IMPACT OF RUMEN FLUID SOURCE AS INOCULANT TAKEN FROM BEEF COWS OF VARYING PRODUCTIVITY AND AT DIFFERENT TIMES POST-PARTUM ON IN VITRO DIGESTION ACTIVITY

by

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(Under the Direction of Mark A. Froetschel)

ABSTRACT

An experiment was conducted to determine variation in digestion activity in vitro using rumen fluid from 10 multiparous Angus cows from a 104 cow-calf pair herd. Cows were selected as having relatively high or low productivity as assessed by their life time weaning weight ratio taken from AIMS records (American Angus Association, Saint Joseph, MO 64506). There was a productivity effect on certain VFAs, like acetate: propionate concentrations both in vivo ($P < 0.1$) and their 24 hour production rate in vitro ($P < 0.1$). There was, however, no productivity effect ($P > 0.1$) on in vitro fiber digestion activity. The interaction effect of productivity and season influenced ($P<0.1$) more digestibility measures in vitro than any interaction effects. Although there are some inferences that suggest these results are related to the diet fed and the intake of the cows, the cause and effect nature of this relationship requires further investigation.

INDEX WORDS: In Vitro Dry Matter Digestibility, In Vitro Neutral Detergent Fiber Digestibility, Indigestible Neutral Detergent Fiber, Weaning Weight Ratio.

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DEDICATION

I dedicate this thesis to my loving and supporting family who has always been there for me as well as to my incredibly charitable uncle Charlie; thank you very much for having given a nephew from Africa the chance to embark upon such an incredible journey and realize a dream. Last but definitely not least, I thank You God for without You, none of these would have even been imaginable!

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CHAPTER ONE

INTRODUCTION

Productivity of cows in a cow-calf herd can be highly variable and is dependent on both genetic and environmental factors. This has spurred interest in knowing the potential causes responsible for this variability for the purpose of using this information to improve overall productivity of the herd.

Calf weaning weight is one of the most important production traits in a cow-calf herd. It is dependent upon multiple factors and has been the subject of several research investigations. One of the most important determining factors of calf weaning weight is milk production of the cow. Marston et al. (1992) found a positive correlation between the cow's genetic potential for milk production, assessed by its expected progeny difference (EPD) for milk production, or its actual milk production and calf weaning weight. It is well recognized in lactating dairy cattle that milk production is a function of the secretory activity of the mammary gland and the animal's nutrient intake required to support lactogenesis.

Geneticists have used a number of variables to refine their assessment of breeding values involved in determining calf weaning weights. Milk production is influenced by age and parity of the dam and these are major factors that are used to adjust the cow's breeding

values for calf weaning weight. Parity of the cow was significantly related to ($P < 0.1$) weaning weight of Angus calves. Age of dam has a positive influence on calf weaning weight until cows reach 7 to 9 years of age (Pell and Thayne, 1978). Greater calf weights were related to the dams consuming more energy (g/Mcal) for Angus, Hereford, Red Poll, or Maine Anjou F1 cows but not in Chianina or Gelbvieh (Jenkins et al., 1991) cows. Nevertheless, inconsistencies still persist in certain breeds, but for the most part, caloric intake of the cow during lactation is the major genetic factor that drives calf weaning weight.

Animal productivity is a combination of genetics as well as management. Within management practices, providing nutrition is one of the more highly important determinants of animal productivity. Even though major improvements have been accomplished in the field of nutrition, in the last 60 years as depicted in the NRC publications, most of these improvements are related to nutrient levels in feeds and forages and animal nutrient requirements. Relatively, much less research has been dedicated to understanding applications to improve microbial digestion efficiency to better ruminant productivity (Sejrsen et al., 2004). The in vitro method has high correlation with forage digestibility in vivo and has proved pivotal in improving productivity. However, little work has been done in using in vitro digestibility data as a potential indicator of animal digestion efficiency and not just in evaluating feed digestibility.

This research was conducted with the objective of studying the nature of the interactions between microbial digestion efficiency using rumen fluid obtained from a group of ten grazing multiparous Angus cows in vitro over three different seasons in one calving cycle; namely spring, summer and fall with their life time weaning weight ratio.

CHAPTER TWO

LITERATURE REVIEW

Variation in digestion studies in vivo:

Dry Matter Digestion:

Ruminant food producing animals provide a substantial amount of high quality protein nutrition for humans. According to a 2007 report by the food and agricultural organization, they account for "all of the world wide milk production and a third of its meat production" (Kebreab et al., 2008). Ruminants are even more important as food producing animals because of their ability to utilize forages and high fiber byproduct feeds because of the rumen microbial digestion activity. This has driven a lot of interest in research towards improving ruminant productivity of forage based diets. Utilization of high fiber forage diets is limited mainly by rumen digestibility. Owing to the complexity of the ruminant digestive system, it is rather difficult to accurately account for rumen digestion in vivo. Ruminant nutritionists have therefore come up with ways of predicting in vivo digestion via rumen kinetic parameters and mathematical models (Huhtanen et. al, 2008).

One of the initial and more widely referenced equations used to determine the extent of rumen degradability is written as: (kd/(kd+kp))PD (Waldo et al., 1972), where kd is the ruminal rate of degradability, kp is the rate of passage and PD is the potentially degradable portion in the feed. This formula has undergone slight adjustments over the years but is still of great importance in ruminant nutrition (Poppi et al., 1981). It is an

ongoing task taken by ruminant nutritionists to improve the accuracy of these predictive equations as almost half of the publications on mathematical ruminant models in the JAS database were published in the last decade (Kebreab et al., 2008). Accompanying procedures like the use of cannulae, internal and external markers have been developed to make this process more reliable but the whole task is subject to multiple sources of variation and is very laborious and time consuming. These hence enabled researchers to account for variations in ruminant DM digestion.

The physiological state of the animal itself could also have an impact on its efficiency at digesting DM. Doreau et al. (1990) found that the digestibilities of DM, OM and CF of the same experimental diet varied before and after calving for dairy cows. They found that rumen fluid sampled from cows three weeks prior to calving gave more DM and OM digestion activity on the same substrate as a pose to rumen fluid taken from the same cows three weeks after calving. This is probably due to the changes in the physiological needs of the newly nursing calf from pregnancy. The stage of maturity for annuals and perennials, as well as the re-growth after last harvest for the latter will also have an impact on their digestibility. There was a linear decrease in apparent digestibility of DM by sheep with an increase ($P < 0.1$) in re-growth age of *Echinocloa* specie rice grass hay (Lima et al., 2007).

Differences in in-vivo dry matter digestibility were also accounted for among different species of ruminant animals. Steers had a greater ($P < 0.1$) DM digestion than deer while sheep and goats had intermediate and statistically insignificant differences for the same

trait (Huston et al., 1986). Also, buffaloes were observed to have a higher organic matter requirement for production of microbial biomass than cows (Sadhana et al., 1992; Puppo et al., 1993). Differences were also observed between not just cattle and other ruminants, but within cattle species as well. In a experiment with six Hereford (*Bos Taurus*) and six Brahman (*Bos indicus*) steers fed Pangola grass (*Digitaria decumbens*) and spear grass (*Heteropogon contortus*) hay ad lib either by itself or with rumen degradable , sulfur and minerals, it was found that the unsupplemented Pangola grass was digested more rapidly in the Brahman steers in situ, resulting in accompanying rumen ammonia concentrations 24 mg/ml higher than that of their Hereford counterparts, 40 vs. 16 mg/l (Hunter et al., 1984). It was also noted that while the digestion rate of the cell-wall-constituent fraction of the unsupplemented spear grass was more rapid in the Brahma steer, supplementation increased Hereford digestion rates. Differences in dry matter digestibility in vivo among animals in the same herd have however not been studied as widely.

Fiber Digestion:

One of the indigestible fractions of the feed or forage DM is its fiber component. Since indigestible fiber components are inversely correlated with digestible components of a diet, it is possible to predict digestion by using fiber components of the feed. Lima et al. (2008) found an inverse relationship between DM digestibility and ADF content. Utilizing fiber components to predict digestibility may lend itself to error as some fiber is digestible. Huhtanen et al. (2008) looked at the use of mathematical models to predict digestibility of the NDF portion of DM and noted that these could have flaws as some models tend to over predict in vivo NDF digestibility, while others under predict it.

These researchers also looked at the use of gas production kinetic models to predict in vivo NDF digestibility and found that these predicted it with precision and accuracy as the models took into consideration the partition of NDF into indigestible and partially digestible portions and also used passage models (Huhtanen et al., 2008). Differences in fiber digestion rates in vivo are also dependent on methods used to quantify the fiber constituent of the feed. Lignin is a structural constituent of fiber that is often related to its indigestibility. Jung and Allen (1996) reported that they found that the lignin content of thirty six forage samples including C3 and C4 grasses and legumes was dependent upon the method of analysis. These researchers found that lignin was always higher when analyzed using sulfuric acid detergent method as compared to the Klason method even though both assay results were positively correlated with each other ($P<0.01$).

It is a practical approach in ruminant nutrition to partition fiber into potentially digestible and indigestible fractions. The indigestible fractions of fiber serve a role as markers and are indispensable to our understanding of the rather complex ruminal digestive system. Lignin is a component of the indigestible fraction that was once used as an indigestible internal marker. A linear decrease in fiber digestibility was observed with an increase in lignin NDF concentration, determined either by sulfuric acid hydrolysis or permanganate oxidation; of 145 forage samples from tropical South America and Florida (Traxler et al., 1998). They also found evidence of many quadratic relationships among NDF, ADF and lignification as determined by the permanganate method among legumes and C3 grasses but not for C4 grasses. Elam and Davis. (1961) found that despite a low recovery rate which could limit its use as a marker, fecal lignin was not significantly influenced by its

retention time and its dietary concentration, which was relatively uniform as compared to chromic oxide in a study where they looked at the excretion pattern of six Hereford heifers supplemented with a total mixed ration ad lib from feces collected at 3 hour intervals for 48 hours of digestion and four Hereford steers fed the same ration twice a day (AM vs. PM). The use of radioactive Carbon 14 fraction of fiber for use in ruminant nutrition is one such innovation developed in the early half of the 20th century (Alexander, 1965). Smith (1989) revised the employment of radioactive intrinsic C14 and rare earth elements to label neutral detergent fiber for the estimation of passage and digestion and suggested that this method could provide an opportunity to better understand ruminal methanogenesis as well as lag time and microbial attachment in relation to forage quality and utilization.

There is a multitude of published research on the effects of various dietary treatments on in vivo digestibility. Nevertheless, there are very few accounts of work done on the measure of fiber digestibility in vivo of animals in the same herd subject to the same nutritional regime to determine the extent of animal variation in rumen microbial digestion activity. It is possible that ruminants have variation in microbial digestion activity as related to differences in the animal's impact on the symbiotic relationship on rumen microbial digestive function.

Protein Digestion:

Protein digestion in the rumen is quite difficult to account for because as dietary protein is being fermented it is being resynthesized into microbial protein. As much as 40 to 80%

of the protein passing out of the rumen and reaching the site of absorption in the duodenum is of microbial origin and this range is dependent on the degradability of the feed protein being supplemented (Owens and Bergen, 1983). An accumulation of amino acid nitrogen was observed in the rumen after feeding indicating that AA uptake by rumen microflora can be the limiting factor for protein degradation in the rumen (Bach et al., 2005). Maeng et al. (1997) observed that ruminal ammonia production exceeds its absorption rate by microbes owing to lack of readily available energy, resulting in increased ammonia absorption through epithelial cells of the rumen. Ammonia absorbed from the rumen is converted to urea by the liver and then is either recycled back to the rumen as a component of saliva or back across the rumen wall or excreted as urine. Despite some inefficiency in converting dietary protein to microbial protein ruminants are endowed with the ability of transforming lower quality proteins and non protein nitrogen into microbial protein that has a protein quality similar to soybean meal (Merchen and Titgemeyer, 1992) the ruminant can then digest to meet its dietary protein needs. Depending upon the predominant species present, microbial protein is highly digestible and can have varying digestibility values. These can range anywhere from 62% for *Bacteriodes succinogenes* to 86% for *Ruminococcus flavefaciens* (Bergen et al. 1967). Whether or not a change in diet affects microbial protein has been polarizing. Warner (1965) found that changes in diet can exert a modifying effect on rumen microbiota. It was, however, noted that a change in the diet did not have an effect on either microbial protein or composition of the bulk of the amino acids as affected by ration (Bergen et al. 1968). They found this in a 4 X 4 latin square design study they conducted on 4 Targee wethers where they fed four experimental diets in 15 days at isocaloric levels once daily

(6% of their metabolic body weight) and analyzed microbial protein in vitro using the methods of Akeson and Stahman (Akeson and Stahman, 1964), and analyzed the results with standard analysis of variance procedures.

