure of SAS was used to separate means that were statistically different. All data are reported as least square means.

Results and Discussion

Brown Midrib Sorghum, Diets and Nutrient Intake

The chemical analysis of the brown midrib forage sorghum used in the study is reported in Table 1. The forage sorghum harvested at an early maturity (early head) was higher in moisture content, slightly higher in crude protein, NDF, ADF and ash than those harvested at a later maturity (milk to soft dough). They also contain 8 percentage units more of water-soluble carbohydrates and have lower lignin content. Comparison of the ratio of the plant parts shows that as the plant matures from EM to LM, the mass of the seed head increases by 21%. Also, the

proportion of leaves and stem were reduced by 7.9 and 13.56 percentage units, respectively. The silage sample was higher in moisture content as well as having higher concentration of crude protein, NDF, ADF, lignin and ash. The EM silage was lower in ash and lignin but higher in NDF than LM silage. In vitro dry matter digestibility conducted for 48-h was higher for EM. In contrast, GE of the *bmr* sorghum silage is higher for the LM silage. The observed changes in chemical composition of the *bmr* forage sorghum as maturity advances coincides with findings of previous studies (Black et al., 1980, Owen and Webster, 1963). As the *bmr* sorghum forage matures, CP likely declines due to decrease proportion of the leaves. The observed lower NDF in the LM silage is the result of the higher proportion of seed head which dilutes NDF concentration. Higher water-soluble carbohydrate concentration in EM forage can also be attributed to the higher proportion of leaves in EM sorghum forage. Lignin is substantially higher in the LM forage because of its known structural function needed to support the growing seed head. The observed increase in the chemical composition of several nutrients in the silage as compared to the forage is likely the result of the loss of sugars during the fermentation process. The chemical composition of the EM silage used in the study is very similar to that of the *bmr* sorghum-sudan hybrid as reported by Dann et. al (2008). The CP, NDF, ADF and lignin composition of the *bmr* forage sorghum in our study were higher than those reported by Bean et al (2000). These authors analyzed the same variety of *bmr* sorghum (Redtop +) and reported that at soft dough maturity, silage contain CP, NDF, ADF and lignin at 6.7, 47.0, 25.97 and 2.03% of dry matter respectively. The higher CP obtained in our silage maybe attributed to the fertilization provided on the forage during the production.

The ingredient composition and chemical analysis of the TMR fed in the experiment are shown in Table 2. Total mix rations (TMR) were 60% silages and 40% concentrate on a DM

basis. Chemical analysis confirmed that the diets were isonitrogenic and isocaloric as formulated. The TMR made with the EM *bmr* silage had more NDF, ADF and fiber bound protein but less lignin and ash than the TMR with LM silage.

Effects of sorghum maturity and level of intake on DM and nutrient intake of the steers are shown in Table 3. Dry matter intake was intentionally higher in steers receiving T2 and T4 than those in T1 and T3 and had a major effect on most parameters evaluated. When DMI is expressed as a percentage of bodyweight, the steers on T1 and T3 consumed 1.92 and 1.99 % while those on T2 and T4 diets consumed 3.09 and 3.06 % respectively. Mathematical estimation of the intake as a function of maintenance caloric requirement of steers with free choice access to feed for 5 hours (T2 and T4) translates to approximately 3X maintenance feeding. Dry matter intake is the same for the steers on T1 and T3. It can be noted that the steers receiving T2 consumed 0.46 kg more of dry matter than those in T4. No significant effects were observed for maturity and interaction on intake of CP, ADF and ash on the test animals. There is however, a maturity and interaction effect on NDF consumption. Steers in T1 and T3 consumed the same amount of NDF (4.77 vs. 4.78 kg) while T2 consumed more than those that are in T4 (7.93 vs. 7.51 kg). A maturity effect was also observed for intake of lignin. Intake of lignin was highest on the steers receiving T4 which consumed 0.62 kg, followed by T2, T3 and T1 which consumed 0.49, 0.39 and 0.30 kg respectively. The data reflects that the increased NDF concentration of the TMR containing the early maturity *bmr* sorghum silage did not appear to restrict the intake of the steers. Although T2 contain more NDF than T4, it is interesting to note that the steers consumed more DM when fed T2 as compared to T4 when meal fed free choice for 5 h. It is generally well established that NDF is the primary component in forages that contributes to physical fill and distention in the reticulorumen and negatively influences voluntary dry matter intake of

ruminants (Allen, 1996, Jung and Allen, 1995, Mertens, 2003, Waldo, 1986). However, the physical fill provided by NDF diminishes as digestibility and passage rate of NDF in the reticulorumen increases (Jung and Allen, 1995). The result of our data is indicative of the phenomenon described by Jung and Allen (1995). The increased intake of steers in T2 suggests that the NDF of the early maturity *bmr* sorghum is highly digestible. Previous study conducted by Aydin et. al (1999) reported high NDF digestibility for *bmr* sorghum silage. Similar results have been obtained by Beek et. al (2007). Dann et. al compared NDF digestibility of *bmr* sorghum-sudan hybrid with corn silage and found higher digestibility of the *bmr* sorghum-sudan hybrid over corn. A 48-h NDF digestibility showed that *bmr* sorghum was greater for *bmr* forage sorghum than for a dual-purpose corn silage hybrid (Oliver et al., 2004). The higher lignin concentration in the TMR with LM silage might have been the factor which influenced the observed numerical difference or lower DMI of the steers in T4 than T2. The steers in T4 consumed less DM but their lignin intake is 26% more than those in T2. Lignin is the primary indigestible component of plant cell walls and its concentration increases as the plant matures. Aside from being indigestible, it also inhibits digestion of cell wall carbohydrate (Aydin et al., 1999, Oliver et al., 2004).

Apparent DE, Dry matter digestibility and Predicted DE

Apparent total tract digestible energy and dry matter digestibility of the diets as determined through using chromic oxide as an external digestibility marker are summarized in Table 4. Results of the analysis show significant effect of intake on the daily consumption of DE of the steers. Intake of digestible energy was higher on those steers receiving T2 as compared to those fed T4 (55.78 vs. 52.90 Mcal/day). In contrast, the steers in T3 consumed slightly higher

DE than those in T1 (35.02 vs. 34.87 Mcal/day). When the apparent DE concentration was measured among treatments, T1 resulted in the highest DE with 3.18 Mcal/kg followed by T3, T2 and T4 with 3.10, 3.07 and 2.98 Mcal/kg respectively. Similar result was obtained when DE concentration was expressed in terms of a percentage. The dry matter digestibility results reflect that the steers fed T1 have the highest dry matter digestibility (63.66%) intermediate for T2 and T3 (63.38 and 61.77%) and lowest for T4 (60.06%). No significant differences of the main effects were observed however in the three parameters tested. The higher DE and % DE observed in the steers fed low intake (T1 and T3) than those fed ad libitum (T2 and T4) may be attributed to longer mean retention time of ruminal contents to be digested and acted upon by rumen microbes for digestion. In high level of intakes, passage rate of ruminal contents increases decreasing microbial action on potentially digestible fiber components (Allen, 1996, Tyrrell and Moe, 1975). The observed higher dry matter digestibility in steers fed early maturity (T1 and T2) over those fed late maturity (T3 and T4) may be influenced by the lower lignin content of the early maturity sorghum silage.

Digestible energy of the diets was predicted using the equations of NRC (2001) and by an in vitro method and results are summarized in Table 5. The NRC predicted the TMR diets in our study to have a total digestible nutrient (TDN) of 67.23 and 66.10% for early and late maturity respectively. Digestible energy was higher for the diet containing the early maturity sorghum silage by 50 kcal/kg as compared to the diet containing the late maturity silage (2.99 vs. 2.94 Mcal/kg). Digestible energy at actual intake was then predicted using the value of TDN to discount DE at multiple level of intake. The predicted actual DE was highest on T1 with a mean of 2.95 Mcal/kg followed by T3, T2 and T4 with means of 2.90, 2.87 and 2.83 Mcal/kg

respectively. Analysis shows significant effect of intake (P<0.024) and no significant effects were observed from maturity and interaction of maturity and intake.

