EARLY LIFE NUTRITION OF DAIRY CALVES AND ITS IMPLICATIONS ON FUTURE MILK PRODUCTION

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

Fernando Soberon

January 2012

EARLY LIFE NUTRITION OF DAIRY CALVES AND ITS IMPLICATIONS ON FUTURE MILK PRODUCTION

Fernando Soberon, Ph. D.

Cornell University 2012

The pre-weaning management of dairy calves over the last 30 years has focused on mortality, early weaning and rumen development. Recent data has demonstrated the potential to influence metabolic programming and consequently alter future performance of dairy cattle through nutrition and management during the first few months of life. Two main management practices have been identified as having the greatest impact in future performance: colostrum management and energy intake pre-weaning. Therefore, the first objective was to investigate the relationship between nutrient intake from milk replacer and pre and post-weaning growth rate with lactation performance. The evaluation of over 1,800 first lactations resulted in a strong relationship between average daily gain (ADG) pre-weaning and milk production so that for every additional kg of ADG pre-weaning first lactation milk production is increased by 850 kg. The relationship is equally strong when assessed by energy intake above maintenance from milk replacer during the pre-weaning period. The second objective of this work was to evaluate the effects of pre-weaning nutrition on mammary gland development. The mammary gland, especially the parenchymal mass were shown to be responsive to nutrient intake such that the parenchymal mass of calves that consumed more nutrients grew 5.6 times faster than that of control calves. This was significant because it demonstrated that allometric growth can be initiated from birth and that the mammary gland is very nutrient responsive, something not readily recognized in dairy cattle. The interaction of colostrum status and pre-weaning nutrient intake was also evaluated and the data suggests that the effects of colostrum management can be amplified or muted depending on nutritional status pre-weaning. Colostrum contains an array of different growth factors that can account for some of the long-term impacts on growth and efficiency; a

preliminary study evaluating hormone levels post-colostrum feeding in newborn calves suggest that hormones present in colostrum may be directly absorbed by the calf and could impact metabolic programming through the “Lactocrine hypothesis”. Protein synthesis and accretion is proposed as the primary signal that enhances future performance of pre-weaned cattle; however, milk protein sources are expensive and feeding high levels of such proteins may not always be cost effective. Therefore, alternative protein sources were evaluated as a way to reduce the cost of milk replacers. In this study, milk replacers containing whey based protein were the most effective milk replacers, with or without a modified amino acid profile.

BIOGRAPHICAL SKETCH

Fernando Soberón was born on December 28, 1978 in México City, México to Fernando Soberón and María del Consuelo Sieiro de Soberón. Fernando is the firstborn of the family- he has one sister and two brothers: Mariana, Pablo and Gerardo from eldest to youngest. Fernando attended Instituto Cumbres, a Catholic school, from Kindergarten throughout high school. He studied sixth grade at Oaklawn Academy in Edgerton, Wisconsin. After graduating from high school, he spent six months working on a dairy farm in Torreón, México, where he developed a taste for dairy cattle management. He studied his undergraduate degree at Instituto Tecnológico de Estudios Superiores de Monterrey Campus Querétaro in Querétaro, México. During his undergraduate studies he attended Iowa State University as an exchange student for one semester. He graduated in 2002 with the degree of Ingeniero Agrónomo Zootecnista. After graduation, Fernando started working for Elanco Animal Health as a sales representative, where he was promoted to territory manager, a job that he carried out until July of 2005 when he moved to Ithaca, New York to attain his Master of Science degree under the guidance of Dr. David M. Galton and Dr Thomas R. Overton. His thesis research was on the effects of frequent milking during early lactation on milk yield, carry over effect and mammary cell proliferation. During his Master's program, he married Melanie A. Schotthofer. After completion of his M.S. degree, he started working on his Ph D degree under the guidance of Dr. Michael E. Van Amburgh. His dissertation work encompasses the effects of early life management and nutrition and its effects on long term productivity. Specific areas of research include proliferation of stem cells in the mammary gland, feed efficiency, colostral transfer of growth factors and hormonal regulators as well as gene expression and epidemiological data analysis. During his Ph D, Fernando became father to Fernando Stephen Soberón in 2009 and Elena Grace Soberón in 2011.

Le dedico esta publicación a mi esposa Melanie, su constante apoyo ha sido imprescindible para poder culminar estos estudios. También le dedico esta publicación a mi hijo Fernando, su jovialidad y tierna disposición han iluminado mis días y su admiración alimenta mis aspiraciones. Esta publicación no sería posible sin el continuo e incondicional apoyo de mis padres y abuelos.

ACKNOWLEDGMENTS

I would like to start by thanking Dr. Michael E. Van Amburgh, his friendship, support and encouragement have been fundamental not only for the culmination of this project, but for personal and professional development and growth.

I also want to thank the other members of my committee, Dr. Yves R. Boisclair, Dr. Qiaoming Long and Dr. Daryl V. Nydam, for their contributions to this work.

I want to properly acknowledge my sponsor, the Consejo Nacional de Ciencia y Tecnologia (CONACyT), who in conjunction with the Graduate School of Cornell University founded my tuition, health insurance and living expenses during my Ph D program.

I also want to thank the multiple companies that contributed either through monetary donations or through product donations to each of the research projects here presented, in no particular order I express my gratitude to Milk Specialties Co., Milk Products Inc. Cargill, APC Inc., Land O'Lakes Inc., DeLaval Inc., Förster Technik.