Digestible Energy Utilization:

Ruminal energy availability and fermentation is a driving factor behind microbial growth and protein synthesis. Energy availability is related to the digestible energy content of the diet. Conventionally, digestible energy is calculated as the difference between the gross energy of the feed and the gross energy of the feces. In regards to animal feeding standards, feed energy can be described as metabolizable energy or net energy. These systems are more accurate in regards to animal feeding and refer to the energy that is either absorbed or utilized for maintenance or productive purposes. Metabolic energy accounts for gaseous and urinary gross energy losses and net energy accounts for losses of heat gross energy. Even though these other systems are more accurate for feeding fecal energy loss used in estimating digestible energy represents the greatest individual gross energy loss factor (Garrett and Johnson, 1983). Ruminal energetics, as is the case with other parameters of rumen digestion activity, is a challenging index to accurately determine as most often it is predicted for practical feeding as based on the negative relationship with fiber and energy concentration. Although digestible energy is less accurate in meeting animal requirements for maintenance and productive purposes it constitutes the greatest proportion of feed energy for the animal. Characteristics of the feed and/or forage have even more influence on digestible energy than animal factors and are the basis for predictive equations used in feed and forage evaluation. The relationship

between energy and nitrogen supply to ruminants is important in improving production. However, this has been a point of disagreement in the findings of researchers. Herrera-Saldana et al. (1990) found that increased microbial flow could be obtained from synchronizing feed energy and nitrogen supply. On the other hand, no response was found from such a experiment (Casper et al., 1990; Henning et al., 1993). This finding is shared by Valkeners et al. (2004) who found Nitrogen retention was not affected by imbalance at 0, 12 and 24 hours via altering the feed pattern in six double-muscled Belgian blue bulls owing to rumen nitrogen recycling that plays a big role in maintaining microflora under dire conditions as well.

Variation in digestion studies in vitro:

Dry Matter Digestion:

Various techniques have been developed to reliably simulate rumen digestion activity in vitro. Early on it was recognized that the value of conducting in vitro digestion studies as the results of in vitro digestibility experiments were qualitatively identical with in vivo results obtained (Heuter et al., 1957). There has been some refinement in in vitro techniques to improve their reliability. Mabjeesh et al. (1999) found that prediction of in vitro dry matter digestibility with multiple samples in polyester bags in one batch culture using the Daisy II method can be achieved with a relatively little variation than the traditional Tilley and Terry method. Utilization of polyester bags to evaluate in vitro digestibility has made it easier for researchers to evaluate the digestibility of multiple samples of ruminant feeds. However, the polyester bag approach does not allow one to quantify changes that occur in the supernatant phase. Assessment of soluble protein in

the supernatant phases during the Tilley and Terry procedure may allow researchers to estimate rumen microbial efficiency or in vitro digestion activity. No variation in cellulose digestion in vitro was observed using rumen fluid from monozygotic twin steers after rumen microbial stabilization following an adaptation season of approximately 56 days (Church and Petersen, 1960).

Since the rumen fluid as a source of microbial inoculant is a principal determinant of feed digestibility in vitro, its collection and preparation methods should be crucial upon its impact on in vitro dry matter digestibility results. According to Johnson (1966) two factors determine the outcome of in vitro dry matter digestion as affected by rumen fluid; namely the donor animal and inoculum preparation methods. Cherney et al. (1992) found that fiber source in donor animal diet as well as method of filtration can affect in vitro dry matter disappearance but not relative forage ranking. Another important determinant of in vitro outcomes is incubation time. Nelson et al. (1975) found a range of 63.01% to 76.14% for in vitro dry matter disappearance for 24 to 84 hours of incubation time respectively. The concentration of rumen fluid to substrate used in vitro can be consequences on the digestibility results obtained. Church and Petersen (1960) found that in vitro dry matter digestibility increased with a concurrent decrease in substrate concentration as its concentration decreased from 18 to 2 grams and the volume of rumen fluid was increased from 20 to 120 milliliters. Other researchers have found that in vitro dry matter digestibility was dependent on the rumen fluid donor animal and the dietary regimen it was fed as well. Hunt et al. (1954) found that rumen fluid obtained from a

steer fed alfalfa hay digested more cellulose than rumen fluid obtained from a steer fed a poor hay diet.

Some researchers have emphasized the need of using rumen fluid from an animal consuming a diet similar to the substrate sample to be studied for their IVDMD experimentation. This precaution was suggested as a method to maintain microflora density as well as activity and reduce potential errors. Warner (1956) suggested the rumen fluid should be from animals fed similar diets in order for in vitro digestibility to compare to in vivo digestion activity results. Cherney et al. (1993) also suggested that it was important to specify the ration ingredients fed to the donor animal as well as chemical composition in in vitro dry matter digestibility reports. They emphasized that comparison of in vitro dry matter digestibility values from different labs even with similar diets should be done cautiously to avoid any erroneous conclusions.

As is the case with other in vitro methods of digestibility, the in vitro dry matter digestibility assay was devised to measure fiber digestibility and not microbial efficiency at digesting fiber. Owing to this, research using the same parameter as an index of animal performance is scarce.

Fiber Digestion:

There is interest in using in vitro gas production to assess fiber digestion rates because it is relatively easy to measure (Schofield and Pell, 1995). However, the gas production system has some inherent problems according to Firkins et al. (1998). It appears that the gas production system used for measuring the in vitro neutral detergent fiber degradation rate from the difference curve of a given amount of feed sample and its NDF digestion is problematic owing to draw backs such as blank correction issues and ammonia impact on in vitro gas kinetics (Serjsen, 2006).

Researchers have used in vitro neutral detergent fiber degradation to assess and screen various chemical treatments of forages and feeds to improve ruminant productivity. Cross et al. (1974) found that in vitro digestibility of cellulose was increased from 66% to 91% in orchard grass and alfalfa hay by degradation of lignin or its chemical removal. This occurred despite the absence of changes in in vitro digestibility or the rate of digestion of the orchard grass or alfalfa cell NDF. Doane et al. (1997) found that the initial time of in vitro fermentation was critical as the NDF ratio to fermentation end products, and the acetate: propionate ratio only varied during the first eight hours of incubation and then only changed by negligible amounts with further time. Selection of the optimal date to harvest forages to maximize their nutrient quality can be substantiated by conducting in vitro fiber disappearance rate studies. Belyea et al. (1978) utilized the in vitro rate of fiber degradation to determine the ideal harvest time for wheat forage as silage for feeding lactating dairy cow's forage to be at the boot to early head stage.

There are many reports and applications using in vitro digestibility to evaluate forage quality. There is considerable variation in fiber digestibility in vitro due to plant and animal factors as reported in the mentioned studies as well as a plethora of other researches. However, few studies have been focused on determining animal variation in in-vitro digestion activity using in-vitro techniques.

Protein Digestion:

Broderick et al. (2004) estimated rates and extents of ruminal protein degradation from the net appearance of total amino acids and ammonia in vitro. They used a model in vitro system that suppressed synthesis of ammonia and degradation of total amino acids using dialysis in an in vitro media with 30 mg/L of chloramphenicol and 1mM hydrazine. Adding protein at amounts of 0.13 mg nitrogen per milliliter of medium did not alter protein degradation, except with casein and solvent-extracted soy bean meal. This experiment was conducted on five different sources of feed protein; namely 1.9 to 2 mg each of a sample of alfalfa hay, two samples of solvent extracted soy bean meal, casein and two different samples of expeller-extracted soy bean meal, 5 ml of warm McDougal buffer and 10 ml of rumen fluid. Broderick (1972) proposed other possible inhibitors of ammonia uptake as well as amino acid deamination. These include toluene, antibiotics, thymol, and hydroxylamine. Also, when used as blockers of ammonia uptake, it was found that hydrazine sulfate had a higher inhibition effect than chloramphenicol alone but that their addition had no significant effect on uptake inhibition (Broderick, 1987). Broderick (1988) also noted a two-fold in situ value for inhibitor in vitro method of degradation in a study on eight soluble protein as well as ten protein meals. The inhibitor

in vitro method was basically a way to measure degradation rate accurately via the use of such inhibitors to quantitatively recover break down products (Broderick et al., 2004). Craig et al. (1984) conducted a protein disappearance study with seven different preparations of rumen inoculant. Three of the inoculant preparations were composed of particle associated microorganisms and yielded in vitro protein degradation rates that were more than two-fold those obtained using conventional methods.

In vitro and in situ measurements of protein degradation are now the basis for estimating rumen undegradable protein as specified by the current NRC publications (Beef 1996, and Dairy 2001).

Microbial Protein Measurement Methodology Variation:

"Ruminal microbial protein is an important source of amino acids for ruminants" (Herejk et al., 2001). It has previously been mentioned that rumen microbial protein has an amino acid composition similar to soybean meal (Merchen and Titgemeyer, 1992) the most widely used protein supplement in the U.S. Microbial protein is one of the most difficult ruminal in vivo parameters to measure. Owens and Bergen (2009) accounted for microbial protein via purine base concentration in vitro in a study on the omasal sampling of four ruminally fistulated steers. Microbial markers such as diaminopimelic acid (DAPA), RNA, ATP, aminoethylphosphonic acid and isotopes are also used in ruminal microbial protein measurement in vivo (MarsHall and Herejk 1979). Herejk and Hall (2001) used trichloroacetic acid precipitable protein as a means to assess microbial protein production in vitro with a semi-purified substrate. Using in vitro cultures

conducted in 4h intervals for 24 h seasons they found that starch as compared to either sucrose and pectin included at a 60:40 substrate ratios with isolated Bermudagrass NDF gave maximal yields of TCA precipitable crude protein.

Intake:

Voluntary intake of forages is one of the most important productivity determinants associated with feeds and feeding (Jung and Allen, 1995). There are many factors that affect intake of ruminants. Some of the more important factors include: physiological state of the animal, environmental temperature, palatability of the feed, physical form and fiber content of the diet. Rotger et al. (2006) found that DM and OM intake of Holstein heifers was higher for corn based over barley based diets. According to Ellis (1978), capacity for the ruminant GIT and less digestible forage bulk are important factors to consider in determining limiting factors to forage intake by ruminant animals. Conrad established a model that depicted the influence of chemostatic and distension as the major factors controlling DE intake of ruminants (Van Soest, 1965). Mertens improved the Conrad model by using NDF to substitute for DE (Mertens, 1987). Metabolic factors are also important as Herd and Arthur (2009) were able to account for three fourth of the residual feed intake in a study on Angus steers by metabolic heat production, physical activity and body composition variation.

Genetics is also believed to play a major role in intake as higher producing cattle have greater intakes to meet their greater nutrient requirements. Although there are several examples where intake limits productivity the cause and effect nature of productivity

requirements and intake are uncertain. Regardless, the impact of intake has outcomes that impact digestibility and associated measures such as passage rate and retention time. Kendall et al. (2009) determined DM intake in early lactation dairy cows as a response to diets different in NDF and NDF digestibility. They found that feeds with higher in vitro digestibilities increased intake as well as production.

Defaunation, Refaunation, Cross and Trans-inoculation:

It has been a topic of disagreement among ruminant nutritionists whether or not defaunation is beneficial to ruminants. Klopfenstein et al. (1966) found no faunation effect on apparent DM and Nitrogen digestion, but reported a decrease ($P<0.01$) in Nitrogen retention whereas the opposite was observed and an increase in both DM and Nitrogen was noted in two experiments where six wethers in each experiment were defaunated and supplemented with antibiotics and two different feeds respectively.

There were attempts made to trans inoculate rumen fluid obtained from goats that are resistant to Mimosine toxicity to non-resistant sheep with the aim of imparting the same quality in the latter (Vaithiyanathan et al., 2005). Nevertheless, these attempts failed and no outcome was obtained. This could be owing to the attempts made with sheep recipients and not goats like the donors. This requires further investigation.

CONCLUSION

There may be possible opportunities to transfaunate ruminants and improve productivity but first we need to better understand if there is variation in rumen microbial digestion activity in animals fed the same diet.

It is more difficult to accurately access intake and nutrient utilization of cattle grazing pasture. Cattle on pasture may be stomach tubed and the in vitro digestion technique developed to determine relationships to intake and nutrient utilization.