Digestible energy when estimated from the in vitro method shows that the diets containing the late maturity *bmr* sorghum silage have more DE than the diets containing early maturity silage. In contrast, the diet with early maturity silage resulted in slightly higher percentage DE and dry matter digestibility (DMD) than the diet with later maturity silage. Actual DE was predicted using the NRC equation to discount DE on multiple levels of intake and the % DE obtained from the in vitro method was used in lieu of TDN. The DE values obtained was highest for T3 with a mean of 3.04 Mcal/kg followed by T1, T4 and T2 with means of 3.01, 2.97 and 2.94 Mcal/kg respectively. No significant differences were observed from the main effects on predicted actual DE.

Comparison of the predicted DE obtained from the NRC equation and the in vitro method shows that the two predictive models underestimate the in vivo or apparent DE measured using the chromic oxide marker. A simple regression analysis (Table 6) was conducted to measure how well the two methods predict the in vivo or actual DE obtained from the feeding trial. Regression analysis exhibits that the two methods (NRC and In vitro) are inaccurate to predict actual DE. Coefficient of determination (R^2) for NRC predicting In vivo was 0.0004 with a corresponding P-value of 0.941 while In vitro vs. In vivo resulted to 0.046 and 0.425 for R^2 and P-value respectively. The slopes were negative and the intercepts were too high (Figure 1 and 2). It is interesting to note however, that the In vitro DE values were correlated with the NRC predicted values (Figure 3). The data obtained R^2=0.82 with a corresponding P-value of 0.0001. Correlation analysis of the DE values of the NRC equation, In vitro method and In vivo (Table 7) shows that the NRC and In vitro predictions are not correlated with the In vivo DE measurement.

Correlation coefficient (ρ) estimates between In vivo and the NRC equation and In vivo and In vitro obtained -0.02023 and -0.2145 with P-values of 0.9407 and 0.4251 respectively. Correlation analysis show that the NRC and In vitro values are highly correlated ($P \leq 0.0001$).

The absence of relationship and the lack of precision and accuracy of the NRC equation and our In vitro method to predict the actual or In vivo apparent DE maybe attributed to the very few observations used in the regression and correlation analysis. In addition, the dairy NRC (2001) reports that their energy prediction model is subject to overestimation or underestimation depending on associative effects and digestive limitation in the rumen. On the other hand, the observed weakness of the In vitro method is that it does not simulate passage. It is difficult however to disregard the strong correlation of the In vitro method with the NRC prediction. It indicates that the in vitro method employed in this study may still have some practical utility provided certain modifications will be done to improve precision and accuracy of prediction.

Fractional Disappearance Rate of Ruminal Components

The effects of maturity of *bmr* sorghum silage and level of feed intake on the pool sizes of ruminal DM, OM, NDF, NDS, and. Ash are summarized in Table 8. The tabular data reflects the pool sizes of ruminal contents determined at specific rumen evacuation times. In general the pool sizes for DM and NDF of ruminal contents was consistently higher for T2, intermediate for T4 and T3, and lower for T1 on the first evacuation time (~6 h after feeding). This effect was less apparent for OM, NDS and ash. The mass of ruminal contents that disappears through the succeeding evacuation times varies for each component and ranges from 16 – 55% of the initial mass of content. Statistical analysis of the data resulted in significant treatment and time effects on the pool size of DM, NDF, ash, and OM. In most instances, the effects of intake were greater

than the effects of maturity at each specified time of emptying. Except for at 12 h with the NDS component there were no significant interaction effects observed (P<.05) for ruminal contents emptied at specific time intervals. Dry matter and OM, however, tended to have an interaction effect observed between level of intake and maturity. Differences in the ruminal NDS component was significant for treatment, time, and interaction effects. Comparison among treatment means of ruminal contents shows that the steers fed ad libitum (T2 and T4) differ with those fed 1.5X maintenance (T1 and T3) especially on the first two evacuation time. Treatment means of ruminal ash content were no longer different after the first two rumen evacuations and the same result was obtained for ruminal NDS on the 24 h rumen evacuation time.

The fractional disappearance rate (FDR) of ruminal contents are summarized in Table 9. The table reflects analysis of FDR as determined by the non-linear procedures of SAS and slope of the logarithmic transformation of the observations during the rumen evacuation of the steers at four periods. For both data, no significant differences were observed for the main effects among treatments on the parameters evaluated. The data measured with the non-linear procedure however shows that there is a tendency for an interaction effect on the FDR of ruminal DM, NDF, neutral detergent soluble (NDS) and organic matter (OM). Fractional disappearance rate of ruminal DM, NDF, NDS and OM was highest for steers receiving T2, intermediate for T3 and T4 and lowest for T1. The FDR of ash however was highest for T3 followed by T4, T2 and T1 with means of 4.48, 4.20, 3.67 and 3.27 %/h respectively. The same result was observed in the data determined using the slope of logarithmic transformation. The observations on the disappearance rate of dry matter from the non-linear procedure were plotted in a graph (Figure 4) for examination. The graph illustrates that the slope of the trend line that seems to diminish at a constant rate and is almost linear and does not account for the disappearance rate of ruminal

contents occurring from the initiation of feeding to the first evacuation time. It was decided that a second model be evaluated which included an estimated 2 h-time representing the time when most of the feed were consumed by the steers especially those fed 1.5X maintenance. Rumen contents at 2 h-time were estimated by adding the ruminal contents measured during the last evacuation time (~24 h after feeding) to the intake of steers in each treatment. The model with 2 h-time was analyzed separately for the steers receiving the same level of intake to evaluate the effect of maturity and using the total data to test all the main effects.

The effect of stage of maturity at harvest on the FDR of ruminal contents for the steers fed 1.5X maintenance are shown in Table 10. The FDR of ruminal contents as determined using the non-linear procedures of SAS reflects that the animals fed diet with EM *bmr* sorghum (T1) have higher FDR of ruminal contents than the steers fed the LM sorghum (T3). The effect of maturity was significant for FDR of all ruminal contents except for ash. In contrast, FDR of ruminal contents as determined using the logarithmic transformation shows that T3 was numerically higher than T1 for most ruminal contents evaluated. Except for ash ($P \leq 0.053$), no significant effect for maturity was observed for all the parameters evaluated. The observed higher FDR of ruminal contents in T1 over T3 is indicative that the EM is more digestible than the LM *bmr* sorghum and might also be a consequence of the higher lignin content of LM bmr sorghum. A plot of the observations representing the fractional disappearance rate of DM estimated from non-linear procedure was plotted in a graph (Figure 5) to establish the trend line from this analysis. The inclusion of the predicted 2 h time point resulted in a smooth curvilinear trend line and different to that observed in Figure 4.

Comparison of the FDR of the ruminal contents of the steers meal fed for 5 h are summarized in Table 11. The data show that for both method of analysis (non-linear procedure

and logarithmic transformation), FDR of ruminal contents was numerically higher, 10 – 15 %, in the steers fed diets with EM (T2) than those fed LM *bmr* sorghum (T4). No significant maturity effect was observed all the FDR parameters evaluated. The result of the analysis is again suggestive that the numerical difference in the FDR of ruminal contents between T2 and T4 may be due to the EM being more digestible as compared to the LM *bmr* sorghum. The FDR measurement is a function of both digestion and passage. At a given level of intake it is presumed that digestion and passage would be related to each other; however, at variable levels of intake digestion and passage could become more inversely related. At lower levels of intake digestion would be higher and passage lower with the inverse occurring at higher levels of intake. The lack of statistically significant effects of maturity on FDR may have resulted from the higher passage rate and lower digestion rate occurring in the animals fed at higher levels of intake. The trend line of the fractional disappearance rate of DM using data of non-linear procedure for this analysis is shown in Figure 6. The observations also obtained a smooth curvilinear trend line similar to that in Figure 5.