This project wouldn't be possible without the constant love and support from my family and friends that have backed me up from the beginning. I'm especially blessed by the love and support of my wife, Melanie Soberón, who among other countless contributions spent many hours correcting my English.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AA = Amino acids

ADC = Average daily change

ADG = Average daily gain

AFC = Age at first calving

BCS = Body condition score

BrdU = bromo-deoxyuridine

bST = Bovine somatotropin

BW = Body weight

CP = Crud protein

DM = Dry matter

DMI = Dry matter intake

E = Enhanced

EPT = Extra parenchyma tissue

FCM = Fat corrected milk

FPT = Failure of passive transfer

GH = growth hormone

HH = Hip height

IGF-1 = Insulin-growth factor-1

IgG = Immunoglobulin G

Mcal = Mega calories

ME = Metabolizable energy

MG = Mammary gland

MPT = Mid parenchyma tissue

MR = Milk replacer

MRP = Enzyme modified proteins

NRC = National Research Council

PCTRL = Positive control

R = Restricted

RT-PCR = Real time Polymerase chain reaction

TDM = Test Day Model

TRT = Treatment

CHAPTER I

LITERATURE REVIEW

INTRODUCTION

It is common for dairy producers to categorize the rearing of calves and heifers as a necessary expense within the dairy operation. However, few producers understand how intimately related the management of these animals is with their future profitability. Many years of research have encouraged dairy producers to adopt low cost management systems and that providing extra nutrients to pre-pubertal calves would have detrimental consequences to future milk production.

The goals for raising replacement heifers should go beyond achieving a specific weight gain at the least possible cost. Given that they are future dairy cows, the final goal of heifer rearing should be to optimize their future milk production potential. This review will identify some of the critical stages of growth of heifers as well as the biological explanation for such critical stages.

The main goal of this work was to link nutrition and management at critical stages of dairy cattle growth with their potential effects on future productivity and profitability.

COLOSTRUM

Colostrum is the first secretion from the mammary gland of female mammals after the birth of their progeny. It is different from milk and even though it varies by species, it usually contains higher solids content and is rich in antibodies in the form of immunoglobulins. Depending on the species, these immunoglobulins can be absorbed by the newborn as a source of passive immunity. In humans, immunoglobulin A is the main source of immunoglobulins, representing 90% of all the immunoglobulin in human colostrum (Stelwagen et al., 2009). While in cattle the main immunoglobulin present in colostrum is immunoglobulin G (IgG), representing 81% to 85% of all the immunoglobulins (Beam et al., 2009; Stelwagen et al., 2009), colostrum also

contains a variety of hormones and growth promoters such as growth hormone, insulin-like growth factor, leptin, prolactin and relaxin (Baumrucker, 1994; Blum and Baumrucker, 2002; Elfstrand et al., 2002; Pinotti and Rosi, 2006). Colostrum is lower in carbohydrates than milk and higher in fat and protein. The production of colostrum by the dam discontinues at birth.

Immunoglobulin concentrations in bovine colostrum have been the standard measure of colostrum quality. The focus on immunoglobulins in colostrum as a measure of quality is derived from the idea that calves depend on these IgG's to support their immune system during the first weeks of life, given that there is no transfer of immunoglobulins through the placenta. So far, most of the benefits of colostrum feeding have been attributed to IgG's and to a lesser extent, to gut development promoted by growth hormone, IGF-1 and other growth factors present in colostrum (Robison et al., 1988; Elfstrand et al., 2002; Blum and Baumrucker, 2002; Gooden, 2008).

In the cattle, the concentration of immunoglobulins in colostrum drastically declines within 6 h post-calving. Moore et al. (2005) sampled colostrum at 2 h, 6 h, 10 h and 14 h postpartum to evaluate the effects of time harvested on colostrum IgG concentration. They found a decrease in IgG content of colostrum corresponding to increased time post-calving; IgG concentration 2 h post calving was 113 g/L and significantly decreased to 94, 82 and 76 g/L at 6, 10 and 14 h respectively. Nardone et al. (1997) reported similar findings when analyzing colostrum at 1, 12, 24 and 36 h post-calving. They also reported a decrease in total protein and fat content and an increase in lactose as time after calving increased.

Many factors can affect the IgG concentration of bovine colostrum, including parity (Moore et al., 2005), heat stress during the pre-calving months (Nardone et al., 1997), vitamin and mineral supplementation as well as immunization status of the dam.

For many years the main purpose of colostrum administration has been to provide passive immunity to the newborn calf; therefore, the goal has been to increase the immunoglobulin levels in the calves' blood. Calves are usually tested between 24 and 48 h after birth and the goal is to have more than 10 mg/mL of IgG in blood plasma. If a calf has less than 10 mg/mL of IgG, it is

considered to have failure of passive transfer (FPT) (Beam et al., 2009). Other studies have suggested that FPT should be considered for calves with less than 12 mg/ mL (Virtala et al., 1999) or even 13.4 mg/ mL (Tyler et al., 1998) given the morbidity observed in calves with these cut-off points for plasma levels of IgG. Some well known factors that have an effect on passive transfer are timing of ingestion of colostrum, the method used to deliver the colostrum, the volume of administered colostrum, the immunoglobulin concentration of colostrum and the age of the dam that provided the colostrum (Weaver et al., 2000). Other factors associated with the absorbance capacity of the calves include the bacterial load in colostrum or the bacterial load ingested by the calf before colostrum was provided (Corley et al., 1977).

The absorption of IgG in calves is possible due to the nonselective ability of the enterocytes of the small intestine to absorb macromolecules by pinocytosis. The cessation of macromolecule absorption is called gut closure, and in the calf, it occurs approximately 24 h post birth. This may be extended up to 36 h if colostrum ingestion is delayed. However, the maximum absorption will occur within the first 4 h of life and after 12 h the absorptive capacity will be reduced (Weaver et al., 2000).

Total amount of colostrum consumed by the calf is a critical factor affecting passive transfer in the calf; in a study comparing three different methods of delivering colostrum to calves, researchers found that when the calves are left alone to nurse from their mothers, 67% of the calves were classified to have failure of passive transfer. If the calves were given colostrum via a bottle or with a tube feeding, 19.3% and 10.8% respectively had failure of passive transfer; within these two groups total volume fed was the factor with the greatest influence (Besser et al., 1991). Chigerwe et al. (2009) conducted a study where calves were offered up to 3 L of colostrum via a nipple bottle within the first 4 h of life; they found that only 17.2% of the calves consumed the 3 L of colostrum. The study showed that there was no effect of amount of colostrum consumed in the first feeding with amount of colostrum consumed 12 h later. For the calves that consumed 3 L of colostrum, the probability of failure of passive transfer was less than 0.05. In a study looking at differences in passive immunity between two different feeding

methods, calves fed 3 L of colostrum exhibited no difference in the apparent efficiency of absorption of IgG (Gooden et al., 2009). A cross-sectional study performed on 394 dairy operations in 2007 demonstrated that almost 20% of dairy calves in the United States were classified with FPT (Beam et al., 2009) suggesting the industry still has more work to do to ensure colostrum absorption is optimized.