The in vitro technique is most useful to predict DM and fiber digestibility as related to intake and nutrient utilization. However, VFA production and microbial protein synthesis are subsequent processes of the microbes that ultimately influence animal productivity. The in vitro technique should be developed further to utilize these other parameters and relate them to animal productivity.

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CHAPTER THREE

IMPACT OF RUMEN FLUID SOURCE AS INOCULANT TAKEN FROM BEEF COWS OF VARYING PRODUCTIVITY AND AT DIFFERENT TIMES POST-PARTUM ON IN VITRO DIGESTION ACTIVITY[1]

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Abstract

Rumen fluid digestion activity was assessed in 10 multiparous Angus cows averaging 8 ± 3 years of age (± SD) from the pure-bred herd at The University of Georgia, Wilkins Beef Research Center (Rayle, GA) using dry matter digestibility, neutral detergent fiber digestibility, soluble protein content, indigestible neutral detergent fiber and volatile fatty acid production in vitro. This was done to examine further modifications to similar previous studies conducted in vitro. Five of the cows were 6% below and 5 were 9.4% above the herd average life time weaning weight ratio (AIMS: American Angus Association, Saint Joseph, MO 64506). The cows grazed mixed specie permanent pastures of primarily bermuda grass and tall fescue. Incubations were conducted as a modification of the two stage technique for the in vitro digestion of forage crops for 24 and 48 hours. There were no significant ($P>0.1$) effects on digestion activity in vitro due to cow productivity as related to productivity. There was, however, a productivity effect on certain VFAs, like acetate: propionate concentrations, both in vitro ($P < 0.1$) and their 24 hour production rate in vitro ($P < 0.1$). Season impacted ($P<0.1$) VFA production rates of certain VFAs in vitro. Neither productivity nor sampling season affected fecal fiber percentages ($P>0.1$). Season influenced digestion activity in vitro more times than productivity. The interaction effects that included season also affected more parameters ($P<0.1$) than those with incubation time and productivity. Further study to look into the possible causes for such differences in vitro is warranted.

INDEX WORDS: In Vitro Dry Matter Digestibility, In Vitro Neutral Detergent Fiber Digestibility, Total Soluble Protein Content, Indigestible Neutral Detergent Fiber, Weaning Weight Ratio.

Introduction

Productivity of cows in a cow-calf herd can be highly variable and is dependent on both genetic and environmental factors. This has spurred interest in determining the potential causes for this variability of specific phenotypic traits in order to improve breeding programs and better select and retain the most productive, efficient and highest quality cattle.

Calf weaning weight is one of the most important production traits in a cow-calf herd. Marston et al. (1992) found a positive correlation between the both the cow's heritability for milk production and actual milk production with weaning weights of calves. It is assumed that cow productivity is ultimately linked to intake as cows that produce higher weaning weight calves produce more milk in response to consuming more nutrients. Lactogenesis is driven by uptake of calories and by the mammary gland in response to its secretory capacity and this is ultimately related to nutrient intake.

Genetic variation of the dam is one of the major causes for variation in calf weaning weights. Age of dam and parity also influence calf weaning weight and are used to adjust its measurements to better understand its heritability. Age of dam is positively correlated with weaning weight until cows reached 7 to 9 years of age and the weaning weight ratio of cows with 5 or more calves is more reflective of their true genetic potential (Pell and Thayne, 1978).

In addition to the age of the dam, another detrimental factor to be considered in cow-calf production systems is breed feed conversion characters. Jenkins et al., (1991) found that greater calf weaning weights are related to their dams' ability to consume more energy (g/Mcal) for certain breeds such as Angus, Hereford, Red Poll, or Maine Anjou F1 cows. This was found in a study where they estimated the cow differences in net energy requirement among different breeds. Cows used were produced during cycle 2 of the Germ Plasm Evaluation Project that were housed in pens and their ME intake calculated from tabular values. However, caloric intake was not as related to weaning weight in other breeds such as Chianina or Gelbvieh cows. Nevertheless, even though inconsistencies still persist in certain breeds, for the most part, caloric intake of the cow during lactation is the major genetic factor that drives calf weaning weight.

The in vitro method for studying the rates of degradation of forage crops by ruminal microbes is one approach that has been developed to assess the digestibility and quality of forages. Variation in rumen digestion activity may be more critical to cow performance since cow-calf production systems rely on pastures for energy.

This research was conducted with the objective of developing an in vitro approach to assess animal variation in rumen fluid digestion activity.

Materials and Methods

Animals:

A total of 10 multiparous 8 year old (± 3) pure-bred registered Angus cows with established production records from AIMS (Angus Information Management Software; American Angus Association, Saint Joseph, MO 64506) chosen from a grazing cow-calf herd of 104 cow-calf pairs on permanent pasture (Bermuda grass 60-70% & Tall fescue 30-40%) were used as experimental animals. The cows were selected from the cow-calf herd at the University of Georgia Wilkins Beef Cattle Research Unit (Rayle, GA) and had life time productivity records that consistently placed them into either a high or low productivity classification based on their weaning weight ratios. Five of the ten cows were chosen that had average weaning weight ratios that were 6 % below the herd average and the remaining five had average weaning weight ratios that were 9.4% above the herd average for their life time parturition records. The productivity of the experimental animals was hence 'high' or 'low' for those cows that were in the upper 9.4% or lower 6% of the herd average weaning weight ratio respectively.

Pasture and diet:

The cows used were rotated on 8 different paddocks of mixed- grass permanent pastures that were approximately thirty acres in size each. The entire herd was rotated onto a new grazing paddock every 10 to 12 days, depending on the herdsman's assessment of pasture availability. The major grass species in the pastures were Fescue and common Bermuda grass. The Fescue was more vegetative in the fall and the Bermuda grass more vegetative in the summer. Other visible grass species in the pastures included volunteer annual rye

and crab grass. The cows were supplemented at a rate of 10 pounds of concentrate per head daily in a fence-line feeder from February to mid April (40% corn, 30% Soybean hulls and 30% corn gluten feed).

Sample collection:

The experimental substrate used was a composite sample of 49 mixed grass species collected manually from the same paddocks from April to June of the spring 2007 grazing season. The samples of vegetative grass were dried to constant weight in a forced air oven (Blue M Stabilitherm Constant Temperature Cabinet, The Blue M Co., Blue Island, IL) at 55 degrees centigrade and ground to pass through a 1mm screen in a Wiley mill (Thomas-Wiley, Model 4 Laboratory Mill, Thomas Scientific, Swedesboro, NJ). The composite so prepared was transferred into 80 polypropylene centrifuge tubes of 50 ml capacity each, whose empty dry weights were immediately previously recorded. In addition to these tubes, 40 additional tubes of the same capacity were also weighed in the same fashion and sorted with the tubes containing the substrate sample at the rate of 4 empty tubes for every 8 sample containing tubes. This was done to designate a total of 12 tubes per cow for the incubation in vitro.

Modified McDougall's buffer was used as the buffer in vitro and consisted of 5 g casein HCl, 2 g Ammonium bicarbonate and 0.625 g cysteine HCl per liter over the stock McDougall buffer (McDougall, 1948 as Modified by Hall and Herejk, 2001). The buffer hence prepared was then poured into the previously prepared tubes at the rate of 20 ml per tube. The 40 tubes that contained only the 20 ml blank were designated as the blanks.

This set up was then transported to the farm for rumen fluid inoculation. in such a way that each half, (i.e.) composed of 60 tubes, contained 4 sample and buffer inoculated tubes and 2 sample-free tubes (only buffer) per cow.

Rumen fluid samples were obtained from all the subjects via a stomach tube using negative vacuum pressure. The stomach tube consisted of a stainless steel strainer connected to a nylon reinforced clear plastic tube (6 meters in length). The tubing was fit with snap-lock connectors that fit into stainless steel rods into a double-hole rubber stopper. This plugged a 500 ml polyethylene bottle connected to a side arm Erlenmeyer flask and an electric vacuum pump fitted with a pressure regulator (Emerson LR39793, Fisher Scientific). A speculum was used to pass the tube with the strainer-end about 1 to 1.5 meters into the reticulo-rumen. Approximately 500 ml of rumen fluid was collected from each cow.

Rumen fluid was immediately measured for pH using a Fisher Accumet AR25 dual channel pH, ion meter (Fisher Scientific Co., Pittsburgh, PA); and then poured while being stirred into 12 tubes per cow at a rate of 10 ml per previously prepared tube according to the designated animal identification. All incubations were immediately gassed with CO_2 then capped with one way valves. All centrifuge tubes were held in racks that were immersed in 39 degree centigrade water in insulated coolers.

A sample of in vivo (0 hour) rumen fluid from the cows was immediately put on ice and transported back to the ruminant nutrition laboratory on campus (~30 miles from research farm) then stored frozen prior to its characterization for fermentation end products including ammonia, volatile fatty acids and pH. Ammonia concentration of rumen fluid was measured using a Fisher Accumet Model 810 pH meter with an Orion electrode (Orion Research Inc, Beverly, MA).

In addition to the rumen fluid samples collected from the subject animals, fecal samples were also collected by rectal palpation. The fecal samples were immediately bagged in zip lock bags and placed on ice to be transported to the lab for analysis of their percentage fiber and dry matter contents. The rumen fluid sampling and the incubation procedures were repeated two more times at approximately three month intervals on June 25 and September 23. This was done to get an idea of seasonal effects of forage nutrient variability, if any, on digestion activity in vitro.

Pasture grass samples were collected by hand from several random locations in the pastures where the cows were grazing. For the purpose of forage analysis, the summer and fall forage samples were manually separated into either mainly Bermuda grass or Fescue or kept as a mixed sample. The spring sample was comprised mainly of fescue with a smaller amount of annual ryegrass and dormant Bermuda grass and was only analyzed as a mixed sample. Also, on one occasion (June 25), we were able to collect a cud sample from one of the cows. This cud sample was kept in a zip lock bag labeled

with the number of the cow, alongside grass samples, for later fiber analysis. Forage samples were placed in gallon ziplock freezer bags and stored frozen until they were dried at 60 degrees centigrade until constant weight, approximately 72 h, in a forced air oven (Grieve forced air drying cabinet, Model SA-350, The Grieve Co., Round Lake, IL). The ratio of the weight of the dried sample to the un-dried pasture sample was used to estimate the farm dry matter concentration of the forages. All samples were ground to pass through a 1 mm screen using a Wiley mill (Thomas-Wiley, Model 4 Laboratory Mill, Thomas Scientific, Swedesboro, NJ). The NDF content of the ground samples was analyzed for percentage neutral detergent fiber (NDF) content in accordance with the technique by Van Soest et al.(1991) using an Ankom 200 Fiber Analyzer (Ankom Co., Fairport, NY). Percentage fiber crude protein of the forage samples was analyzed using a Leco Model FP-528 Nitrogen/Protein Determinator (Leco Co., St Joseph, MI). Percentage farm dry matter was calculated as the percentage difference in weight of the dried forage sample from its wet sample. The nutrient contents of the forage samples are shown in table 1.

These in vitro incubation centrifuge tubes were immediately transported to the lab in thermally insulated boxes that were filled with water warmed to 39 degrees C. The distance from the farm to the lab is approximately 25 miles and required an estimated travel time of approximately 30 minutes. At the lab, the test tubes in racks were immediately incubated in two water baths (Blue M Constant Temperature Bath, Model no. MW-1140A-1, The Blue M Co., Blue Island, IL) set at 40˚C for 24 and 48 h. This was arranged in such a way that 4 sample inoculated test tubes and 2 blank test tubes per

animal were incubated in the water bath for 24 and 48 hours each, replicating the total number of incubations equally for each incubation time,(i.e.) 60. The centrifuge tubes were swirled frequently at approximately 4-6 h intervals during the incubation.

The experimental cows calved from December 15, 2007 to February 12, 2008 and the calves were weaned on August 25, 2008. The spring sampling season was carried out when most of the cows were at early lactation; the summer season, mid to late lactation and the fall; after weaning.

The incubations were done as per the Tilley and Terry procedure for assaying the in vitro digestion of forage crops (J.M.A Tilley and R.A. Terry, 1963) with some minor procedural changes. The substrate sample, a composite of pasture grass samples, was 0.6 g instead of 0.5 g and the volume of rumen fluid was 10 ml and the volume of buffer used was 20 ml instead of 10 and 40 ml, respectively. This is with the aim keeping the incubation volume within the capacity of the 50 ml centrifuge tube while still providing additional buffering to the ground up forage sample which we are introducing in the rumen fluid samples, as these have not undergone mastication the digesta in the rumen fluid already has.