The FDR of ruminal contents using the data for all treatments are summarized in Table 12. Except for OM, the fractional disappearance rate for ruminal DM, NDF, NDS and ash were significantly influenced by intake effect. There is tendency for a stage of maturity effect on the FDR of NDF and OM when data were fit to a non-linear procedure. Using the model with 2 h-time FDR of rumen components were increased suggesting that FDR of ruminal components are highest from feeding time (0-time) to the first rumen evacuation time (~5-6 h after feeding) especially on the steers fed at ad libitum intake (T2 and T4). As discussed earlier, this may be due to the higher passage rate as a result of the high level of intake which corresponds to the observed numerically lower digestibility of energy and DM reported on Table 4 for the diets fed

at higher intakes. Comparing the disappearance rate of NDF between T1 and T3 it appears steers fed the silage harvested at an early maturity exhibited greater NDF digestibility than when fed the silage harvested at a later stage of maturity probably as related to their differences in lignin concentration. A curvilinear trend line was also observed when the disappearance rate of the ruminal dry matter was plotted in a graph (Figure 7). The trend line obtained in Figures 5, 6, and 7 representing the disappearance rate of DM on the three different analysis conducted were graphed in Figure 8. The graph shows that the intercept, a summative function of intake and the rumen contents 24 h after feeding, was highest for steers fed at higher levels of intake (21.61 kg), intermediate using the total data (17.90 kg), and lowest for the analysis conducted on the steers fed at 1.5X maintenance (14.87 kg). The slope seems to show similar pattern of response as with that of the intercept.

Effects of harvest maturity of *bmr* sorghum silage and level of feed intake on the pool sizes of volatile fatty acids (VFA) at the different rumen evacuation time points are shown in Table 13. The data reflects that all VFA except for isovalerate, were influenced by treatments ($P<.05$) and isovalerate showed a tendency to be influenced by treatment. The mean VFA concentration was consistently higher for the steers fed high intake (T2 and T4) than for those fed 1.5X maintenance (T1 and T3) during the first evacuation time (~ 6 h after feeding). The steers fed EM (T1 and T2) have numerically higher mean isovalerate pool size than those fed LM *bmr* sorghum (T3 and T4). The mean VFA pool size declines on subsequent rumen evacuation and varies among treatment approximately 14 - 55% of the initial concentration. Analysis of the data shows significant effect of treatment, time and interaction on the mean pool size of acetate, butyrate, and valerate. The mean pool size of propionate was significantly influenced by treatment and time but there is no interaction effect. A significant time effect and a

trend for treatment were observed for isobutyrate and isovalerate. Comparison among treatment means show that the pool sizes of acetate, propionate, butyrate, and valerate was different between the steers fed 1.5X maintenance (T1 andT3) and those fed ad libitum (T2 and T4) on the first three rumen evacuation times but the difference tend to diminish on the fourth evacuation time. The branch VFA's are not that different among treatments and diminishes after the second evacuation for isobutyrate and after the first evacuation for isovalerate. Analysis of the mean total VFA (Table 14) shows significant effects of treatment, time, and interaction. Comparison of treatment means shows the effect of intake rather than maturity on differences of means. Steers fed the ad libitum level of intake (T2 and T4) have higher total VFA than those fed 1.5X maintenance and this was consistent on all evacuation times.

The effects of harvest maturity of *bmr* sorghum and level of intake on ruminal fluid pH, ammonia and volatile fatty acids of steers are shown in Table 15. The ruminal pH of the steers is not different among the treatments and no significant differences were exhibited from the main effects. In contrast, ammonia concentration was significantly influenced by intake. Ammonia concentration was highest for the steers receiving T1 followed by T3, T2 and T4 with means of 30.89, 28.53, 25.51 and 25.04 mg/dl respectively. The total volatile fatty acid concentrations in rumen fluid of the steers were also significantly affected by intake. Specifically, concentration of acetate, propionate, butyrate and valerate were significantly different due to intake effect. Concentration of isobutyrate and isovalerate were not significantly different among treatments. When VFA is expressed as molar percentage, proportion of acetate, propionate, and valerate do not differ in steers across all treatments. The proportion of isobutyrate and isovalerate however were statistically significant due to intake effect. Molar proportions of butyrate were influenced

by maturity of silage and tended to be influenced by intake ($P \geq 0.074$). Acetate to Propionate ratio (A:P) of steers do not differ among treatments.

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Table 1. Chemical analysis of experimental brown midrib forage sorghum.[a]

Item	Brown Midrib Forage Sorghum		SE
	Early Maturity	Late Maturity	
Green Chop			
DM	24.22	26.77	0.51
CP	9.15	8.84	0.11
NDF	60.74	56.74	0.74
ADF	33.25	31.79	0.41
LIGNIN	4.11	5.12	0.25
ASH	7.16	6.18	0.45
WSC	19.19	11.05	1.47
Plant Part Ratio			
Leaf	31.26	23.36	2.07
Stem	59.80	46.24	4.18
Seed head	8.94	30.40	5.32
Silage			
DM	22.56	25.10	0.56
CP	13.76	13.01	0.55
NDF	64.05	61.91	0.59
ADF	36.92	35.97	0.46
LIGNIN	4.28	5.57	0.29
ASH	9.25	10.04	0.76
GE (Mcal/kg)	4.484	4.542	0.04
48-h IVDMD (%)	70.31	69.75	0.13

[a]Values for chemical analysis except for DM and GE are expressed in percentage of Dry Matter

Table 2. Ingredient composition and chemical analysis of experimental diets.[a]

Item	Diets[b]		SE
	Early Maturity	Late Maturity	
Composition, %			
Bmr Sorghum Silage	60.0	60.0	-
Ground Corn	32.0	32.0	-
Soybean Meal, 48%	7.5	7.5	-
Trace Mineral Salt[c]	0.5	0.5	-
Analysis, %			
CP	16.31	15.86	0.36
NDF	43.59	42.31	0.41
ADF	23.36	22.79	0.28
LIGNIN	2.70	3.48	0.17
NDICP	7.78	7.60	0.86
ADICP	5.51	5.05	0.42
ASH	7.69	8.16	0.47
Ether Extract[d]	3.59	3.59	-
Gross Energy (Mcal/kg)	4.56	4.59	0.02

[a] Dry Matter basis

[b] Mean of four periods

[c] Contain a minimum of the following: 95% NaCl, 10.35% Ca, 5% Cu, 5% Fe, 12% Mn, 12% Zn, 600 ppm of Co, 2500 ppm of I, and 600 ppm of Se

[d] Estimated using NRC tabular values

Table 3. Dry matter and nutrient intake of the steers.[a]

	Early maturity		Late maturity			*P*- value		
Items	T1	T2	T3	T4	SEM	Mat	Int	Mat x Int
BDMI, %	1.92	3.09	1.99	3.06	0.051	0.725	<.0001	0.332
DMI, kg	10.95	18.20	11.29	17.74	0.223	0.800	<.0001	0.123
CP	1.78	2.96	1.79	2.82	0.050	0.218	<.0001	0.205
NDF	4.77[b]	7.93[c]	4.78[b]	7.51[d]	0.077	0.034	<.0001	0.030
ADF	2.56	4.25	2.58	4.04	0.063	0.117	<.0001	0.119
LIGNIN	0.30[b]	0.49[c]	0.39[d]	0.62[e]	0.019	0.001	<.0001	0.488
ASH	0.84	1.40	0.93	1.45	0.095	0.511	0.0013	0.850

[a]Values are least square means are each estimated from four observations , BDMI = intake as % of BW

[b, c, d, e]Least square mean without a common superscript differ (*P*<.001)

Table 4. Effects of harvest maturity of *bmr* sorghum (early vs. late) and level of feed intake (1.5X vs. Ad libitum) on the In vivo digestible energy and DM digestibility of the steers.[a]