The IgG concentration in colostrum is crucial in determining the total amount of colostrum needed to provide proper passive immunity to the calves. Chigerwe et al. (2008) determined that in order to obtain proper passive immunity, the calves had to consume at least 153 g of colostral IgG before 2 h of life, and more if colostrum feeding was delayed; in this study, calf birth weight was not a significant factor. Good quality colostrum has been recognized as colostrum containing more than 50 g of IgG/L (Gooden, 2008). Therefore, calves would require at least 3 L of good quality colostrum within the first 2 h of life to acquire proper passive transfer.

There are many methods used to evaluate the IgG concentration of colostrum. The standard test is a laboratory test known as the radial immunodiffusion assay, which directly measures the IgG content of colostrum. However, it takes up to 24 h to get results, making it impractical for farm implementation (Bielmann et al., 2010). Cow-side tests have been used to estimate the IgG content of colostrum; for example, the colostrometer uses the specific gravity of colostrum to estimate the IgG content. However, other factors such as fat content of colostrum as well as total solids and temperature of colostrum affect the specific gravity. Pritchett et al. (1994) reported that 2 of every 3 samples of colostrum with low IgG counts can be mislabeled as good quality with this method so they suggest adjusting the reading depending on the amount of colostrum that is being fed to the calves.

Other cow side tests include the Brix refractometer instruments. These instruments measure the total solids in colostrum but in contrast with the colostrometer, they are not affected by temperature. The Brix instrument, when compared with the radial immunodiffusion assay, had a correlation coefficient between 0.71 and 0.74 (Bielmann et al, 2010). Commercial laboratories have developed other cow side tests such as the Colostrum Bovine IgG Quick Test Kit from

Midland Bio-Products, which will provide an answer in about 20 minutes that will classify colostrum as ‘good’ or ‘poor’ quality but will not give an actual concentration of IgG.

For many years, it has been believed that colostrum from first lactation cows is of lower quality than that from multiparous cows. However, this assumption cannot be made blindly since colostrum IgG content has a normal distribution across multiple samples and in the study by Grusenmeyer et al. (2006) first lactation cattle had IgG concentrations equal to multiparous cattle. First lactation cows, as well as second lactation cows, had a lower mean concentration of IgG than cows in their third and greater lactation, but most of the colostrum still tested above the cut-off point of 50 g/L of IgG, determining it to be of good quality (Bielmann et al., 2010; Tyler et al., 1999; Pritchett et al., 1991). A more accurate analysis will conclude that colostrum should be discarded based on a determination of the IgG concentration rather than on parity of the cow.

Correlations between FPT and post-weaning growth performance have been made. Robison et al. (1988) reported a significant correlation between serum IgG levels at 24 h post birth and average daily gain (ADG) from birth to 180 d. A year later, FPT was linked to decreased milk and fat production during first lactation (DeNise et al., 1989). In this study, there was a linear correlation such that every unit of increase in IgG in blood serum at 24 h of life was associated with a first lactation milk yield increase of 8.5 kg.

The effects of colostrum on life productivity were clearly described by Faber et al. (2005) in a study where Brown Swiss cattle were fed either 2 L or 4 L of colostrum at birth and managed in the same way thereafter. The researchers observed increased ADG in the calves fed 4 L of colostrum, consistent with previous reports. They also observed an increase in the survival rate through the end of the second lactation, and of the surviving cows, the group of cattle that received 4 L of colostrum produced 1,027 kg more milk during the first two lactations.

The increased in feed efficiency and performance observed in these studies, may be partially explained by differences in intestinal absorption capacity. Hammon and Blum (1997) conducted and experiment where they fed calves colostrum for 3 d, 1 d or fed milk replacer instead of colostrum and tested their xylose absorption at 5 d of life. Researchers observed that calves that

consumed colostrum had a greater rise in plasma xylose after dosage than calves that received only milk replacer. Glucose basal levels were also higher for colostrum fed calves compared to milk replacer fed calves. These findings are similar to those from Hadorn et al. (1997), who tested the effects of colostrum feeding during the first day and observed that colostrum fed calves had higher glucose, globulin, insulin and IGF-1 plasma levels up to d 7 of life.

In addition to immunoglobulins, colostrum contains insulin-like growth factors and other bioactive factors that both promote gut development and have systemic targets in the newborn calf. Colostrum intake in piglets and calves strongly influence the activity of digestive enzymes as well as the secretion of gastric and pancreatic hormones; the intestinal absorptive capacity is also modified by these bioactive substances (Hadorn et al., 1997; Blum and Baumrucker, 2002). Whole milk and formulas intended to replace or supplement colostrum lack some or all of these bioactive substances. When compared with colostrum, colostrum intake increased circulating concentrations of total protein, albumin, IgG, urea, amino acids, fatty acids, glucose, triglycerides, phospholipids and cholesterol as well as the endogenous secretion of IGF-1, IGFBPs, insulin, glucagon and cortisol (Blum and Baumrucker, 2002). Colostrum ingestion in piglets was shown to stimulate protein synthesis in skeletal muscle (Fiorotto et al., 2000) demonstrating that the other factors in colostrum are bioactive and can impart other actions on the developing neonate.

NUTRIENT REQUIREMENT OF THE CALF

The calf has a requirement for maintenance. Only once maintenance requirements have been met can growth be achieved, provided sufficient nutrients and the proper balance of nutrients are available to the calf. Therefore, it is important to understand the maintenance requirements of calves. The nutrient requirements of the calf have been described in the current Nutrient Requirements of Dairy Cattle 7th edition (NRC, 2001) publication. The requirements are very useful for diagnosing the impact of temperature on the maintenance requirements of the calf through the computer program that accompanies the publication.