The first stage of the Tilley and Terry procedure, the fermentation stage, was conducted at both 24 and 48 hours, after which the incubation tubes were immediately taken out the

water bath, un-stoppered and sealed with parafilm and placed in frozen storage over night to cease further microbial activity

After a season of frozen storage, samples were taken out of the freezer and allowed to thaw at room temperature. After making sure they were properly thawed, the samples were thoroughly mixed by vortexing and then spun down in a clinical centrifuge (IEC Model CU-5000 Centrifuge, Needham Hts., MA) at 2,415 g X 5 min. The supernatants were collected and stored in plastic vials subsequent to soluble protein assay using the Lowry assay (Lowry et al., 1951). The pellets were re-suspended with 20 ml of water and the tubes were centrifuged again at 2,415 g X 5 min. The supernatant from the re-suspension in water was discarded. The re-suspension step and centrifugation were repeated again for all tubes before the pellets were ready for the second or pepsin digestion stage of the Tilley and Terry procedure.

The second stage of the Tilley and Terry IVDMD procedure involves using 20 ml of a pepsin solution (0.002% 0.1 N HCl) that was poured into each of the tubes containing the rinsed IVDMD pellet. These were placed in test tube racks, capped with one-way valves and placed in a water bath set to 39 C. The samples were left in the water bath for 24 hours and were all taken out at the same time and placed in frozen storage until they could be further processed.

Processing the second stage pepsin digestion involved taking the tubes out of the freezer, and allowing them to thaw at room temperature. The pellets were re-suspended by vortexing and then re-centrifuged at 2,415 g X 5 min. for five minutes. The incubation tubes were then taken out of the centrifuge and the supernatants collected into labeled 20 ml vials and placed in frozen storage until subsequent analysis of LP. The remaining pellets were then rinsed twice in the centrifuge with 20 ml warm (~40 degrees C) water per tube as described before for the processing of the first stage supernatant fluid. After re-suspending and re-centrifuging the contents of the second stage of in vitro digestion, the rinse supernatant fluid was discarded and the tubes with pellets were placed in a forced air oven (Blue M Stabilitherm Constant Temperature Cabinet, The Blue M Co., Blue Island, IL) for DM determination at 55 C. The samples were dried until they reached constant weight (approximately 72 h). They were then removed from the drying oven and allowed to air equilibrate and re-weighed before being transferred into weighed and tared fiber bags in preparation for the NDF assay. The supernatant fluids were then analyzed for their soluble protein content using a Milton Roy spectrophotometer (Milton Roy Company., Rochester, NY).

It is assumed that the Total Soluble Protein (TSP) assay would provide an estimate of rumen microbial protein. This is as rumen microbes should not just be attached to feed particles but also be solubilized after freezing the incubation after the first phase and thus crystallizing microbial protein and facilitating its solubilization into the supernatant after thawing. Furthermore, the pepsin incubation during the second phase of digestion should also facilitate solubilization of microbial protein. The Lowry protein assay was used to

qualify differences in dry matter and fiber degradability in terms IVDMD or IVNDFD in grams per unit gram of in vitro soluble protein. The inverse relationship was also considered as an estimate of the efficiency of microbial protein synthesis expressed as total soluble protein per in vitro dry matter digested in grams (TSPg/IVDMDg).

The Lowry method for determining soluble protein (Lowry et al., 1951) is typically used to qualify enzyme kinetic data and it is also known as the Folin-Ciocalteu test. It is a colorimetric reaction with the color formed caused by the reaction of protein with alkaline copper in the reagent as in the biuret test and the reduction of the phosphomolybdate-phosphotungstate salts in the reagent by tyrosine and tryptophan residues in the protein. The color yield per mg of protein depends upon the content of these two amino acids that vary in different protein sources considerably. We used bovine serum albumin (BSA) as the standard for the LP assay. In the future, a rumen bacterial protein isolate should be used to establish the standard curve. The Lowry protein measurement in this study is meant as a qualitative indicator and its use as such should be refined in future experiments. Mixed rumen bacteria contain approximately 2.9% tryptophan and 3.2 to 4.5% tyrosine expressed as a percentage of their dry weight. Bovine serum albumin reportedly contains 1.7 to 1.9% Tryptophan and 3.2 to 3.6% Tyrosine expressed as a percentage of their protein. This assay is also relatively simple and can be conducted at room temperatures. Furthermore, this assay is easily reproducible and requires simple equipments and was hence chosen over other protein assays such as the Bradford assay (Bradford, 1976). It also has the advantage of being more sensitive than the UV VIS spectrophotometer and can hence be cheaper to carry

out, without compromising reliability.

The in vitro rate of volatile fatty acid production was also estimated as the differences of the 0 hour VFA concentrations from the 24 and 48 hour concentrations of individual as well as total volatile fatty acids. The VFA concentrations were measured using a Varian 2400 Gas Chromatograph (Varian Instrument Group, Walnut CA).

The fecal samples were analyzed for their percentage NDF content, indigestible NDF content, potentially digestible NDF content and dry matter. With the exception of the percentage indigestible NDF content, fecal NDF content and its percentage dry matter content were determined directly from fecal samples in accordance with the technique by Van Soest et al.,(1991) on samples dried and ground to 2mm texture using a Wiley mill (Thomas-Wiley, Model 4 Laboratory Mill, Thomas Scientific, Swedesboro, NJ) and on the basis of dry to wet weight respectively. The percentage indigestible NDF content was determined from the in vitro disappearance rate of fecal neutral detergent fiber after 72 hours of incubation in composited rumen fluid sample from all the experimental animals in equal proportions collected on the last date of rumen fluid collection (September 23) as per the first step of the Tilley and Terry procedure.

Statistical Analyses:

All data were analyzed linearly using the PROC CORR and PROC GLM model procedures in SAS (SAS, 2003). The main parameters tested in this model were in vitro dry matter digestion (IVDMD), in vitro neutral detergent fiber digestion (IVNDFD), supernatant protein concentrations (S1, S2 and LP), indigestible neutral detergent fiber (INDF), in vitro neutral detergent fiber digested, in grams (IVDMDg), in vitro neutral detergent fiber digested, in grams (IVNDFDg), total gram Lowry protein per unit gram of DM digested in vitro (TLP/IVDMDg), in vitro dry matter digested in grams per unit gram Lowry protein (IVDMDg/TSP) and in vitro neutral detergent fiber digested in grams per unit gram soluble protein (IVNDFDg/TSP), as shown below:

$$Y_{ijk} = \mu + P_i + S_j + T_k + (P*S)_{ij} + (S*T)_{jk} + (T*P)_{ki} + (P*S*T)_{ijk} + e_{ijk}$$

Where, μ= overall mean;

P_i= fixed effect of productivity level (i= 1 or 2);

S_j= fixed effect of season (j= 1, 2 or 3);

T_k= Fixed effect of incubation time (k= 24 or 48);

$(P*S)_{ij}$= random effect of the interaction of P_i and S_j;

$(S*T)_{jk}$= random effect of the interaction of S_j and T_k;

$(T*P)_{ki}$= random effect of the interaction of P_k and W_i;

$(P*S*T)_{ijk}$= random effect of the interaction of W_i, P_j and T_k

and e_{ijk}= random residual error, assumed to be normally distributed.

None of the interaction effects were correlated with each other and had a Pearson correlation value of (*r=0*) for all three. The same approach was used for fecal indigestible NDF. Since the in vitro digestibility assay for the fecal samples was conducted over a single span of time (72 hours), the effects of grouping, season and their interactions were the only class variables. The remaining percentage fiber analyses of the fecal samples, namely their neutral detergent fiber, dry matter and potentially digestible neutral detergent fiber contents were also statistically analyzed using the same model.

$$Y_{ijk} = \mu + P_i + S_j + (P*S)_{ij} + e_{ijk}$$

Where, μ= overall mean;

P_i= fixed effect of weaning weight ratio treatment level (i= 1 or 2);

S_j= fixed effect of season (j= 1, 2 or 3);

$(P*S)_{ij}$= random effect of the interaction of P_i and S_j;

and e_{ijk}= random residual error, assumed to be normally distributed.

Results and Discussions

The data set for all digestion parameters conducted in vitro over 24 and 48 hours is showed in tables 1 and 2 respectively. These were conducted on experimental cows that maintained their life time weaning weight productivity records with the exception of only one of the high producing cows falling to the lower half of the herd for the experimental season.

The crude protein (CP), neutral detergent fiber (NDF) and dry matter (DM) content of the forage samples collected from several sites representative of the grasses in the particular paddock the cows were grazing at each season of rumen fluid collection are shown in table 3. The pasture sample collected during September 23 had the highest CP content and was approximately 34% higher than the mean of the March 14 and June 25 pasture sample CP contents. The June 25 forage samples had the highest NDF content (approx. 20% higher than the mean NDF of the March 14 and September 23 forage samples). The CP content of the forage samples collected was lowest for June 25. This is probably owing to the predominance of bermuda grass (Approx 60-70% vs. 30-40 % tall fescue) in all 8 paddocks, which was in its vegetative phase during June 25 and its CP content being lesser than that of tall fescue in the cooler season. The cud sample taken from the cow mentioned in the 'Materials and Methods' section was also analyzed for its NDF content (not shown). It was found to have a DM content of 14.96%, an NDF content of 68.43% and a CP content of 15.16%. The cud sample had approximately 75% less DM, and 10% more NDF and 45% more CP than the mean forage samples collected during the June 25.

This could indicate one of two things: The cows were more selective and grazed pasture higher in CP than was collected as being representative of grazed forage. A second and more remote possibility for the increase in CP could be microbial or mucosal protein.

The major factors that contribute to animal productivity can be characterized as being either environmental or genetic. Since certain traits are selected in animals on the basis of their heritability, it might be interesting to determine if the more productive cattle in a genetically improved herd would have differences in their rumen fluid digestion activity. Genetic selection for cow productivity could affect a number of factors that would affect rumen fluid digestion activity due to differences in intake, gastrointestinal physiology and nutrient metabolism. These factors all can affect the rumen environment and its microbial population and activity as Church and Petersen (1960) found no variation in cellulose digestion in vitro using rumen fluid from twin steers (assumed to be monozygotic) after rumen microbial stabilization following an adaptation season of approximately 56 days.

The in vitro fiber digestion activity as influenced by inoculation with rumen fluid from cows of varying productivity as compared to their herd mates is shown in Tables 4 and 5 respectively. There were no significant effects of productivity observed on both 24 and 48 hour in vitro digestibility parameters measured ($P > 0.1$). The rate of in vitro digestion was much faster in the first 24 hours with as much as approximately 80%, of the 48 hour in vitro dry matter digestion having taken place with the first 24 h ($P < 0.1$). This could be related to both substrate availability or fermentation end product feedback inhibition of microbial activity. Nelson et al. (1976) reported that 63% to 76% of the 48 h

in vitro digestion occurred within the first 24 hours. Soluble protein in the liquid supernatant phases of the in vitro incubation could represent either microbial protein or substrate protein solubilization or both. It is assumed that the soluble protein is more representative of microbial protein.

The VFA and ammonia concentrations in rumen fluid (in vivo) as sampled from the individual cows and used to inoculate the in vitro fermentations in terms of productivity and season of rumen fluid collection are showed in Tables 6 and 7 respectively. These were in accordance with that of cows consuming high forage ration as expected of grazing pasture. The in vivo rumen fluid isovalerate concentration (Table 6) was 26% higher in the rumen fluid collected from the high productivity cows ($P<0.1$) and the acetate: propionate ratio was 7% lower ($P < 0.1$). The acetate: propionate ratio of the in vivo rumen fluid was 4.46 and was typical for cattle fed high forage diets. The total VFA of the in vivo rumen fluid that s averaged 109.6 µmoles /ml. The in vivo rumen fluid VFA concentration added to the in vitro tubes as inoculant was substracted from the 24 and 48 h in vitro VFA concentration to provide results that correspond to in vitro VFA production.