	Early maturity		Late maturity			*P*- value		
Items	T1	T2	T3	T4	SEM	Mat	Int	Mat x Int
DE Intake, Mcal/day	34.87	55.78	35.02	52.9	1.36	0.357	<.0001	0.311
DE, Mcal/kg	3.18	3.07	3.1	2.98	0.08	0.341	0.198	0.983
DE, %	69.83	67.25	67.49	65.99	1.56	0.19	0.154	0.983
DMD, %	63.66	63.38	61.77	60.06	1.4	0.113	0.505	0.628

[a]Values are least square means estimated from four observations periods

Table 5. Digestible energy as predicted by NRC and In vitro method.[a]

	Early maturity		Late maturity			*P*- value		
Items	T1	T2	T3	T4	SEM	Mat	Int	Mat x Int
NRC Prediction								
TDN, %	67.23		66.10		0.82	-	-	-
DE, Mcal/kg	2.99		2.94		0.04	-	-	-
DE, Mcal/kg[a]	2.95	2.87	2.90	2.83	0.025	0.158	0.024	0.872
In Vitro Prediction								
DMD, %	75.93		74.75		0.53			
DE, %	66.37		66.33		0.75	-	-	-
DE, Mcal/kg	3.01		3.04		0.04	-	-	-
DE, Mcal/kg[a]	3.01	2.94	3.04	2.97	0.045	0.533	0.150	1.00

[a]Values are least square means estimated from four periods.

Table 6. Regression analysis of DE measured from In vivo, NRC and In vitro method.[a]

	Regression Coefficient					
Item	Slope	SE	Intercept	SE	R^2	*P*
In vivo vs. NRC	0.213	0.195	2.44	0.631	0.08	0.294
In vivo vs. In vitro	0.026	0.231	3.05	0.676	0.0005	0.912
NRC vs. In vitro	1.034	0.128	0.215	0.373	0.82	0.0001

[a]Values are least square means estimated from four periods (n=16)

Table 7. Correlation analysis of DE measured from In vivo, NRC and In vitro method.[a]

Variables	In vivo	NRC	In vitro
In vivo	1.0000	-0.2023 0.9407	-0.2145 0.4251
NRC	-0.2023 0.9407	1.0000	0.8603 <.0001
In Vitro	-0.2145 0.4251	0.8603 <.0001	1.0000

[a]Values are least square means estimated from observations at four periods.

Table 8. Effect of harvest maturity and level of intake on the mean pool sizes of rumen contents at evacuation time points

Items	Early Maturity		Late Maturity		SE	Trt	Time	Trt x Time
	T1	T2	T3	T4				
	---------- kg ----------							
Dry Matter								
6 h	8.86	12.63	10.01	12.04	0.395	<.0001	<.0001	0.078
12 h	7.08	9.46	8.09	9.65				
18 h	5.81	7.22	6.23	7.86				
24 h	4.26	5.46	4.35	5.24				
Organic Matter								
6 h	7.80	11.44	8.79	10.67	0.380	<.0001	<.0001	0.074
12 h	6.23	8.35	7.22	8.63				
18 h	5.11	6.46	5.53	7.00				
24 h	3.70	4.82	3.83	4.63				
NDF								
6 h	5.62	7.81	6.39	7.49	0.262	<.0001	<.0001	0.256
12 h	4.67	6.26	5.37	6.41				
18 h	3.89	4.87	4.34	5.35				
24 h	2.96	3.78	3.04	3.66				
NDS								
6 h	3.24	4.82	3.62	4.56	0.174	<.0001	<.0001	0.042
12 h	2.41	3.20	2.73	3.24				
18 h	1.92	2.36	1.89	2.51				
24 h	1.30	1.68	1.31	1.57				
Ash								
6 h	1.06	1.19	1.21	1.37	0.075	0.01	<.0001	0.610
12 h	0.85	1.11	0.88	1.02				
18 h	0.70	0.77	0.70	0.86				
24 h	0.56	0.64	0.53	0.60				

[a]Values are means estimated from observations at four periods over four evacuation times.
[b, c, d] Least square means without a common superscript differ ($P< 0.05$).

Table 9. Effect of harvest maturity of *bmr* sorghum (early vs. late) and level of feed intake (1.5X vs. adlib) on the fractional disappearance rate of ruminal dry matter, NDF, NDS, ash and organic matter.[a]

	Early maturity		Late maturity			*P*- value		
Items	T1	T2	T3	T4	SEM	Mat	Int	Mat x Int
Evacuation Time Model	----------%/h----------							
Non-Linear								
Dry Matter	3.80	4.74	4.32	4.14	0.267	0.889	0.204	0.082
NDF	3.32	4.07	3.76	3.51	0.310	0.847	0.449	0.161
NDS	4.71	5.98	5.54	5.44	0.316	0.649	0.111	0.074
ASH	3.27	3.67	4.48	4.20	0.644	0.227	0.928	0.615
OM	3.87	4.87	4.30	4.13	0.252	0.543	0.15	0.059
Log transformed								
Dry Matter	1.70	2.04	1.97	1.92	0.125	0.554	0.303	0.163
NDF	1.48	1.79	1.74	1.66	0.144	0.683	0.478	0.218
NDS	2.11	2.49	2.44	2.43	0.123	0.297	0.183	0.160
ASH	1.42	1.67	1.89	1.83	0.219	0.201	0.687	0.514
OM	1.73	2.08	1.98	1.92	0.126	0.744	0.302	0.162

[a]Values are least square means estimated from observations at four periods over four sampling times; model contains data collected during rumen evacuation time points.

Table 10. Effect of harvest maturity of *bmr* sorghum (early vs. late) on the fractional disappearance rate of ruminal dry matter, NDF, NDS, ash and organic matter of the steers fed at 1.5X maintenance.[a]

	Early maturity	Late maturity		*P*- value
Items	T1	T3	SEM	Maturity
With 2h-Time Model	----------%/h----------			
Non-Linear				
Dry Matter	6.63	5.30	0.278	0.043
NDF	4.52	3.57	0.185	0.036
NDS	11.30	9.05	0.866	0.163
ASH	4.51	4.14	0.375	0.538
OM	8.01	5.61	0.453	0.033
Log transformed				
Dry Matter	2.21	2.27	0.034	0.255
NDF	1.72	1.72	0.026	0.917
NDS	2.97	3.15	0.088	0.228
ASH	1.66	1.91	0.058	0.053
OM	2.39	2.38	0.086	0.879

[a]Values are least square means estimated from observations at four periods over four sampling times; model includes predicted 2 h time.

Table 11. Effect of harvest maturity of *bmr* sorghum (early vs. late) on the fractional disappearance rate of ruminal dry matter, NDF, NDS, ash and organic matter of the steers fed ad libitum for 5 h.[a]

	Early maturity	Late maturity		*P*- value
Items	T2	T4	SEM	Maturity
With 2h-Time Model	----------%/h----------			
Non-Linear				
Dry Matter	7.86	7.09	0.611	0.441
NDF	5.42	4.64	0.492	0.344
NDS	13.08	12.70	1.109	0.824
ASH	5.68	5.60	0.348	0.889
OM	7.84	6.84	0.883	0.484
Log transformed				
Dry Matter	2.64	2.49	0.205	0.647
NDF	2.10	1.90	0.189	0.519
NDS	3.46	3.40	0.205	0.853
ASH	2.12	2.13	0.192	0.983
OM	2.64	2.45	0.242	0.629

[a]Values are least square means estimated from observations at four periods over four sampling times; model includes predicted 2 h time.