The maintenance requirements estimated by 2001 NRC appear to be accurate in their reflection of field observations for overcoming negative energy balance brought about by cold stress conditions. Example requirements are demonstrated in Table 1.1 based on body weight and ambient temperature. However, the user needs to remember that these values are the basal requirements for energy to maintain core body temperature, not considering wind or other environmental conditions, which would exacerbate the requirements. The long-term consequences of not altering these values will be discussed later.

Once maintenance requirements are met, growth can occur. During the pre-pubertal phases of growth, when a balanced diet is provided, the body of all mammals will favor lean tissue growth. As puberty approaches, the composition of growth changes and adipose tissue starts increasing relative to lean tissue. As the animal approaches its mature body size, lean tissue growth is limited and most of the changes in body weight will be a consequence of adipose tissue. Therefore, body composition is directly related to growth rate, diet composition and stage of maturity at the time the growth occurred (Fox et al, 1999; Soberon and Van Amburgh, 2011).

Calves are born with about 4% body fat, of which about 50% can be mobilized; much of that is brown adipose tissue needed for thermogenesis (Diaz et al., 2001). This provides the calf with up to four days of energy reserves depending upon the ambient conditions. Once depleted, the calf has to rely on either dietary intake or body protein if nutrient intake is below maintenance requirements, to generate heat and mount an immune response. This creates a situation that encourages failure of the immune system unless additional calories from protein, carbohydrates and fat are provided (Keusch, 1977; Baracos et al., 1986; Romanyukha et al., 2006).

Body protein reserves are very low in neonatal calves and are not good sources of calories for maintaining body heat and mounting immune responses. An additional factor to be considered is the source of carbon for lipid deposition in the pre-weaned calf. Data from several studies demonstrate that calves cannot make fat from carbohydrate very effectively if at all; thus, any increase in adiposity must be from dietary fat intake (Tikofsky et al. 2001; Joost et al., 2007).

Table 1.1. The amount of milk replacer or milk dry matter (kg) required to meet the maintenance requirements of calves at varying temperatures. The calculations assume 5.4 Mcal ME per kg of dry matter (Modified from Van Amburgh and Drackley, 2005).

	Temperature, degrees C						
Body Weight, kg	20	10	0	-10	-15	-20	-30
27	0.27	0.36	0.41	0.45	0.50	0.54	0.63
36	0.36	0.41	0.50	0.59	0.63	0.68	0.77
45	0.45	0.50	0.59	0.73	0.77	0.82	0.91
54	0.50	0.59	0.68	0.77	0.86	0.91	1.04

Joost et al. (2007), using stable isotopes of glucose and fatty acids, demonstrated that adipose tissue in milk fed calves originates only from dietary fat and not carbohydrate. This has significant implications for dietary strategies for calves fed under conditions of cold stress. Under cold stress conditions or situations where feed intake is compromised due to illness, the only way to provide greater calories and energy reserves is through the increased intake of dietary fat. Milk fat is approximately 30-35% on a dry matter basis, thus milk replacers should be adjusted to a higher fat content to not only be closer to whole milk, but also to match the energy requirements of calves below their lower critical temperature. Compared to most milk replacers, this is likely why calf managers see significant increases in calf performance when whole milk is fed, especially in cold weather conditions.

New data that allows for better understanding of the components and requirements for growth of cattle have been generated (Bartlett, 2006, Diaz et al., 2001, Tikofsky et al., 2001; Bascom et al., 2007; Blome et al., 2002; Brown et al, 2005a; Meyer, 2006b; Mills, 2009). Table 1.2 summarizes the current knowledge about the requirements for growth of the calf based on the body composition data derived since the 2001 NRC was published (Van Amburgh and Drackley, 2005).

These values are consistent with the current publication (NRC, 2001), but have slightly lower energy requirements per unit of gain because the original equations were based on heavier veal type calves fed higher fat diets and depositing more fat per unit of weight gain (Van Amburgh and Drackley, 2005). The predictions for energy requirements in Table 1.2 are consistent with dairy replacement calves being fed diets more typical of our system. The protein requirements are higher than the NRC (2001) publication because of updated data on the efficiency of use of absorbed protein. These values represent the dynamic relationship between energy and protein given that protein requirements are very energy dependent; for example, the more energy they consume, the greater the potential protein synthesis, and the higher the protein requirement (Van Amburgh and Drackley, 2005).

Table 1.2. Energy and crude protein requirements of calves from birth to weaning (Van Amburgh and Drackley, 2005)

Rate of gain, kg/d	Dry matter intake, kg/d	Metabolizable energy, Mcal/d	Crude protein, g/d	Crude protein, % DM
0.20	0.54	2.4	94	18.0
0.41	0.63	2.9	150	23.4
0.60	0.77	3.5	207	26.6
0.80	0.91	4.1	253	27.5
1.00	1.09	4.8	307	28.7

These requirements reinforce the idea that what the cow would normally provide to the calf is a more appropriate combination of protein and energy required by the calf. Thus, many milk replacers are not really replacing milk because they do not contain equal nutrient levels and they are rarely fed to equal the nutrient intake of whole milk. It further suggests that least cost milk replacer formulations would not be able to provide much beyond maintenance energy supply and the feeding of such milk replacers at previously recommended levels might exacerbate the lack of immune system responsiveness and energy reserves needed to support an illness event. Again, dietary fat levels will be dependent upon the ambient temperatures. The body composition data would indicate that 15% fat milk replacer is adequate when the calves are not under cold stress conditions, and that as temperatures decrease, fat needs to increase to offset the fatty acid oxidation for thermogenesis (Jaster et al., 1990; Jaster et al., 1992; Van Amburgh and Drackley, 2005). In addition, attention should be made to the inclusion of essential fatty acids in the diet of neonatal and weaned calves since it appears traditional calf diets have been deficient in essential fatty acids required for proper growth (Hill et al., 2009). However, as new data indicates, newborn calves have requirements beyond those for maintenance and growth. Emerging data suggest that factors such as colostrum status and nutrient intake and therefore, growth rates, up to at least 8 weeks of age have lifetime effects that can be measured in the first lactation. Just like other neonates, it appears that early life events may serve as a catalyst for metabolic programming, generating epigenetic changes in the calves that will remain with them for their entire life; therefore "compensatory mechanisms" are not present for this stage of development.