Isobutyrate concentration (Table 7) was approximately 50% higher in rumen fluid collected during the June 25 ($P < 0.1$). Butyrate concentration was approximately 40% lower in r rumen fluid collected during the September 23 ($P < 0.1$). Isovalerate concentration was approximately 50% higher in rumen fluid collected during the June 25. Valerate concentration was approximately 38% higher for rumen fluid collected during

the June 25 ($P < 0.1$). In vivo acetate: propionate ratio was highest in rumen fluid collected during the September 23 (4.71 vs. 4.22 & 4.46 µmoles/ml). Ammonia concentration was 55 % lower in rumen fluid collected during the June 25 and September 23 collections as compared to the March 14 ammonia concentration ($P < 0.1$). This could be due to soluble protein intake or digestible organic matter intake resulting in more ammonia being incorporated into microbial protein during this season as the total soluble protein in vitro is highest in the June 25 collection as well. One possible reason for this could be the abundance of vegetative bermuda. This capacity of microbes is stated by Maeng et al. (1997) who, among many other researchers, mention the capacity of rumen microbes to capture nitrogen from ammonia that is produced from non protein nitrogen and protein to synthesize their protein.

In vitro production of VFA after 24 and 48 hour as affected by the source of rumen fluid being from cows of varying productivity is reported in Tables 8 and 9. In vitro VFA production is expressed in µmoles per ml of the supernatant fluid from the first phase 0f the fermentation step of the Tilley and Terry procedure corrected for the VFA added at the start of the incubation as a result of adding rumen fluid inoculants.

In vitro production of propionate, butyrate and the acetate: propionate ratio was influenced by cow productivity in 24 hour fermentations (Table 8). The 24 hour propionate production rate was 9% higher in 24 h fermentations inoculated with rumen fluid from more productive ($P < 0.1$). Also, 24 hour butyrate production rate was 14% higher in more productive cows ($P<0.1$). The acetate: propionate ratio was 10% lower (P

< 0.1) in 24 h fermentations that were incubated with rumen fluid from higher producing cows. This result is in accordance with Dove and Milne (1994) who found a relationship between microbial protein and rumen acetate: propionate concentration in that the supply of metabolizable energy becomes the limiting step to microbial protein production once the ratio becomes higher than 3 in a study they conducted on ruminally and abomasally canulated ewes during early lactation in spring and after the end of lactation in fall.Higher propionate production could be related to greater cow productivity as related to its metabolic role. Firkins et al. (2006) have stated the importance of Propionate in influencing milk protein percentage. Also, Bergman (1990) has stated that propionate is the “only” VFA that makes a significant contribution to net glucose synthesis and it is quantitatively the most important single precursor of glucose. This glucose could be used by the cow to meet its lactational energy requirement. Even though this the study of Dove and Milne used ewes and not cattle, Kitessa et al. (1999) state that “Predictive equations generated using sheep and cattle as a source of in vivo data have generally been used interchangeably”. They also mention that this is not without any differences in the fiber break down capacities of the two species.

Higher acetate: propionate ratio reflects a fermentation that does not generate as much microbial ATP to drive microbial protein synthesis. The in vitro fermentations contained one third rumen fluid and two thirds buffer so the total VFA concentration added to the in vitro fermentations upon inoculation averaged 36.1µmoles/ml. Once corrected for the VFA concentration of the initial rumen fluid inoculation, the VFA production in vitro averaged 52.6 µmoles/ml, representing a 45.6% increase in production after 24 hours.

The 48 hour isovalerate production rate was 12% lower (P<0.1) in the high producing cows (Table 9). A closer look at these two tables, 6 and 7, indicates that most of the volatile fatty acid production in vitro takes place in the first 24 hours of fermentation, 53 µmoles/ml vs. 65 µmoles/ml of total VFA produced after another 24 hours. Also, the contained conditions of the in vitro batch cultures likely select for reducing the acetate: propionate because of the lack of turnover and VFA absorption**.** Furthermore, grinding the substrate may have may have increased its energy availability and influenced the in vitro acetate: propionate ratio. The capacity of the rumen fluid of higher productive cows to produce more propionate suggests that these cows have a different population of rumen microorganisms. This could be a function of these cows having different patterns of intake than cows with low productivity. It was also interesting to note that the acetate: propionate ratio (Table 6), in both the 24 and 48 hour cultures was less (~40%) than that found in the rumen fluid used as inoculants.

Table 10 shows the impact of sampling season on 24 hour in vitro digestibility parameters measured. In vitro dry matter digestibility was 10% lower with rumen fluid inoculants from the March 14. The highest value for percentage IVDMD was obtained with rumen fluid collected during the June 25 (June 25) (P < 0.1). It is difficult to ascertain the reason for the season effect on in vitro rumen fluid digestion activity in this study as there are seasonal differences in cow productivity due primarily to lactation performance and there are seasonal changes in pasture nutrient availability. The cool and warm season grasses in the mixed grass pastures mature at different times during the

sampling three seasons of the study. The predominant grasses would be cool season varieties in the March 14 (early spring) and then change to being predominantly warm season grasses in season two and three (mid and late summer). Depending on grazing pressure these grasses would mature and change in forage mass and nutrient availability. This is in accordance with Burton et al. (1963) who state that the digestibility of coastal bermuda grass decreases with age and drops rapidly in grass over six weeks old. The bermuda grass sample collected on June 25 is 3- 4 months old.

Soluble protein in the supernatant fluid after the pepsin stage of in vitro digestion was approximately 28% higher during the second collection season ($P < 0.1$). Total soluble protein in vitro was approximately 30% higher during the June 25 ($P < 0.1$). These results imply that the rumen fluid sampled during the June 25 contained a microbial population that was able to grow more efficiently per unit of organic matter digested. The reason for season differences rumen fluid activity could be due to seasonal differences in animal productivity and seasonal differences in pasture nutrient availability. This could be related to the maturity of the predominant grass type in the pasture, which ultimately drives intake and promotes productivity. Lima et al. (2008) found a linear decrease in apparent digestibility of DM by sheep with an increase in the re-growth age of *Echinocola* specie rice grass hay. Since microflora used in vitro are indicative of their fiber degrading activities in vivo, even though these results are from in vivo studies, they can shed some light on the influence of forage maturity. It is difficult to account for the specific reason for IVDMD being lower with in vitro fermentations inoculated with season one rumen fluid, which occurred when the cows were 64 days post partum.

Accordingly, they had to have their highest energy requirements and intake. Also, the cows were being provided additional supplemental feed (40% corn, 30% soybean hulls and 30% corn gluten feed) fed at the rate to provide approximately 10 pounds per head using a fence line feeder from February to mid April. Supplemental feed was not used in seasons 2 and 3 as there was more than adequate pasture available from the growth of bermuda grass in response to warmer temperatures.

Another possible explanation for a high digestion activity in vitro during the June 25 could be the physiological stage of the cows. Since the cows were inseminated at the end of March and the beginning of April and had to support the growing fetus in addition to suckling their calves, they could have been eating more which supports microbial population and is therefore reflected in its activity in vitro. This finding is in agreement with Broderick et al., (2004) who found that using rumen fluid from lactating cows gave more rapid casein degradation rates than rumen fluid from non lactating cows with lower feed intake. Also, Doreau et al. (1990) found that the digestibilities of DM, OM and CF of the same experimental diet varied before and after calving for dairy cows, in that digestibility was higher ($P < 0.1$) in late pregnancy than in early lactation. IVNDFD was not affected by the time season that rumen fluid was sampled (Tables 8 and 9). The amount of both DM and NDF digested in vitro was influenced by rumen fluid sampling season when these parameters were qualified as a function of soluble protein concentration and this effect was more statistically apparent in the 24 h fermentations. The amount of total NDF digested in grams per gram of total soluble protein obtained in vitro was approximately 25% lower during the June 25 ($P < 0.1$).

The impact of sampling season on 48 hour in vitro digestibility parameters measured is shown in table 11. With the passage of time, it is yet again seen that the rate of in vitro digestion is faster in the first 24 hours of fermentation. In vitro DM digestibility was 8% lower for the March 14 ($P < 0.1$). Soluble protein post fermentation was approximately 31% higher during the June 25 ($P < 0.1$) and soluble protein post pepsin digestion was approximately 22% higher during the June 25 ($P<0.1$). Total soluble protein was approximately 7% higher during the June 25 of rumen fluid collection ($P < 0.1$). The amount of NDF digested in grams per gram total soluble protein concentration in vitro was 27% significantly lesser ($P < 0.1$) for the June 25.

The impact of season of sampling rumen fluid on 24 hour in vitro VFA production rate is shown in table 12. Acetate production rate was approximately 10% lower using rumen fluid collected during the September 23 ($P<0.1$). Propionate production rate was approximately 20% lower for rumen fluid collected during the September 23 ($P < 0.1$). It was also interesting to see that the concentration of the mean total 24 hour soluble protein (Table 8) was higher during the June 25. This sheds light on the relationship between propionate and in vitro protein digestion. Isobutyrate production rate was approximately 37% lower for June 25 ($P < 0.1$), which was also the case with isovalerate production rate which was approximately 34% lower during the June 25 ($P < 0.1$). It was interesting to note that concentrations of the branched chain VFA were lowest for the June 25 while the total soluble protein concentration in the supernatant fluid was the highest. This may be due to branched chain VFA utilization by the microflora and its subsequent conversion

into microbial protein during that season. This is in accordance with Yang (2002) who stated that ruminal microorganisms utilize branch chain VFA as a source of carbon skeleton to synthesize branched chain amino acids (Allison et al., 1962 as cited by Yang, 2002).

The impact of rumen fluid sampling season on 48 hour VFA production rate is shown in table 13. The 48 hour acetate production rate was approximately 21% lower for rumen fluid collected during the September 23 ($P < 0.1$). Propionate production rate was approximately 30% lower for rumen fluid collected during the September 23 ($P < 0.1$). The rate of propionate production (both over 24 and 48 hours of incubation) was found to be highest for June 25 rumen fluid collection. It is noted that the rumen fluid (in vivo) used to inoculate the in vitro fermentations was higher in acetate concentration during the same season. In other words, the initial concentration of acetate in the rumen fluid used in vitro was highest for rumen fluid collected on the 25th of July. This is in accordance with the findings of Peters et al., (1989) who found greater propionic acid production in vitro at high vs. low initial acetic acid concentrations using rumen fluid collected from four beef steers fed a hay-concentrate supplement once daily. Isovalerate production was approximately 16% lower for rumen fluid collected during the second rumen fluid sampling season ($P < 0.1$). The amount of the total VFAs produced at 24 hours equaled 80% of the total VFA produced in vitro at 48 hours. This agrees with the findings of Barth et al., (1972) who found that approximately 80% of the total VFA produced at 36 hours of in vitro incubation was present at 24 hours. These researchers used the Tilley and Terry procedure in an experiment where they used rumen fluid from 16 yearling

steers randomly assigned to experimental pastures of orchard grass-ladino clover and fescue-lespedeza from the end of April to mid October. In our experiment, total VFA production rate was 20% lower for rumen fluid collected during the September 23 ($P < 0.1$). The total amount of VFAs produced was highest for the first and June 25s of rumen fluid collection ($P < 0.1$). This finding is likely related to both animal and feeding variables that impact nutrient intake, rate of passage and select for particular rumen microflora. These variables include stage of lactation requirements of the cows; concentrate supplementation in the March 14, availability and seasonal nutrient availability of the pasture. It is well recognized that diets that are higher in digestible energy result in greater VFA production and a lower proportion of acetate: propionate. Lana et al., (1998) reported that cattle fed only forage had a lower ruminal VFA concentration than cattle fed a 90% concentrate diet in a experiment where they fed four steers a timothy: concentrate diet with an increasing amount of concentrate (cracked corn grain and soybean meal) and a decreasing amount of forage (timothy hay). They also found that when mixed ruminal bacteria were incubated in vitro with amino nitrogen, the specific activities of deamination were positively correlated with acetate: propionate ratio in vivo using the Lowry assay to measure microbial protein.

The fiber and indigestible fiber concentrations of fecal samples as related to cow productivity are reported in Table 13. There was a trend ($P=0.12$) for the NDF concentration to be lower in the more productive cows. Since fiber is the least digestible component of the diet less fiber in the feces suggests that there is more digestible material

in the feces. This could be due to a number of factors including intake, intake selectivity, rate of passage and endogenous nutrient production.

The fecal indigestible NDF content was determined from Tilley and Terry pellets after 72 hours of incubation as renewal of the fermentation medium was necessary to obtain maximum extent of digestion (>96 hours) in vitro over 72 hours (Mertens. 1973, as cited by Traxler et al. 1998). Impact of sampling season on total and indigestible fiber concentrations of fecal samples are shown in table 15. There were no significant season effects on fecal fiber content ($P > 0.1$). The fecal indigestible NDF measurement is typically used as a measure of internal digestibility. Higher indigestible NDF would be indicative of greater fiber digestion.