Table 12. Effect of harvest maturity of *bmr* sorghum (early vs. late) and level of feed intake (1.5X vs. adlib) on the fractional disappearance rate of ruminal dry matter, NDF, NDS, ash and organic matter.[a]

	Early maturity		Late maturity			*P*- value		
Items	T1	T2	T3	T4	SEM	Mat	Int	Mat x Int
With 2h-Time Model	----------%/h----------							
Non-Linear								
Dry Matter	6.15	7.86	5.78	7.09	0.399	0.205	0.009	0.638
NDF	4.25	5.42	3.83	4.64	0.279	0.076	0.012	0.542
NDS	10.26	13.08	10.09	12.70	0.745	0.725	0.011	0.893
ASH	4.11	5.68	4.54	5.60	0.413	0.686	0.019	0.568
OM	7.29	7.84	6.33	6.84	0.465	0.08	0.299	0.973
Log transformed								
Dry Matter	2.21	2.64	2.27	2.49	0.108	0.730	0.024	0.360
NDF	1.72	2.10	1.72	1.90	0.104	0.393	0.035	0.374
NDS	2.97	3.46	3.16	3.40	0.116	0.594	0.02	0.327
ASH	1.67	2.12	1.91	2.13	0.144	0.402	0.056	0.424
OM	2.40	2.64	2.38	2.45	0.114	0.407	0.209	0.500

[a]Values are least square means estimated from observations at four periods over four sampling times; model includes predicted 2 h time.

Table 13. Effects of harvest maturity and level of intake on mean pool sizes of VFA at evacuation time points.[a]

	Early Maturity		Late Maturity					
Items	T1	T2	T3	T4	SE	Trt	Time	Trt x Time
	----------*M*----------							
Acetate								
6 h	3.91[b]	5.50[c]	3.72[b]	5.20[c]	0.17	<.0001	<.0001	0.0172
12 h	2.90[b]	4.58[c]	3.22[b]	4.24[c]				
18 h	2.43[b]	3.34[c]	2.31[b]	3.19[c]				
24 h	1.45[b]	2.03[c]	1.42[b]	1.87[bc]				
Propionate								
6 h	1.27[b]	1.79[c]	1.18[b]	1.76[c]	0.08	<.0001	<.0001	0.1093
12 h	0.81[b]	1.29[c]	0.89[b]	1.30[c]				
18 h	0.57[b]	0.84[c]	0.56[b]	0.88[c]				
24 h	0.31	0.47	0.31	0.47				
Isobutyrate								
6 h	0.07[bc]	0.10[b]	0.06[c]	0.08[bc]	0.01	0.092	<.0001	0.7509
12 h	0.06[b]	0.10[c]	0.08[bc]	0.08[bc]				
18 h	0.04	0.06	0.06	0.05				
24 h	0.04	0.04	0.05	0.04				
Butyrate								
6 h	0.81[b]	1.29[c]	0.90[b]	1.28[c]	0.04	<.0001	<.0001	0.0005
12 h	0.54[b]	0.98[d]	0.71[c]	0.99[d]				
18 h	0.34[b]	0.56[c]	0.37[b]	0.57[c]				
24 h	0.17[b]	0.29[bc]	0.22[bc]	0.31[c]				
Isovalerate								
6 h	0.11[bc]	0.11[c]	0.08[bd]	0.07[d]	0.01	0.0854	0.04	0.5270
12 h	0.08	0.10	0.08	0.09				
18 h	0.09	0.10	0.08	0.08				
24 h	0.07	0.07	0.06	0.08				
Valerate								
6 h	0.13[b]	0.20[d]	0.12[b]	0.16[c]	0.01	<.0001	<.0001	0.0017
12 h	0.07[b]	0.11[c]	0.08[b]	0.11[c]				
18 h	0.04[b]	0.07[c]	0.04[b]	0.07[c]				
24 h	0.02	0.03	0.02	0.03				

[a]Values are means estimated from observations at four periods over four sampling times.

[b, c, d] Least square mean without a common superscript differ ($P < 0.05$).

Table 14. Effects of harvest maturity and level of intake on mean total pool sizes of VFA at evacuation time points[a]

	Early Maturity		Late Maturity					
Items	T1	T2	T3	T4	SE	Trt	Time	Trt x Time
	----------mM----------							
Total								
6 h	6.25[b]	8.93[c]	6.03[b]	8.52[c]	0.26	<.0001	<.0001	0.0030
12 h	4.44[b]	7.12[c]	5.02[b]	6.77[c]				
18 h	3.48[b]	4.93[c]	3.40[b]	4.81[c]				
24 h	2.04[b]	2.92[c]	2.07[b]	2.81[c]				

[a]Values are means estimated from observations at four periods over four sampling times.
[b, c, d] Least square mean without a common superscript differ ($P < 0.05$).

Table 15. Effects of harvest maturity of *bmr* sorghum (early vs. late) and level of feed intake (1.5X vs. Adlib) on ruminal pH, ammonia and volatile fatty acids in steers.[a]

	Early maturity		Late maturity			*P*- value		
Items	T1	T2	T3	T4	SEM	Mat	Int	Mat x Int
pH	6.53	6.55	6.64	6.60	0.06	0.234	0.818	0.661
Ammonia, mg/dl	30.89	25.51	28.53	25.04	2.01	0.508	0.07	0.656
VFA, m*M*								
Acetate	52.26	59.03	49.77	55.92	1.52	0.096	0.004	0.835
Propionate	14.10	16.56	13.72	16.73	0.79	0.900	0.014	0.737
Isobutyrate	1.05	1.11	1.11	0.96	0.05	0.343	0.371	0.074
Butyrate	8.86	11.55	9.88	11.76	0.39	0.166	0.001	0.340
Isovalerate	1.11	1.07	1.05	0.95	0.04	0.091	0.167	0.528
Valerate	1.23	1.49	1.20	1.35	0.05	0.175	0.007	0.308
Total	78.60	90.81	76.73	87.68	2.18	0.294	0.002	0.783
VFA, % mol								
Acetate	67.2	65.8	65.68	64.41	0.739	0.093	0.125	0.948
Propionate	17.47	17.82	17.28	18.6	0.591	0.633	0.210	0.448
Isobutyrate	1.41	1.24	1.54	1.16	0.072	0.706	0.009	0.198
Butyrate	10.91	12.26	12.45	13.11	0.465	0.042	0.074	0.492
Isovalerate	1.53	1.29	1.56	1.22	0.083	0.828	0.013	0.552
Valerate	1.48	1.55	1.48	1.49	0.033	0.363	0.283	0.335
A:P Ratio	3.94	3.76	3.92	3.55	0.155	0.506	0.123	0.554

[a]Values are least square means estimated from observations at four periods over four sampling times.

Figure 1. Relationship between NRC DE values and In vivo DE as fit a linear regression equation.