MAMMARY GLAND DEVELOPMENT AND MILK YIELD

Bovine mammary gland growth was first described as isometric with body weight during the pre-weaning stage, followed by an allometric stage of growth until puberty, followed by another isometric stage of growth from puberty to conception, and finally, an allometric growth phase

during pregnancy (Sinha and Tucker, 1969). However, when these studies were done, there was no consideration to nutrient intake prior to weaning.

When researchers started examining the effects of nutrition on mammary gland development, they focused on what was known as the allometric stage of growth. The seminal work by Sejrsen et al. (1982; 1983) describing the effect of high energy intake on mammary development and the relationship with circulating growth hormone negatively linked the relationship between pre-pubertal growth, mammary development and future milk yield. The primary goal of this work was to provide a mechanism to explain why rapid growth during the pre-pubertal phase resulted in reduced milk production in the first lactation.

The observation of reduced mammary development with higher pre-pubertal daily gains could be repeated in almost every experiment (Pritchard et al., 1972; Petitclerc et al., 1984; Mäntysaari et al., 1995; Capuco et al., 1995; Meyer et al., 2006ab) making it one of the most repeatable experiments but not answering the question of what is the mechanism surrounding the effect. Most studies concluded that when nutrient intake, especially energy intake, was elevated from 3 to 4 mo of age until puberty, the fat pad of the mammary gland grew faster than the epithelium. Therefore, they concluded that higher levels of nutrition were detrimental to mammary gland development. These repeatable observations led to the conclusion that high energy intakes reduced mammary development through altered hormone status or signaling processes. However, Meyer et al. (2006a,b) demonstrated that the real effect was time and not energy intake and that the mammary gland grew at a constant rate independent of nutrient intake, thus younger heifers at harvest always contained less parenchymal tissue or DNA content. Further, that data explained most of the variation in mammary development observed in the previous studies strongly indicating that the real effect was simply time and that the mammary epithelial cells grows at a genetically predetermined rate between post-weaning and puberty and not in response to nutrient intake (Meyer, 2005).

The long-term impact of mammary development on milk yield seemed intuitive after the publication of the Sejrsen data (1982, 1983). However, when milk yield was evaluated against

mammary development differences in studies that evaluated pair fed animals as they entered lactation the link could not be sustained. Capuco et al. (1995) observed a 52% decrease in mammary development at puberty in heifers fed for higher rates of pre-pubertal gain, but in the pair fed animals, there was no significant difference in milk yield (Waldo et al., 1998). Smith (2002) fed a calcium salt of conjugated linoleic acid (Ca-CLA) and measured differences in body composition and pre-pubertal mammary development and followed pair-fed animals into lactation and measured milk yield. In this study, mammary development was reduced by approximately 60% in heifers fed Ca-CLA, however there was no significant difference in milk yield of the pair-fed heifers.

In studies by Radcliff et al. (1997; 2000), bST was administered from 125 to 336 kg of body weight to enhance pre-pubertal mammary development. In the tissue harvest study, mammary development was enhanced approximately 48% by the use of growth hormone (Radcliff et al., 1997). Milk yield from the heifers treated pre-pubertally with growth hormone did increase by approximately 5.9%, but that was not significant and not correlated with the increase in mammary parenchyma development (Radcliff et al., 2000). Thus, mammary development, measured as DNA content of the parenchyma at puberty, varied by about 100% (+48 to -60%) with no significant difference in milk yield. This strongly suggests that mammary development when measured as DNA content at puberty is not a good indicator of future milk yield.

Meyer et al. (2006ab) were the first to recognize that mammary development was not reduced by high energy intake; instead, the time to reach puberty and the associated signals to change allometric mammary growth were altered. Further, Meyers et al. (2006b) observed that when calves were provided with higher levels of nutrition prior to weaning, cell proliferation in the mammary epithelium was higher than in calves given traditional levels of nutrients. Since then, two additional studies have observed similar responses to nutrient intake prior to weaning (Brown et al., 2005b). Meyers et al. (2006b) also showed that post-weaning cell proliferation was not affected by nutrient intake, but was directly related to age. Therefore, when the mammary

gland growth was adjusted for age, differences in gland development of most published papers were explained (Meyer, 2005)

For the most part, efforts to correlate pre-pubertal mammary gland development with future productivity have proven unsuccessful, and this might be explained by the normal development of the mammary gland. In virgin heifers, the epithelium of the mammary gland is composed exclusively of ducts and stoma tissue. Secretory cells are not present in the mammary gland of non-pregnant mammals, and as previously mentioned, nutrient intake did not have an effect on epithelium growth from weaning to puberty. It is during pregnancy that all of the secretory tissue differentiates and the alveolar structures form. Therefore, changes in the proliferation or total mass of the mammary parenchymal tissue pre-breeding, fail to correlate with milk production (Waldo et al., 1998; Radcliff et al., 2000; Smith, 2002). However, it is now clear that pre-weaning is the stage of life where mammary epithelium is responsive to nutrient intake (Meyers et al., 2006b; Brown et al., 2005b). Studies that focused on the correlation of nutrient intake or average daily gain (ADG) pre-weaning, with respect to future milk production, have shown significant milk yield increases with increased ADG prior to weaning. This topic will be discussed in more detail later.

These concepts do not diminish the importance of mammary development, but rather provide opportunity to consider specific cell types instead of gross measurements such asusing DNA as a proxy for cell number (Sinha and Tucker, 1969; Ballagh et al., 2008).