The interaction effects of season, productivity and incubation time on parameters measured in vitro are shown in tables 16- 18. Table 16 shows that the interaction effect of productivity and season had an influence ($P< 0.1$) on pepsin soluble protein, production rates of propionate, isobutyrate, butyrate, isovalerate and total volatile fatty acids.

Parameters influenced ($P< 0.1$) by the interaction effects of season and incubation time are shown in table 17. It was interesting to see that none of the measurements of fiber digestion activity in vitro were affected by this interaction. All parameters in vitro affected were VFA production rates measured in vitro. Table 18 shows that acetate, propionate, valerate and total volatile fatty acid production rates in vitro were influenced ($P< 0.1$) by the interaction effects of productivity and incubation time. The three way

interaction effect of class variables did not influence (P>0.1) any of the parameters measured in vitro.

Whether or not these effects are related to differences in cow productivity or seasonal variations in forage quality or incubation time or all three requires further study.

Table 1: Digestion measures over 24 hours in vitro for the three incubation seasons and productivity levels.

Season*	%DMD	% NDFD	FSP	PSP	TSP	DMDg	NDFDg	TSPE[1]	DMDA[2]	NDFDA[3]	Ac	Pr	IB	Bu	IV	Va	TVFA	Prod.[4]	Ac:Pr
Spr	39.4	38.1	24.6	26.2	50.8	0.2	0.1	0.2	4.7	2.7	29.4	10.0	3.1	5.9	2.3	2.2	53.0	2	2.9
Spr	34.8	33.5	17.2	14.7	32.0	0.2	0.1	0.2	6.6	3.8	35.1	9.4	1.9	4.2	1.9	2.0	54.6	1	3.7
Spr	39.5	37.5	26.9	25.2	52.1	0.2	0.1	0.2	4.6	2.6	31.9	10.3	1.6	4.1	1.6	2.8	52.3	2	3.1
Spr	33.0	30.6	20.4	17.1	37.5	0.2	0.1	0.2	5.4	2.9	29.8	9.0	1.8	4.9	1.8	2.4	49.7	1	3.3
Spr	40.3	40.4	23.8	19.4	43.2	0.2	0.1	0.2	5.6	3.3	33.9	9.8	1.8	4.6	2.0	3.2	55.4	1	3.4
Spr	42.6	40.7	33.4	14.0	47.4	0.3	0.1	0.2	5.5	3.1	32.4	7.6	1.4	2.7	1.6	2.6	48.2	1	4.3
Spr	39.9	37.5	28.0	24.1	52.2	0.2	0.1	0.2	4.6	2.6	42.5	13.3	2.4	6.4	2.5	3.1	70.2	2	3.2
Spr	36.1	34.1	25.4	20.9	46.3	0.2	0.1	0.2	4.7	2.6	36.9	12.0	2.4	6.6	2.6	3.1	63.5	2	3.1
Spr	36.8	35.8	20.1	19.9	40.0	0.2	0.1	0.2	5.7	3.3	29.5	10.6	1.7	4.8	2.2	3.0	51.8	2	2.8
Spr	35.9	33.2	18.9	18.4	37.3	0.2	0.1	0.2	6.0	3.3	29.5	10.4	2.1	4.9	2.1	2.4	51.4	1	2.8
Sum	40.6	35.2	27.5	36.1	63.6	0.3	0.1	0.3	3.9	2.0	30.0	11.2	1.5	4.4	0.4	2.7	50.2	2	2.7
Sum	40.2	36.2	47.2	17.9	65.1	0.2	0.1	0.3	3.8	2.0	29.4	9.1	0.6	2.5	1.5	3.2	46.5	1	3.2
Sum	43.6	40.9	36.9	20.4	57.3	0.3	0.1	0.2	4.7	2.6	28.5	10.6	1.6	3.9	0.9	2.5	48.1	2	2.7
Sum	41.7	38.7	20.8	34.4	55.2	0.3	0.1	0.2	4.6	2.5	30.5	10.5	1.9	4.7	1.3	2.6	51.4	1	2.9
Sum	42.0	38.5	35.3	27.9	63.2	0.3	0.1	0.2	4.1	2.2	35.6	11.7	1.8	4.7	0.9	3.0	57.6	1	3.0
Sum	42.8	40.7	41.3	28.0	69.3	0.3	0.1	0.3	3.8	2.1	37.4	12.5	1.6	4.2	1.1	2.3	59.1	1	3.0
Sum	43.6	43.1	32.4	25.9	58.3	0.3	0.2	0.2	4.6	2.7	24.7	9.4	0.6	4.1	1.3	2.1	42.3	2	2.6
Sum	40.5	37.1	33.4	30.6	64.0	0.2	0.1	0.3	3.8	2.1	35.4	11.2	0.9	5.4	1.9	3.0	57.9	2	3.2
Sum	42.2	36.6	32.5	33.2	65.7	0.3	0.1	0.3	3.9	2.0	41.7	13.3	1.3	6.4	2.0	3.9	68.5	2	3.1
Sum	41.9	37.4	39.0	24.6	63.7	0.3	0.1	0.2	4.0	2.1	33.4	9.9	0.9	6.7	2.2	2.9	56.0	1	3.4
Fal	44.6	39.3	22.4	14.2	36.6	0.3	0.1	0.1	7.4	3.9	31.2	9.6	1.1	4.8	2.3	3.1	52.1	2	3.3
Fal	43.7	39.9	18.0	13.7	31.7	0.3	0.1	0.1	8.3	4.5	27.7	7.5	1.4	4.0	2.0	2.9	45.6	1	3.7
Fal	41.9	34.8	18.7	18.2	36.9	0.3	0.1	0.1	6.9	3.4	28.0	9.4	1.2	4.6	2.2	2.6	47.9	2	3.0
Fal	42.1	35.8	25.8	25.1	50.9	0.3	0.1	0.2	5.0	2.5	28.8	7.9	1.2	4.2	1.9	2.6	46.6	1	3.6
Fal	42.6	37.2	20.7	26.4	47.1	0.3	0.1	0.2	5.5	2.8	32.1	10.0	2.4	5.1	2.0	2.3	54.0	1	3.2
Fal	38.8	32.7	26.2	23.6	49.8	0.2	0.1	0.2	4.7	2.4	27.5	8.2	1.2	3.7	1.8	2.6	45.1	1	3.4
Fal	41.9	36.0	35.1	19.2	54.3	0.3	0.1	0.2	4.7	2.4	28.2	9.4	1.7	6.2	2.1	2.6	50.3	2	3.0
Fal	41.0	33.6	31.4	16.0	47.4	0.3	0.1	0.2	5.3	2.6	31.4	8.9	1.7	5.8	2.0	2.5	52.3	2	3.5
Fal	36.1	26.4	30.9	20.7	51.6	0.2	0.1	0.2	4.2	1.8	25.1	8.0	1.1	4.9	2.0	2.1	43.2	2	3.1
Fal	45.6	39.0	22.6	16.7	39.3	0.3	0.1	0.1	7.0	3.5	31.2	9.6	1.6	5.8	1.9	2.7	52.8	1	3.3

[1]TSPE= TSP/IVDMDg
[2] DMDA= IVDMD/TSP
[3]NDFDA= IVNDFD/TSP
[4]Prod: High productivity=2, low productivity=1
*Spr= March 14, 2008; Sum= June 25, 2008 & Fal= September 23, 2008.
VFA production rates are expressed in µmoles/ml.

Table 2: Digestion measures over 48 hours in vitro for the three incubation seasons and productivity levels.

Season*	%DMD	% NDFD	FSP	PSP	TSP	DMDg	NDFDg	TSPE[1]	DMDA[2]	NDFDA[3]	Ac	Pr	IB	Bu	IV	Va	TVFA	Prod.	Ac:Pr
Spr	50.4	50.7	29.1	21.3	50.4	0.3	0.2	0.2	6.1	3.6	42.4	12.4	2.2	5.4	2.2	3.8	68.4	2.0	3.4
Spr	51.8	53.6	30.6	16.3	46.9	0.3	0.2	0.1	6.7	4.1	42.4	13.7	2.2	5.0	2.7	4.2	70.1	1.0	3.1
Spr	49.8	49.5	37.9	22.2	60.1	0.3	0.2	0.2	5.0	2.9	43.8	13.8	1.7	4.7	2.2	4.4	70.6	2.0	3.2
Spr	47.6	50.3	38.3	17.9	56.2	0.3	0.2	0.2	5.2	3.2	48.7	14.8	2.1	5.9	2.6	4.6	78.6	1.0	3.3
Spr	44.5	45.7	32.0	17.4	49.4	0.3	0.2	0.2	5.4	3.3	43.7	12.5	1.9	5.5	2.5	4.4	70.5	1.0	3.5
Spr	49.9	52.2	27.9	12.7	40.6	0.3	0.2	0.1	7.5	4.6	45.7	15.1	1.5	5.3	3.5	4.4	75.6	1.0	3.0
Spr	44.3	44.7	29.7	22.1	51.8	0.3	0.2	0.2	5.2	3.1	52.8	18.3	2.6	8.5	3.2	4.7	89.9	2.0	2.9
Spr	46.1	47.5	33.8	15.6	49.4	0.3	0.2	0.2	5.7	3.5	46.9	16.0	2.4	7.3	3.0	4.1	79.7	2.0	2.9
Spr	47.8	49.2	11.8	11.3	23.1	0.3	0.2	0.1	12.9	7.8	34.7	10.7	1.2	3.5	2.6	3.8	56.4	2.0	3.2
Spr	55.7	56.9	17.9	7.0	24.9	0.4	0.2	0.1	14.4	8.7	32.8	13.0	2.0	4.4	2.2	3.5	57.9	1.0	2.5
Sum	54.1	50.4	30.1	10.2	40.4	0.3	0.2	0.1	8.2	4.5	32.0	8.9	0.4	1.1	0.3	2.7	45.4	2.0	3.6
Sum	54.2	51.7	52.7	14.7	67.5	0.3	0.2	0.2	4.9	2.8	47.8	16.6	2.4	8.1	3.0	4.1	82.1	1.0	2.9
Sum	53.4	51.0	49.8	27.1	76.9	0.3	0.2	0.2	4.2	2.4	38.3	11.9	0.3	3.9	2.0	3.9	60.3	2.0	3.2
Sum	51.9	49.4	33.8	25.1	59.0	0.3	0.2	0.2	5.4	3.0	45.7	15.1	2.2	5.5	1.9	4.1	74.5	1.0	3.0
Sum	54.1	52.0	35.0	24.6	59.6	0.3	0.2	0.2	5.5	3.1	47.0	15.6	1.7	7.5	2.7	3.9	78.3	1.0	3.0
Sum	53.8	52.7	50.4	23.4	73.8	0.3	0.2	0.2	4.5	2.6	50.3	17.9	2.6	7.7	2.3	3.7	84.6	1.0	2.8
Sum	54.6	53.1	40.3	23.0	63.3	0.3	0.2	0.2	5.3	3.0	32.4	12.9	0.9	5.1	1.8	3.2	56.3	2.0	2.5
Sum	51.2	47.4	37.6	28.0	65.6	0.3	0.2	0.2	4.8	2.6	49.7	16.8	2.5	8.1	2.2	3.8	83.0	2.0	3.0
Sum	51.5	47.3	41.4	25.6	67.0	0.3	0.2	0.2	4.7	2.5	45.4	12.9	1.3	6.6	2.3	4.1	72.6	2.0	3.5
Sum	53.3	50.3	47.6	20.0	67.6	0.3	0.2	0.2	4.9	2.7	39.3	12.9	1.1	7.0	2.4	3.9	66.6	1.0	3.0
Fal	53.6	51.3	27.5	11.9	39.4	0.3	0.2	0.1	8.4	4.7	33.4	9.7	1.1	4.4	2.4	3.4	54.4	2.0	3.4
Fal	54.2	53.4	22.1	9.9	32.0	0.3	0.2	0.1	10.3	6.0	31.3	8.1	0.9	4.1	2.3	3.2	49.9	1.0	3.9
Fal	51.8	48.6	23.3	20.5	43.8	0.3	0.2	0.1	7.2	4.0	31.1	10.9	2.2	4.5	2.2	3.1	53.9	2.0	2.9
Fal	66.7	65.6	32.2	22.0	54.2	0.4	0.2	0.1	7.5	4.4	39.4	11.1	1.3	5.0	2.4	3.2	62.3	1.0	3.6
Fal	51.7	49.2	30.0	23.5	53.4	0.3	0.2	0.2	5.8	3.3	37.4	12.5	2.7	5.7	2.4	3.3	64.0	1.0	3.0
Fal	51.8	50.1	40.2	20.2	60.4	0.3	0.2	0.2	5.2	2.9	30.3	9.4	1.2	4.4	2.0	3.0	50.3	1.0	3.2
Fal	55.9	52.8	37.7	23.4	61.1	0.3	0.2	0.2	5.6	3.1	28.4	10.5	1.9	6.1	2.1	2.9	52.0	2.0	2.7
Fal	52.3	48.2	36.2	17.4	53.6	0.3	0.2	0.2	6.0	3.2	36.0	10.7	1.7	5.8	1.9	2.9	59.0	2.0	3.4
Fal	51.3	47.7	29.8	19.0	48.8	0.3	0.2	0.2	6.4	3.5	33.2	10.9	1.6	5.9	2.3	3.0	56.9	2.0	3.0
Fal	42.4	34.8	27.5	14.3	41.8	0.3	0.1	0.2	6.1	3.0	32.3	10.0	1.7	6.5	2.3	2.8	55.7	1.0	3.2

[1]TSPE= TSP/IVDMDg
[2] DMDA= IVDMD/TSP
[3]NDFDA= IVNDFD/TSP
[4]Prod: High productivity=2, low productivity=1
***Spr= March 14, 2008; Sum= June 25, 2008 & Fal= September 23, 2008.**
VFA production rates are expressed in µmoles/ml.