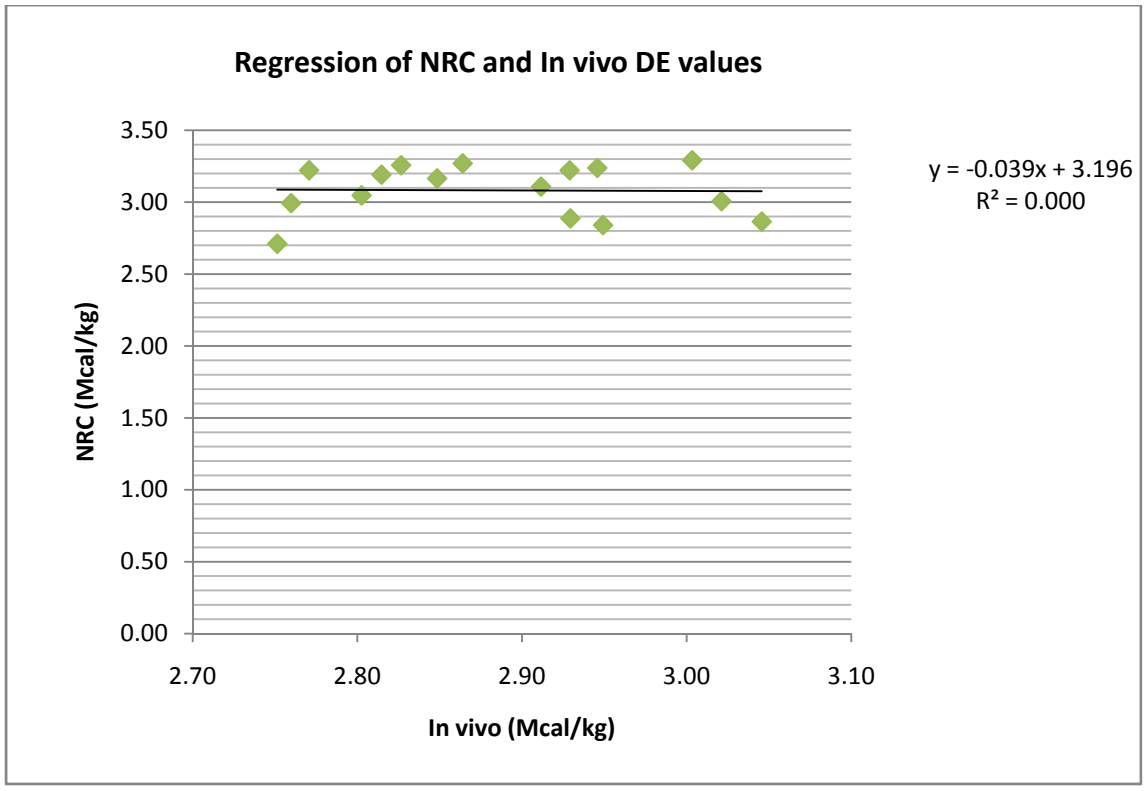


Figure 2. Relationship between In vivo DE values and In vivo DE as fit a linear regression equation.

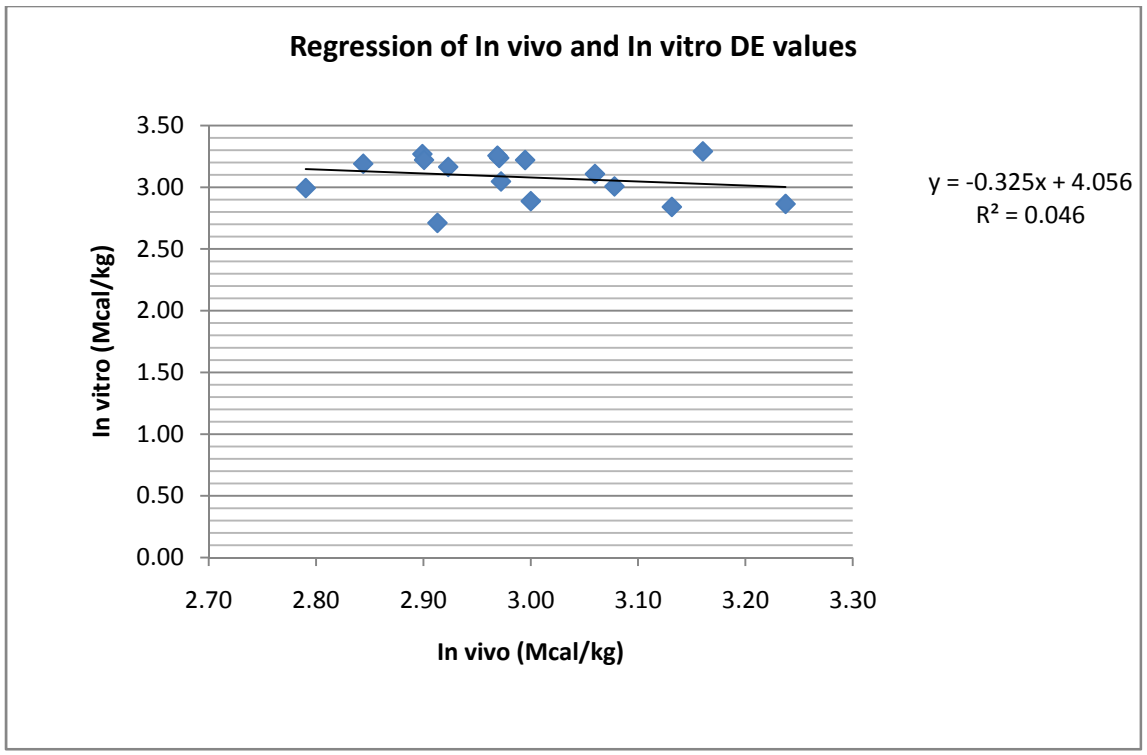


Figure 3. Relationship between NRC DE values and In vitro DE as fit a linear regression equation.

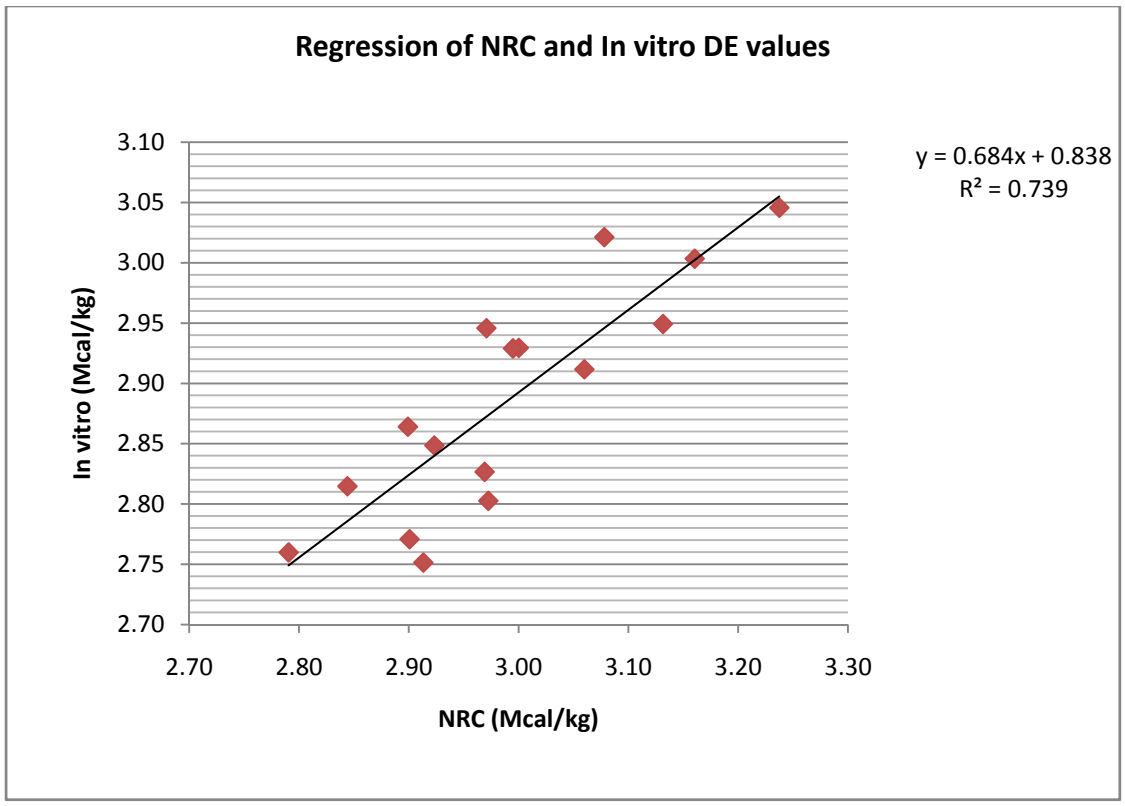


Figure 4. Effect of harvest maturity of *bmr* sorghum (early vs. late) and level of feed intake (1.5X vs. adlib) on the fractional disappearance rate of ruminal DM at evacuation time points.