The cell type that has received most attention has been mammary stem cells or mammary progenitor cells. This type of cells provides for growth and maintenance of the gland (Capuco et al., 2009). In an effort to increase the stem cell population of the mammary gland Capuco et al. (2009) infused xanthosine into half of the glands of lactating cattle and used bromo-deoxyuridine (BrdU) to labeled proliferating cells; after 40 d of last BrdU injection animals were harvested and BrdU labeled retaining cells were detected by Immunohistochemistry. The infusion of Xanthosine doubled the amount of labeled retaining cells from 0.4% on control half to 0.8% on

treated half. This experiment provides evidence of a responsive population of mammary stem cells within the mammary gland.

Increasing proliferation of stem cells in the mammary parenchyma of pre-weaned calves through nutrient intake has also been tested, but researchers found no differences in BrdU labeled retaining cells. However, they observed a positive correlation between mammary parenchyma growth and telomerase activity, another marker for stem cell population (Daniels et al., 2009).

Another population of cells that has been studied due to its possible role in altering the allometric growth rate of the mammary gland in growing cattle are the myoepithelial cells. Ballagh et al. (2008) reported that pre-pubertal ovariectomized dairy calves had an abundance of smooth muscle cells while intact calves had minimal presence of these cells until puberty. Myoepithelial cells are known to limit parenchymal cell proliferation, but the effect on specific cell types within the epithelium has not been investigated.

EPIGENETICS

The term epigenetics was proposed by Waddington in 1940, to describe the interaction of genes with the environment. Epigenetics was proposed to be responsible for phenotypic expression (Waddington, 1940). The term has since evolved to more accurately describe changes in gene expression that are independent of changes in DNA sequence. More importantly, the definition of epigenetics includes the ability to transfer such patterns of gene expression to the next generation through the inheritance of gene expression rather than gene sequence (Bartol et al., 2008).

The environment can influence gene expression in many different ways. Some environmental conditions generate immediate and short lived changes in gene expression while others generate long-lasting effects. Factors that affect developmental stages, especially those with long-lasting or heritable effects are known as epigenetic elements of the developmental program (Jirtle and Skinner, 2007). Within the developmental programming category of epigenetic changes,

nutritional programming and maternal programming are of special interest to those in animal production.

Although the terms “nutritional programming” and “imprinting” are relatively new (Plagemann, 2005), the concept was first described in 1966 by Dubos et al. Their work focused on the long-term impact of perinatal environment on human body weight and they named it “Biological Freudialism” (Dubos et al., 1966).

The term maternal programming refers to changes in development or gene expressions triggered by maternally derived signals. Maternal programming influences the development of embryonic, fetal and perinatal tissue. The maternal influence on developmental programming post-partum is most likely carried through the colostrum and milk in a system that has been recently described as the “Lactocrine hypothesis” (Bartol et al., 2008).

The long-lasting effects of maternal programming have been described in multiple species (Baumrucker and Blum, 1993; Donovan and Odle, 1994; Burrin et al., 1997; Blum and Hammon, 2000; Rauprich et al. 2000). In swine, maternal relaxin present in sow colostrum and milk during the first few days post-partum has been directly linked with the development of the female reproductive tract, by acting on specific receptors in the uterus (Bartol et al., 2008). Several other groups have also made a direct connection from a milk-born factor to a developmental function at the tissue or organ level (Nusser and Frawley, 1997; Bagnell et al., 2005).

In rats, maternal behavior was proven to alter the normal response of pups to stressors later in life through epigenetic mechanisms. Although this is not accomplished directly through nutrition or metabolic signals, it demonstrates that environmental conditions during the early post-natal period are capable of epigenetic imprinting which has long lasting implications (Weaver et al., 2004).

There is a considerable amount of human studies linking the effects of maternal under-nutrition and maternal diabetes during pregnancy with adult onset of obesity, metabolic syndrome and diabetes in progeny (Plagemann, 2005; Cottrell and Ozanne, 2007). Other reports

describe detrimental effects of over-nutrition during fetal and early life with disease outcomes in later in life (Armitage et al., 2005).

In animal production, many reports have associated early life parameters with future productivity. Even though the influence of epigenetic mechanisms was not studied, the outcome of such regulation has been observed (Robison et al., 1988; DeNise et al., 1989; Faber et al. 2005).

MILK PRODUCTION ASSOCIATED WITH NUTRITION

Over 60 years ago, researchers were already considering the relationship between heifer raising and future milk production. In 1957, Reid et al. (1957) reported advances in a study designed to compare the effects of under- and over-nutrition during the rearing period on future milk production and reproductive performance. Researchers reported numerically lower first lactation milk yields for heifers reared at 65% of the nutrient recommendations when compared to heifers reared at nutrient recommended levels. Heifers fed at 140% of recommendation had first lactation milk yields in between the other two groups. Second lactation milk yields were considerably lower for heifers fed 140% of requirements during the rearing period. Researchers did not report the starting time of the nutritional treatments.

The concept of accelerated growth during the rearing period has consistently been confounded with the effect of over-conditioning or fattening dairy heifers. Even though some researchers have clearly specified the fattening effect of such diets, as is the case with a study conducted by Swanson (1960) where the effect of rapid growth with fattening of dairy heifers was studied in twin animals, other researchers have failed to separate the effects of fattening vs. rapid growth on first lactation.

Heifers between 3 to 12 mo of age fed higher energy levels to achieve rapid growth and fattening and fed such diets until first calving, produced 84% less milk than their twin sisters fed at standard rates (Swanson, 1960). Fattened heifers in this study lost 14% of their body weight within two wk post-partum, while their twin sisters only lost 6% of their body weight post-

calving. Nine months post-calving; fattened heifers had not recovered their post-partum weight while their twin sisters had gained 64 kg of body weight 9 months post-calving. Researchers did not report BCS of cattle at any stage but inferences can be made given their post-partum behavior.