Table3: Nutrient analysis of representative samples of mixed grass pasture grazed by the cows used in the experiment.

Date	Sample ID	%DM	%NDF	%CP
3/14/2008	Mixed pasture sample (Mainly vegetative Tall fescue)	30.59	54.57	15.14
	Hay	91.57	76.95	14.42
6/25/2008	Vegetative Bermuda	45.40	73.37	9.25
	Dormant Fescue	74.51	80.40	7.23
	Mixed	52.15	76.30	8.83
9/23/208	Mainly Bermuda	20.49	57.55	18.50
	Mainly Fescue	22.34	56.59	16.72
	Mixed	21.77	58.59	18.33
In vitro composite*		92.03	59.3	12.14

*Composite sample of dried and ground mixed grass species from March to September of 2007 from Wilkins Farm pastures used as substrate for in vitro fermentations.

Table 4: Impact of rumen fluid source used as inoculant from cows selected according to productivity (weaning weight ratio) on 24 hour in vitro dry matter (DM), neutral detergent fiber (NDF) digestibility and soluble protein.[1]

In Vitro Digestion Parameter	Cow Productivity			
	LWWR[2]	HWWR[3]	SEM[4]	P > F
DM digestibility (%)	40.53	40.52	0.92	0.99
NDF digestibility (%)	36.97	36.4	1.23	0.64
Soluble protein post fermentation (mg)	27.38	28.42	2.20	0.64
Soluble protein post pepsin digestion (mg)	21.46	23.39	1.67	0.26
Total soluble protein (mg)	48.84	51.81	2.29	0.21
Total soluble protein/ DM digested (g/g)	0.20	0.21	0.01	0.24
DM digested/Total soluble protein (g/g)	5.34	4.93	0.34	0.23
NDF digested/Total soluble protein (g/g)	2.88	2.61	0.2	0.18

[1] Each mean was estimated from thirty observations corresponding to 4 repeat in vitro incubations from rumen fluid collected by stomach tube from five multiparous Angus cows at 3 seasons post partum.

[2] Low herd weaning weight ratio (5 cows with life time weaning weight records 6 % below herd average).

[3] High herd weaning weight ratio (5 cows with life time weaning weight records 9.4% above herd average).

[4] Standard error of the means.

[5] Sum of protein measured in the supernatant fermentation and pepsin digestion phases of the Tilley and Terry procedure using the Lowry assay.

Table 5: Impact of rumen fluid source used as inoculants from cows selected according to productivity (weaning weight ratio) on 48 hour in vitro dry matter (DM), neutral detergent fiber (NDF) digestibility and soluble protein.[1]

In Vitro Digestion Parameter	Cow Productivity			
	LWWR[2]	HWWR[3]	SEM[4]	P > F
DM digestibility (%)	52.24	51.21	1.54	0.51
NDF digestibility (%)	51.19	49.30	1.87	0.32
Soluble protein post fermentation (mg)	34.56	33.07	2.84	0.61
Soluble protein post pepsin digestion (mg)	17.94	19.92	1.92	0.31
Total soluble protein (mg)	52.49	52.98	4.10	0.90
Total soluble protein/ DM digested (g/g)	0.16	0.17	0.01	0.64
DM digested/Total soluble protein (g/g)	6.62	6.36	0.86	0.76
NDF digested/Total soluble protein (g/g)	3.85	3.63	0.52	0.69

[1] Each mean was estimated from thirty observations corresponding to 4 repeat in vitro incubations from rumen fluid collected by stomach tube from five multiparous Angus cows at 3 seasons post partum.

[2] Low herd weaning weight ratio (5 cows with life time weaning weight records 6 % below herd average).

[3] High herd weaning weight ratio (5 cows with life time weaning weight records 9.4% above herd average).

[4] Standard error of the means.

[5] Sum of protein measured in the supernatant fermentation and pepsin digestion phases of the Tilley and Terry procedure using the Lowry assay.

Table 6: Fermentation end products in rumen fluid (in vivo) of cows selected according to productivity .[1]

Volatile Fatty Acid	Cow Productivity			
	Low WWR[2]	High WWR[3]	SEM[4]	P > F
Acetate (µmoles/ml)	77.13	69.97	6.55	0.28
Propionate (µmoles/ml)	16.90	16.42	1.7	0.78
Isobutyrate (µmoles/ml)	2.36	2.65	0.25	0.27
Butyrate (µmoles/ml)	12.94	13.60	1.22	0.59
Isovalerate (µmoles/ml)	2.08	2.81	0.42	0.09
Valerate (µmoles/ml)	1.07	1.23	0.23	0.48
Total (µmoles/ml)	112.48	106.68	9.67	0.55
Acetate:Propionate	4.62	4.30	0.13	0.02
pH	7.31	7.40	0.12	0.45
NH3	6.56	6.18	0.63	0.69

[1] Each mean was estimated from a single sample stomach tubed at 0 hours from each of ten multiparous Angus cows per experimental season . The three sampling seasons were on March 14, June 25, 2008 and September 23 and averaged 64, 167 and 257 days post partum.

[2] Low herd weaning weight ratio (5 cows with life time weaning weight records 6 % below herd average).

[3] High herd weaning weight ratio (5 cows with life time weaning weight records 9.4% above herd average).

[4] Standard error of the means.

Table 7: Fermentation end products in rumen fluid (in vivo) as influenced by season of sampling post-partum .[1]

In Vitro Digestion Parameter	March 14	June 25	September 23	SEM[2]	P > F
Acetate (µmoles/ml)	72.66	75.90	72.09	8.42	0.88
Propionate (µmoles/ml)	17.20	17.21	15.56	2.11	0.66
Isobutyrate (µmoles/ml)	2.02[a]	3.96[b]	1.54[a]	0.32	<.0001
Butyrate (µmoles/ml)	13.70[a]	16.57[a]	9.54[b]	1.44	0.0004
Isovalerate (µmoles/ml)	1.88[a]	3.99[b]	1.47[a]	0.49	<.0001
Valerate (µmoles/ml)	0.97[a]	1.62[b]	0.87[a]	0.26	0.02
Total (µmoles/ml)	108.43	119.25	101.06	12.13	0.32
Acetate:Propionate	4.22[a]	4.46[a,b]	4.71[b]	0.18	0.02
pH	7.42	7.23	7.42	0.15	0.36
NH3	9.87[a]	4.92[b]	4.32[b]	0.79	<.0001

[1] The FEPs and pH were determined from the rumen fluid of the cows at 0 hour. The three sampling seasons were March 14, June 25, 2008 and September 23 and averaged 64, 167 and 257 days post partum.
[2] Standard error of the means.
[a.b] Means in a row with different superscripts are different.

Table 8: Impact of rumen fluid used as inoculant from cows selected according to productivity (weaning weight ratio) on 24 hour in vitro volatile fatty acid (VFA) production.[1]

Volatile Fatty Acid	Cow Productivity			
	Low WWR[2]	High WWR[3]	SEM[4]	P > F
Acetate (µmoles/ml)	31.63	31.63	1.52	1.00
Propionate (µmoles/ml)	9.55	10.48	0.44	0.04
Isobutyrate (µmoles/ml)	1.56	1.60	0.17	0.82
Butyrate (µmoles/ml)	4.47	5.22	0.38	0.06
Isovalerate (µmoles/ml)	1.74	1.89	0.14	0.31
Valerate (µmoles/ml)	2.65	2.77	0.15	0.45
Total (µmoles/ml)	51.60	53.58	2.31	0.40
Acetate:Propionate	3.35	3.02	0.11	0.01

[1] Each mean was estimated from thirty observations corresponding to one of 4 repeat in vitro incubations from rumen fluid collected from five multiparous Angus cows at 3 seasons post partum. VFA production was determined as the difference between the individual VFA concentrations of the post fermentation supernatant fluids and the VFA concentrations of the rumen fluid of the corresponding cow at 0 hour, corrected for the concentration of McDougall buffer. The three sampling seasons were on March 14, June 25, 2008 and September 23 and averaged 64, 167 and 257 days post partum.

[2] Low herd weaning weight ratio (5 cows with life time weaning weight records 6 % below herd average).

[3] High herd weaning weight ratio (5 cows with life time weaning weight records 9.4% above herd average).

[4] Standard error of the means.

Significant differences between LWWR and HWWR observed within 24 hours of incubation are presented, $P \leq 0.05$, or as shown. The 24 hour volatile fatty acid production values were all significantly different from the 48 hour incubation results, $P \leq 0.05$, with the exception of Isobutyrate and Acetate:Propionate.

[a,b] Means in a row with different superscripts are different, $P \leq 0.05$, or as shown..

Table 9: Impact of rumen fluid used as inoculant from cows selected according to productivity (weaning weight ratio) on 48 hour in vitro volatile fatty acid (VFA) production.[1]

Volatile Fatty Acid	Cow Productivity			
	Low WWR[2]	High WWR[3]	SEM[4]	P > F
Acetate (µmoles/ml)	40.94	38.70	2.01	0.28
Propionate (µmoles/ml)	13.21	12.48	0.74	0.33
Isobutyrate (µmoles/ml)	1.84	1.60	0.22	0.28
Butyrate (µmoles/ml)	5.84	5.39	0.56	0.43
Isovalerate (µmoles/ml)	2.48	2.17	0.17	0.09
Valerate (µmoles/ml)	3.75	3.59	0.13	0.23
Total (µmoles/ml)	68.06	63.92	3.45	0.24
Acetate:Propionate	3.14	3.13	0.12	0.92

[1] Each mean was estimated from thirty observations corresponding to one of 4 repeat in vitro incubations from rumen fluid collected from five multiparous Angus cows at 3 seasons post partum. VFA production was determined as the difference between the individual VFA concentrations of the post fermentation supernatant fluids and the VFA concentrations of the rumen fluid of the corresponding cow at 0 hour, corrected for the concentration of McDougall buffer. The three sampling seasons were on March 14, June 25, 2008 and September 23 and averaged 64, 167 and 257 days post partum.

[2] Low herd weaning weight ratio (5 cows with life time weaning weight records 6 % below herd average).

[3] High herd weaning weight ratio (5 cows with life time weaning weight records 9.4% above herd average).

[4] Standard error of the means.

Significant differences between LWWR and HWWR observed within 48 hours of incubation are presented, $P \leq 0.05$, or as shown. The 48 hour volatile fatty acid production values were all significantly different from the 24 hour incubation results, $P \leq 0.05$, with the exception of IsoButyrate and Acetate:Propionate.

[a,b] Means in a row with different superscripts are different, $P \leq 0.05$, or as shown..