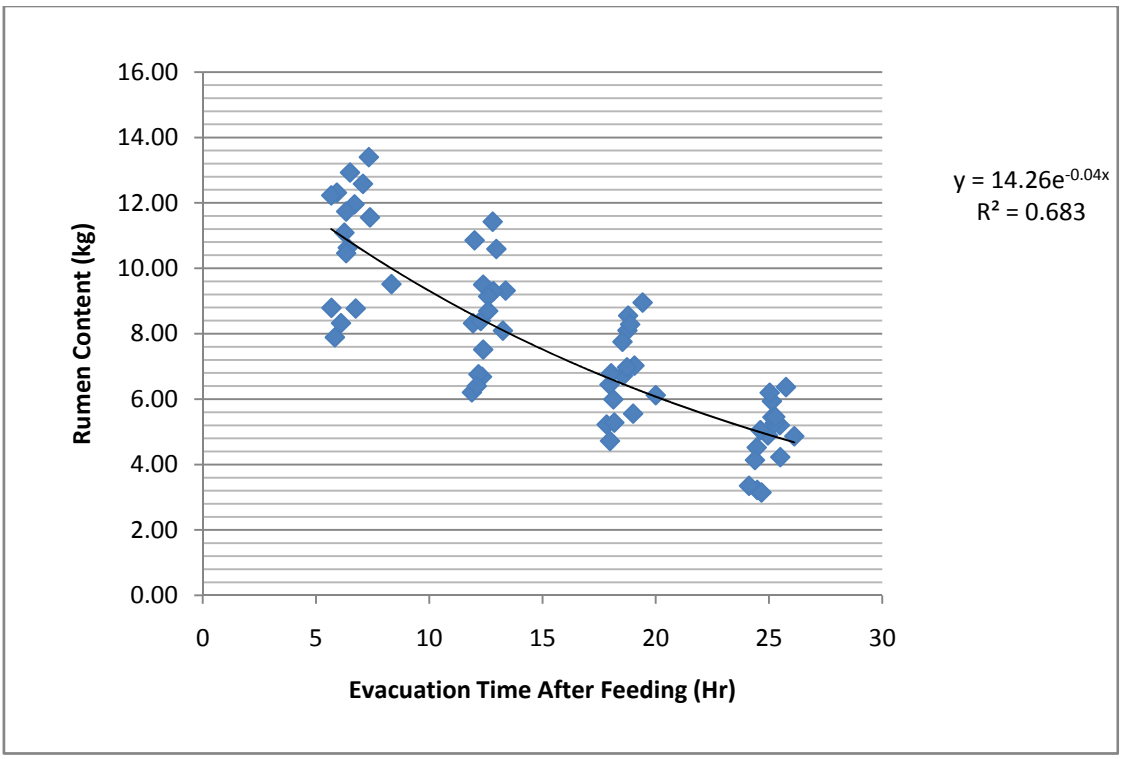


Figure 5. Effect of harvest maturity of bmr sorghum (early vs. late) on the fractional disappearance rate of ruminal DM of steers fed 1.5X maintenance using the model with predicted 2 h time.

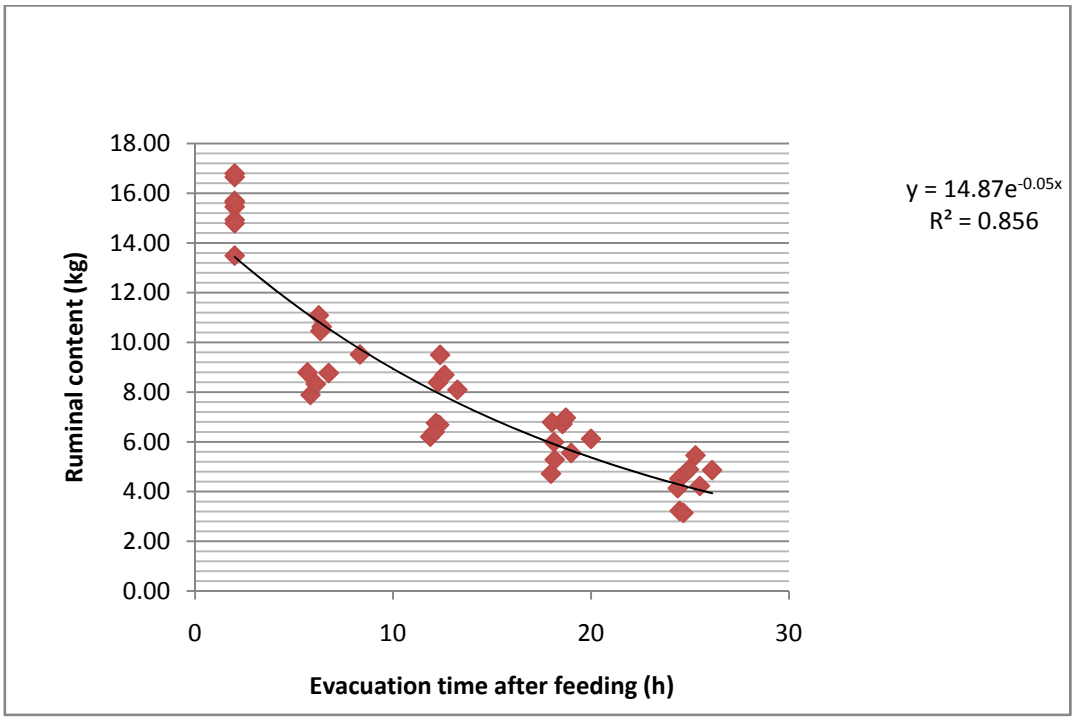


Figure 6. Effect of harvest maturity of bmr sorghum (early vs. late) on the fractional rate of disappearance of ruminal DM of steers fed ad libitum for 5 h using the model with predicted 2 h time.

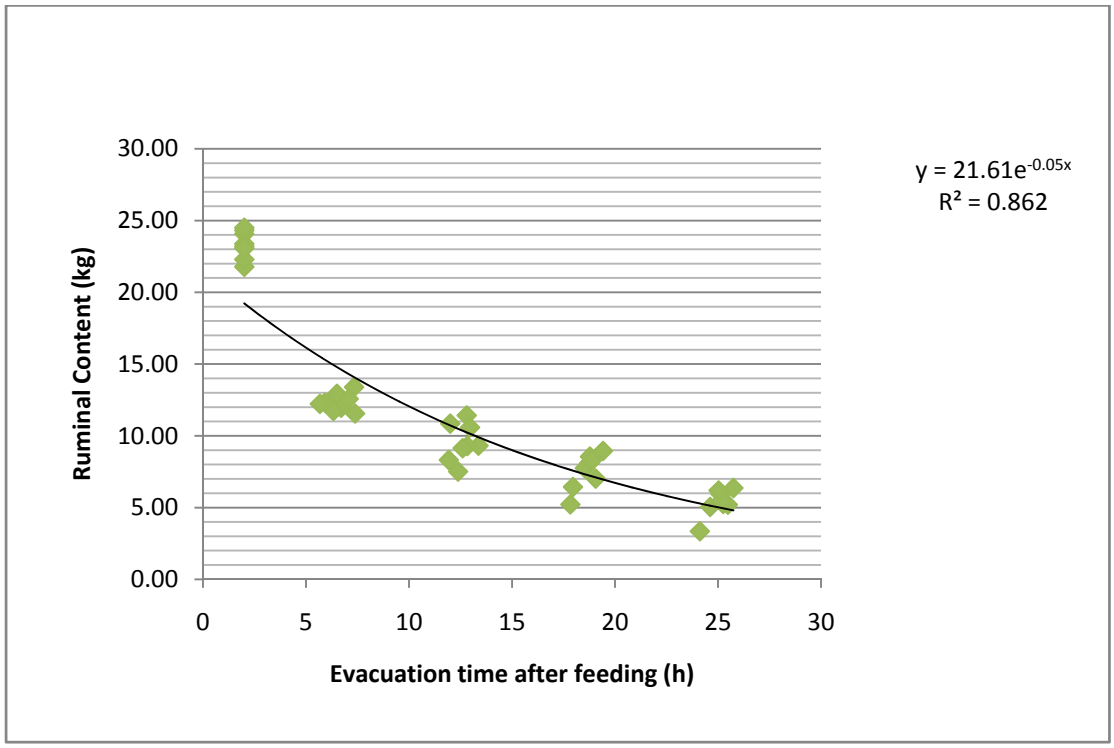


Figure 7. Effect of harvest maturity of *bmr* sorghum (early vs. late) and level of feed intake (1.5X vs. adlib) on the fractional disappearance rate of ruminal DM using the model with predicted 2 h time.