Few studies have evaluated the effects of nutritional management from birth on future milk production. In 1960, a study designed to evaluate the effects of nutritional management from birth to 44 wks and from 44 wks to 2 mo before first calving on milk yield throughout three lactations, observed no differences in milk yield or length of lactation among any of the four treatments (Crichton et al., 1960b). Growth rates for the low treatment during the first 44 wks of life were 0.44 kg/d compared with 0.68 kg/d for the high group; during the second part of the growing period, ADG were 0.55 kg and 0.84 kg for the low and high treatment, respectively. In this study, all cattle were bred by age regardless of body weight; therefore, heifers varied in body weight 3 mo post-calving from 370 kg to 429 kg (Crichton et al., 1959; Crichton et al., 1960ab).

When under-nutrition was examined, researchers observed reduced milk production of 285 kg of FCM on heifers fed 25% under the recommended feeding level from 4 to 24 months when compared to twin sisters fed at recommendation (Swanson and Hinton, 1964). The underfed twins had not matched their counterparts in body weight after three lactations, suggesting a permanent stunting on their growth potential due to under-nutrition during the rearing period.

In 1988, Johnsson conducted a review of all the studies that had evaluated the effects of nutrition during the rearing period on subsequent milk yield. In this review, the author pointed out the conflicting findings of different studies and identified possible sources of discrepancy among the results. Two of the most important sources of discrepancy of results were the definition of low versus high nutritional levels, which researchers have paid attention to and is no longer as big of a concern when comparing among different studies; the second source of variation was the age, weight, and plane of nutrition of the heifer calves at the time they started the study. Even though Johnsson identified this as a source of discrepancy in the results in 1988, the vast majority of the studies comparing pre-pubertal growth rates and future productivity

initiated their treatments between 3 to 5 months of age without accounting for previous nutritional management.

Pre and post-pubertal rates of gain have the potential to modify the composition of the gain, resulting in heifers with different body composition or BCS. The first report of pre-pubertal growth that acknowledged this effect reported that heifers grown at 0.94 kg/d from 90 to 320 kg of BW produced less milk than heifers grown at 0.68 kg/d during that same period. However, researchers noted that heifers differed in post-calving BW and BCS (Hohenboken et al., 1995). A regression analysis with their data revealed that pre-pubertal rate of gain explained little of the variation; in contrast, post-calving BW explained more of the variation in milk yield among treatments (Van Amburgh et al., 1998). The concept of composition of the gain in growing heifers was further explored by Soberon and Van Amburgh (2011). Using mathematical equations to predict the composition of the gain and therefore, the body composition at calving, of heifers reared at different rates, they were able to explain differences in milk yields among different studies evaluating milk yield responses to heifer growth rates (Soberon and Van Amburgh, 2011).

Exogenous growth hormone in well-fed animals is known to promote protein accretion and mammary development; Radcliff et al. (2000) evaluated the effect of a high protein, high energy diet during the pre-breeding stage with a daily dose of bovine somatotropin (bST). Heifers grown at 0.8 kg/d from 120 d until pregnancy produced more milk than heifers grown at 1.1 kg/d during that same period; milk production of heifers grown at 1.2 kg/d that received a daily dose of bST was in between the other two treatments. Increased protein synthesis in heifers administered daily bST might have resulted in differences in body composition, which could have contributed to the non-significant differences in the observed milk yield response – leaner animals at calving or animals containing more lean tissue would partition less nutrients towards growth and more towards milk yield.

In a few studies, researchers evaluated the effect of nutritional status on the post-pubertal heifer. Hoffman et al. (1996) compared the lactation performance of heifers reared at 969 g/d and

bred to calve at 21 mo versus heifers reared at 792 g/d and bred to calve at 24 mo. Pre-partum body weights of cattle were not different among treatments; however, wither height and post-partum body weight was lower for heifers reared at higher rates of gain. The heifers grown at higher rates post-puberty produced 2.5 kg/d less milk than control animals. Some of the reduced performance of this group of heifers can be attributed to the reported higher BCS at calving.

One of the first reported studies evaluating the effects of early life nutrition on milk performance was conducted by Foldager et al. (1997). Researchers evaluated the effects of feeding restricted amounts of whole milk (4.6 kg/d) for 42 d versus ad libitum whole milk for 42 or 87 d. Pre-weaning ADG were higher for ad libitum-fed calves (660 versus 960 g/d). From d 43 to 87, calves allowed to drink ad libitum milk had higher ADG than all other calves (600 versus 1,036 g/d). Beyond 87 d, all calves had similar ADG until calving. Calves fed milk ad libitum reached puberty a month earlier than calves fed restricted amounts of whole milk. Age at first calving was not reported, but heifers from all TRT had similar post-partum body weights. Heifers that were fed ad libitum whole milk for 42 d produced 1.6 kg/d more milk than calves fed restricted amounts of milk and 2.5 kg/d more milk than calves fed ad libitum for 87 d (25.3, 26.9 and 24.4 kg/d for restricted, ad libitum 42 d and ad libitum 87 d respectively; $P < 0.05$).

Similar findings have been reported by other researchers. Shamay et al. (2005) evaluated the long-term effects of feeding restricted amounts of milk replacer with those of feeding free access whole milk 2 times a day. In addition to the pre-weaning treatment, half of the calves from each treatment were supplemented with an additional 2% crude protein from 180 to 270 d. During lactation, calves fed free choice milk produced 1.2 kg/d more fat corrected milk than calves fed restricted amounts of milk replacer; furthermore, when the calves fed free choice milk were supplemented with additional protein pre-puberty, they produced 2.8 kg/d more fat corrected milk than calves fed restricted milk replacer, not supplemented with pre-pubertal protein. Findings from this study suggest that the benefits of early life nutrition can be maximized or silenced by their nutritional status pre-puberty. Moallem et al. (2010) observed a similar response to diet supplementation with crude protein during the post-weaning period of heifers

that were offered free choice whole milk during the pre-weaning period (3.5 kg/d of additional milk during first lactation). Moallem et al. (2010) did not observe differences in milk yield of calves offered free choice milk replacer versus calves offered free choice whole milk during the first 60 d of life indicating that milk replacer nutrient content and quality might be important in setting the animal up for the long-term response. Moallem et al., (2010) suggested that milk replacer did not contain the same bioactive growth factors available in milk and thus milk replacer should not be expected to stimulate the long-term impact observed in milk fed calves.