Table 10: Impact of rumen fluid used as inoculants as influenced by season of sampling post-partum on 24 hour in vitro dry matter (DM), neutral detergent fiber (NDF) digestibility and soluble protein.[1]

In Vitro Digestion Parameter	March 14	June 25	September 23	SEM[2]	P > F
DM digestibility (%)	37.83[a]	41.90[b]	41.83[b]	1.13	0.002
NDF digestibility (%)	36.13	38.46	35.46	1.49	0.20
Soluble protein post fermentation (mg)	23.87[a]	34.64[b]	25.18[a]	2.70	0.001
Soluble protein post pepsin digestion (mg)	19.99[a]	27.90[b]	19.38[a]	2.04	0.0004
Total soluble protein (mg)	43.86[a]	62.53[b]	44.57[a]	2.81	<.0001
Total soluble protein/ DM digested (g/g)	0.19[a]	0.24[b]	0.18[a]	0.01	<.0001
DM digested/Total soluble protein (g/g)	5.36[a]	4.13[b]	5.92[a]	0.41	0.001
NDF digested/Total soluble protein (g/g)	3.02[a]	2.24[b]	2.98[a]	0.24	0.01

[1] Each mean was estimated from twenty observations corresponding to 4 repeat in vitro incubations from rumen fluid collected by stomach tube from five multiparous Angus cows at 3 seasons post partum. The three sampling seasons were on March 14, June 25, 2008 and September 23 and averaged 64, 167 and 257 days post partum.

[2] Standard error of the means.

[3] Soluble Protein as measured in the supernatant from the the fermentation and pepsin digestion phases of the Tilley and Terry procedure using the Lowry protein assay.

[a.b] Means in a row with different superscripts are different, $P \leq 0.05$, or as shown.. Season differences in in vitro digestion activity between post calving seasons within 24 hours of incubation time are shown above. These values were all significantly different from the 48 hour incubation results, $P \leq 0.05$, with the exception of NDF digested per supernatant protein.

Table 11: Impact of rumen fluid used as inoculant as influenced by season of sampling post-partum on 48 hour in vitro dry matter (DM), neutral detergent fiber (NDF) digestibility and soluble protein.[1]

In Vitro Digestion Parameter	March 14	June 25	September 23	SEM[2]	P > F
DM digestibility (%)	48.79[a]	53.22[b]	53.17[b]	1.89	0.04
NDF digestibility (%)	50.04	50.53	50.17	2.21	0.91
Soluble protein post fermentation (mg)	28.90[a]	41.88[b]	30.66[a]	3.48	0.002
Soluble protein post pepsin digestion (mg)	16.39[a]	22.18[b]	18.20[a,b]	2.35	0.06
Total soluble protein (mg)	45.29[a]	64.06[b]	48.86[a]	5.02	0.002
Total soluble protein/ DM digested (g/g)	0.15[a]	0.20[b]	0.15[a]	0.02	0.03
DM digested/Total soluble protein (g/g)	7.40[a]	5.23[b]	6.83[a,b]	1.07	0.10
NDF digested/Total soluble protein (g/g)	4.49[a]	2.93[b]	3.81[a,b]	0.64	0.07

[1] Each mean was estimated from twenty observations corresponding to 4 repeat in vitro incubations from rumen fluid collected by stomach tube from five multiparous Angus cows at 3 seasons post partum. The three sampling seasons were on March 14, June 25, 2008 and September 23 and averaged 64, 167 and 257 days post partum.

[2] Standard error of the means.

[3] Soluble Protein as measured in the supernatant from the the fermentation and pepsin digestion phases of the Tilley and Terry procedure using the Lowry protein assay.

[a.b] Means in a row with different superscripts are different, $P \leq 0.05$, or as shown.. Season differences in in vitro digestion activity between post calving seasons within 48 hours of incubation time are shown above. These values were all significantly different from the 24 hour incubation results, $P \leq 0.05$, with the exception of NDF digested per supernatant protein.

Table 12: Impact of rumen fluid used as inoculant as influenced by season of sampling post-partum on 24 hour in vitro volatile fatty acid (VFA) production.[1]

Volatile Fatty Acid	March 14	June 25	September 23	SEM[2]	P > F
Acetate (µmoles/ml)	33.09[a]	32.67[a,b]	29.12[b]	1.86	0.08
Propionate (µmoles/ml)	10.26[a]	10.95[a]	8.84[b]	0.53	0.002
Isobutyrate (µmoles/ml)	2.02[a]	1.26[b]	1.46[b]	0.21	0.004
Butyrate (µmoles/ml)	4.91	4.71	4.92	0.47	0.88
Isovalerate (µmoles/ml)	2.07[a]	1.35[b]	2.03[a]	0.18	0.001
Valerate (µmoles/ml)	2.68	2.83	2.61	0.19	0.51
Total (µmoles/ml)	55.02[a]	53.76[a,b]	48.98[b]	2.83	0.10
Acetate:Propionate	3.26[a]	2.98[b]	3.31[a]	0.13	0.04

[1] Each mean was estimated from thirty observations corresponding to one of 4 repeat in vitro incubations from rumen fluid collected from five multiparous Angus cows at 3 seasons post partum. VFA production was determined as the difference between the individual VFA concentrations of the post fermentation supernatant fluids and the VFA concentrations of the rumen fluid of the corresponding cow at 0 hour, corrected for the concentration of McDougall buffer. The three sampling seasons were on March 14, June 25, 2008 and September 23 and averaged 64, 167 and 257 days post partum.

[2] Standard error of the means.

a.b Means in a row with different superscripts are different, $P \leq 0.05$, or as shown.. Season differences in in vitro volatile fatty acid production between post calving seasons within 24 hours of incubation time are shown above. These values were all significantly different from the 48 hour incubation results, $P \leq 0.05$, with the exception of IsoButyrate and Acetate:Propionate.

Table 13: Impact of rumen fluid used as inoculant as influenced by season of sampling post-partum on 48 hour in vitro volatile fatty acid (VFA) production.[1]

Volatile Fatty Acid	March 14	June 25	September 23	SEM[2]	P > F
Acetate (µmoles/ml)	43.39[a]	42.79[a]	33.28[b]	3.23	0.001
Propionate (µmoles/ml)	14.02[a]	14.14[a]	10.37[b]	0.91	0.0004
Isobutyrate (µmoles/ml)	1.97	1.55	1.63	0.27	0.26
Butyrate (µmoles/ml)	5.54	6.06	5.24	0.68	0.4885
Isovalerate (µmoles/ml)	2.65[a]	2.09[b]	2.24[a,b]	0.21	0.04
Valerate (µmoles/ml)	4.19[a]	3.75[b]	3.08[c]	0.16	<.0001
Total (µmoles/ml)	71.78[a]	70.36[a]	55.84[b]	4.23	0.001
Acetate:Propionate	3.11	3.06	3.23	0.14	0.48

[1] Each mean was estimated from thirty observations corresponding to one of 4 repeat in vitro incubations from rumen fluid collected from five multiparous Angus cows at 3 seasons post partum. VFA production was determined as the difference between the individual VFA concentrations of the post fermentation supernatant fluids and the VFA concentrations of the rumen fluid of the corresponding cow at 0 hour, corrected for the concentration of McDougall buffer. The three sampling seasons were on March 14, June 25, 2008 and September 23 and averaged 64, 167 and 257 days post partum.

[2] Standard error of the means.

a.b Means in a row with different superscripts are different, $P \leq 0.05$.. Season differences in in vitro volatile fatty acid production between post calving seasons within 48 hours of incubation time are shown above. These values were all significantly different from the 24 hour incubation results, $P \leq 0.05$, with the exception of isobutyrate and acetate:propionate.

Table 14: Impact of cow productivity on fecal % neutral detergent fiber (NDF), % indigestible neutral detergent fiber (INDF), % potentially digestible neutral detergent fiber (PDNDF) and % dry matter (DM) contents.[1]

In Vitro Digestion Parameter	Cow Productivity			
	LWWR[2]	HWWR[3]	SEM[4]	P > F
NDF content (%)	63.82	56.78	4.84	0.12
Indigestible NDF content (%)	30.87	27.80	2.38	0.29
Potentially digestible NDF content (%)	32.945	28.97	2.82	0.13
DM content (%)	10.66	10.30	1.04	0.84

[1] Each mean was estimated from ten observations corresponding to 4 repeated in vitro incubations from fecal samples collected from ten multiparous Angus cows grouped as having weaning weight ratios higher or lower than the average life time herd weaning weight ratio, dried and ground to 2 mm texture and incubated in a composite rumen fluid sample obtained from the same cows by stomach tube at 3 seasons post partum. The three sampling seasons were March 14, June 25, 2008 and September 23 and averaged 64, 167 and 257 days post partum. 5 of the cows were 6% below this average and 5 of them were 9.4% above it.

[2] Low herd weaning weight ratio (5 cows with life time weaning weight records 6 % below herd average).

[3] High herd weaning weight ratio (5 cows with life time weaning weight records 9.4% above herd average).

[4] Standard error of the means.

Table 15: Impact of sampling season on fecal % neutral detergent fiber (NDF), % indigestible neutral detergent fiber (INDF), % potentially digestible neutral detergent fiber (PDNDF) and % dry matter (DM) contents.[1]

In Vitro Digestion Parameter	March 14	June 25	September 23	SEM[2]	P > F
NDF content (%)	60.67	59.32	60.71	10.24	0.97
Indigestible NDF content (%)	32.54	27.06	28.39	2.29	0.15
Potentially digestible NDF content (%)	28.13	32.80	32.32	3.51	0.34
DM content (%)	11.14	9.95	10.24	1.30	0.44

[1] Each mean was estimated from ten observations corresponding to 4 repeated in vitro incubations from fecal samples collected from ten multiparous Angus cows grouped as having weaning weight ratios higher or lower than the average life time herd weaning weight ratio, dried and ground to 2 mm texture and incubated in a composite rumen fluid sample obtained from the same cows by stomach tube at 3 seasons post partum. The three sampling seasons were March 14, June 25, 2008 and September 23 and averaged 64, 167 and 257 days post partum. 5 of the cows were 6% below this average and 5 of them were 9.4% above it.

[2] Standard error of the means.

Table 16: Significant interaction effects of productivity and season on parameters measured in vitro.

Parameter[1]	Interaction	SEM	P< F
PSP (mg)	Productivity * Season	4.41	0.1
Pr (µmoles/ml)	Productivity * Season	1.49	0.07
IB (µmoles/ml)	Productivity * Season	0.48	0.06
Bu (µmoles/ml)	Productivity * Season	1.17	0.06
IV (µmoles/ml)	Productivity * Season	0.39	0.10
TVFA (µmoles/ml)	Productivity * Season	7.2	0.08

[1]Parameters are measured over 24 and 48 hours of incubation and values refer to means of 4 repetitions per incubation time.

Table 17: Significant interaction effects of season and incubation time on parameters measured in vitro.

Parameter[1]	Interaction	SEM	P< F
Ac	Season * Incubation time	4.37	0.09
Pr (µmoles/ml)	Season * Incubation time	1.49	0.1
Va (µmoles/ml)	Season * Incubation time	0.35	0.01
TVFA (µmoles/ml)	Season * Incubation time	7.20	0.09

[1]Parameters are measured over 24 and 48 hours of incubation and values refer to means of 4 repetitions per incubation time.

Table 18: Significant interaction effects of productivity and incubation time on parameters measured in vitro.

Parameter[1]	Interaction	SEM	P< F
Pr (µmoles/ml)	Productivity * Incubation time	1.49	0.06
IV(µmoles/ml)	Productivity * Incubation time	0.39	0.05
Bu (µmoles/ml)	Productivity * Incubation time	1.17	0.08
Ac: Pr	Productivity * Incubation time	0.27	0.05

[1]Parameters are measured over 24 and 48 hours of incubation and values refer to means of 4 repetitions per incubation time.

Conclusion

It was found that animal productivity did not have a significant effect on in vitro digestion activity ($P > 0.1$). It was, however, found that animal productivity significantly affected 24 hour in vitro production rates of propionate ($P < 0.1$), butyrate ($P<0.1$) and acetate: propionate ($P < 0.1$). In vitro digestion activity and VFA production were influenced by the season post partum that rumen fluid was sampled. The effect of rumen fluid sampling season is likely due to seasonal differences in intake due to either changing animal requirements or forage nutrient availability and supplemental feeding.

Results from this study indicate that rumen fluid from individual animals may possess some variability in its microbial activity as related to animal productivity but that this is much less evident than differences in microbial activity due to sampling season postpartum. The differences in rumen fluid microbial activity in vitro as related to cow productivity were found with propionate production and the acetate: propionate ratio. Although differences in cow productivity in this study were based on established production records these may not have been of the magnitude to influence microbial digestion activity as much as the impact of sampling season.

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