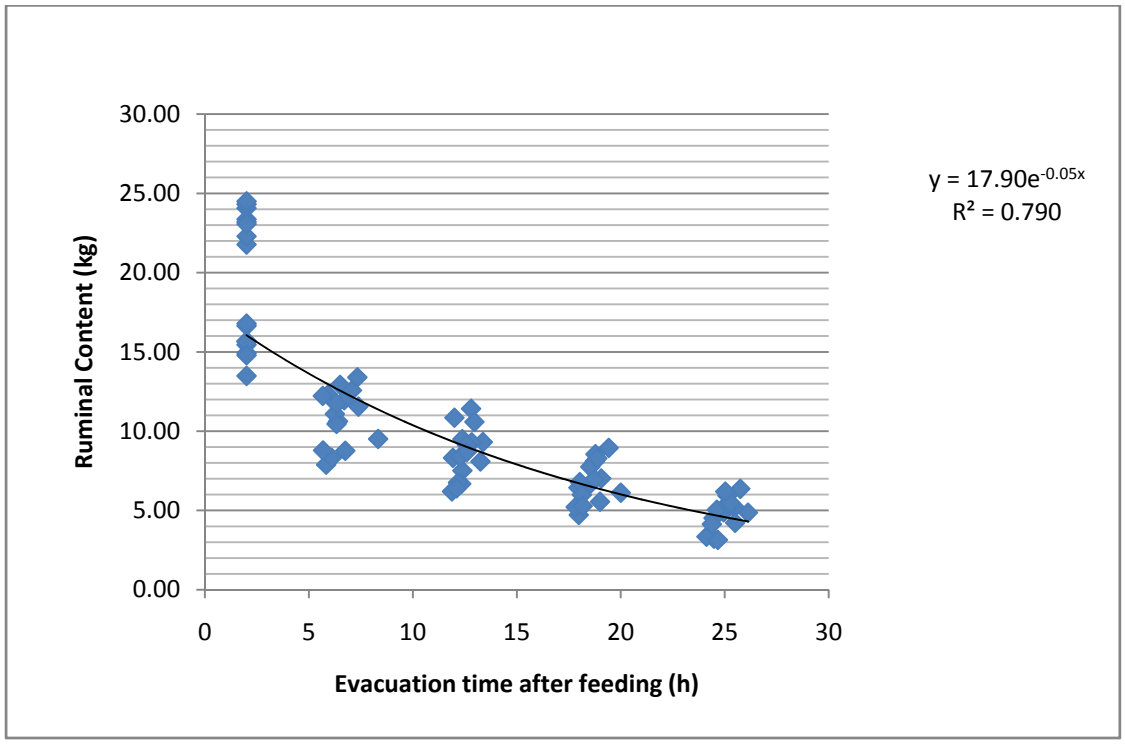
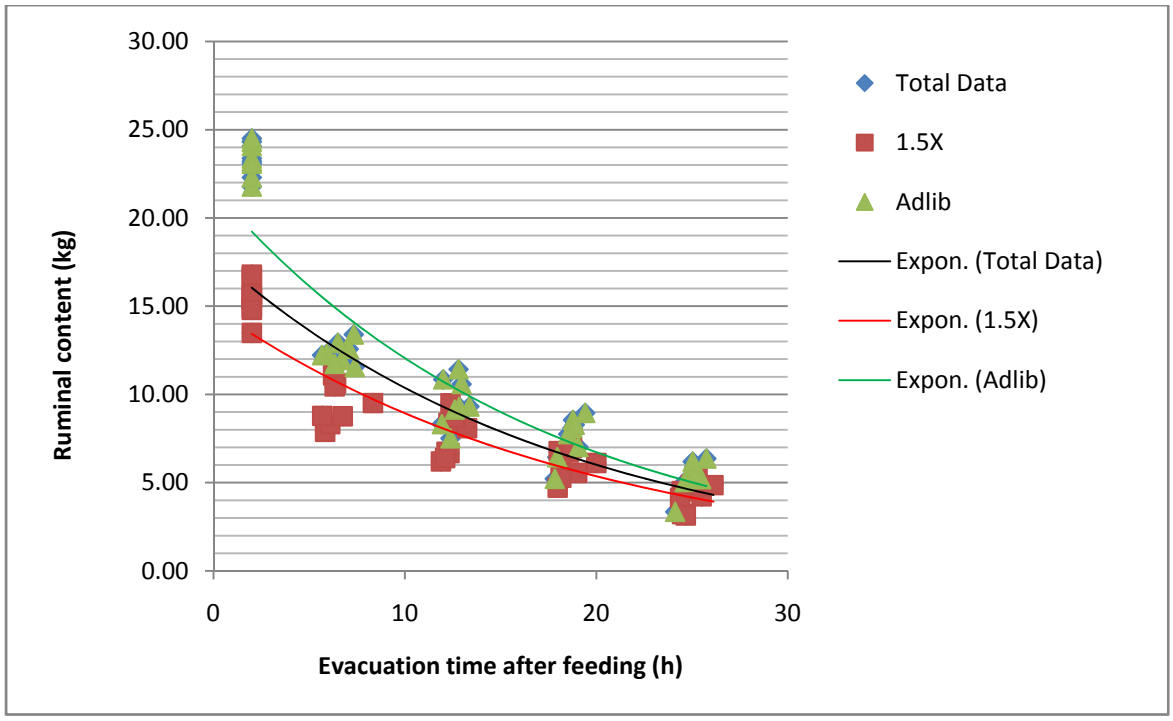


Figure 8. Graph showing the different trend lines of the fractional disappearance rate of ruminal DM measured using the model with predicted 2 h time.



CHAPTER 3

CONCLUSIONS

Understanding the relationship of forage maturity and level of intake provides valuable information on how the animal responds to specific feeding conditions. The *bmr* sorghum harvested at an early head stage of maturity differed from that harvested in the later milk to soft dough stage of maturity in NDF and lignin concentration. These components of forage are of importance to dairy production because of their direct negative impact on voluntary dry matter intake of ruminants. Both NDF and lignin are negatively related to the caloric content of the forage and responsible for the physical fill in the reticulorumen of cattle. Some feed testing laboratories utilize these values with negative coefficients in prediction equations to estimate the energy and feeding value of forage.

This research indicates that brown midrib sorghum contain highly digestible NDF and a lower concentration of lignin which is consistent to the findings of previous researchers. In accordance with its chemical composition a better quality of silage is obtained when *bmr* sorghum is harvested at an early head and have the potential for utility in dairy because of high digestibility of NDF, DM, DE and low lignin content. The actual in vivo determination of DE indicates that stage of maturity and and level of intake do not negatively impact *bmr* sorghum as much as non *bmr* sorghum varieties.

Energy prediction is an important tool in assessing the caloric value of forages. Although the in vitro DE method did not appear to correlate with in vivo values, it may still be useful because it is simple, easy, and highly repeatable and it predicts NRC values well. More research

is necessary to modify the procedure in order to improve its accuracy and precision for predicting DE in forage. Further testing of the in vitro DE method is needed with a greater number and variety of samples that had already been evaluated for actual in vivo DE content. Modifications for improved accuracy and precision may be done by adjusting the incubation time and other related factors such as ratio of buffer to rumen fluid and amount of substrate.

Finally, the data obtained in the rumen evacuation in this study provides a better view as to when would be the best time to conduct rumen evacuation to estimate fractional disappearance rate of rumen contents. In addition, data obtained regarding fermentation end products suggest some feasibility to utilize such in predicting intake in ruminant animals.