Other studies have observed numerical milk yield differences ranging from 416 to 718 kg during first lactation for calves fed either restricted or more appropriate diets during the pre-weaning period (Terré et al., 2009; Raeth-knight et al., 2009; Davis Rincker et al., 2011). Even though these studies did not have enough animals to achieve significant differences in lactation performance, their observations are consistent with other published data evaluating the effects of pre-weaning nutrition on future performance.

CONCLUSIONS

There is a growing amount of evidence both in dairy cattle as well as in other species that suggests that the metabolism of mammals is influenced in a permanent manner by their perinatal environment. Factors influencing the development of the newborn vary from in utero nutrient supply, post-natal hormonal regulation through the lactocrine effect through their mothers' colostrum or milk, early life nutrition and even early life non-nutritional nurturing. All of these different factors affect the expression of their genetic material and as far as we know today, are not able to be compensated later in life. However, future environmental conditions can maximize these changes. Understanding the metabolic adaptations that take place during post-natal life in mammals will allow researchers, physicians and producers to maximize performance, health and profitability of production animals as well as health of humans and companion animal species.

REFERENCES

Armitage J. A., P. D. Taylor, L. Poston. 2005. Experimental models of developmental programming: consequences of exposure to an energy rich diet during development. J Physiol. 565:3-8.

Bagnell, C.A., W. Yan, A.A. Wiley, F.F. Bartol. 2005. Effects of relaxin on neonatal porcine uterine growth and development. Ann. NY Acad. Sci. 1041:248-255.

Ballagh K., N. Korn, L. Riggs, S. L. Pratt, F. Dessauge, R. M. Akers, and S. Ellis. 2008. *Hot Topic:* Prepubertal ovariectomy alters the development of myoepithelial cells in the bovine mammary gland. J. Dairy Sci. 91:2992–2995.

Baracos V. E., W. T. Whitmore, R. Gale. 1986. The metabolic cost of fever. Can. J. physiol. Pharmacol. 65:1248-1254.

Bar-Peled U., B. Robinzon, E. Maltz, H. Tagari, Y. Folman, I. Bruckental, H. Voet, H. Gacitua, A. R. Lehrer. 1997. Increased weight gain and effects on production parameters of Holstein heifer calves that were allowed to suckle from birth to six weeks of age. J. Dairy Sci. 80:2523–2528.

Bartlett, K. S. F. K. McKeith, M.J. Vandehaar, G. E. Dahl, and J. K. Drackley. 2006. Growth and body composition of dairy calves fed milk replacers containing different amounts of protein at two feeding rates. J. Animal Sci. 81:1641-1655.

Bartol F. F., A. A. Wiley, C. A. Bagnell. 2008. Epigenetic programming of porcine endometrial function and the lactocrine hypothesis. Reprod. Dom. Anim. 43 (Suppl. 2):273-279.

Bascom, S. A., R. E. James, M. L. McGilliard, and M. Van Amburgh. 2007. Influence of dietary fat and protein on body composition of Jersey bull calves. J. Dairy Sci. 90:5600–5609.

Baumrucker, C. R. and J.W. Blum 1993. Secretion of insulin-like growth factors in milk and their effect on the neonate. Livestock Prod. Sci. 35:49-72.

Baumrucker, C. R., Hadsell, D. L., Blum, J. W. 1994. Effects of dietary insulin-like growth factor-1i on growth and insulin-like growth-factor receptors in neonatal calf intestine. Journal of Animal Science, 72:428-433.

Beam A. L., J. E. Lombard, C. A. Kopral, L. P. Garber, A. L. Winter, J. A. Hicks, J. L. Schlater. 2009. Prevalence of failure of passive transfer of immunity in newborn heifer calves and associated management practices on US dairy operations. J. Dairy Sci. 92:3973-3980.

Besser T. E., C. C. Gay, L. Pritchett. 1991. Comparison of three methods of feeding colostrum to dairy calves. J. Am. Vet. Med. Assoc.798:419-22.

Bielmann V., J. Gillan, N. R. Perkins, A. L. Skidmore, S. M. Godden, K. E. Leslie. 2010. An evaluation of Brix refractometry instruments for measurement of colostrum quality in dairy cattle. J. Dairy Sci. 93:3713-3721.

Blome, R. J. K. Drackley, F. K. McKeith, M. F. Hutjens, and G. C. McCoy. 2002. Growth, nutrient utilization, and body composition of dairy calves fed milk replacers containing different amounts of protein. J. Anim. Sci. 81:1641-1655.

Blum J. W. and C.R. Baumrucker. 2002. Colostral and milk insulin-like growth factors and related substances: Mammary gland and neonatal (intestinal and systemic) targets. Domestic Animal Endocrinology 23:101–110.

Blum, J.W. and H. Hammon. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. Livestock Prod. Sci. 66:151-159.

Brown, E. G., M. J. VandeHaar, K. M. Daniels, J. S. Liesman, L. T. Chapin, D. H. Keisler, and M. S. Weber Nielsen. 2005a. Effect of increasing energy and protein intake on body growth and carcass composition of heifer calves J. Dairy Sci. 88: 585-594.

Brown, E. G., M. J. VandeHaar, K. M. Daniels, J. S. Liesman, L. T. Chapin, J. W. Forrest, R. M. Akers, R. E. Pearson, and M. S. Weber Nielsen. 2005b. Effect of increasing energy and protein intake on mammary development in heifer calves J. Dairy Sci. 88: 595-603.

Burrin, D. G., T. A. Davis, S. Ebner, P.A. Schoknecht, M.L. Fiorotto, P.J. Reeds. 1997. Colostrum enhances the nutritional stimulation of vital organ protein synthesis in neonatal pigs. J. Nutr. 127:1284-1289.

Capuco A. V., J. J. Smith, D. R. Waldo, and C. E. Rexroad. 1995. Influence of prepubertal dietary regimen on mammary growth of Holstein heifers. J. Dairy Sci. 78:2709-2725

Chigerwe M., J. W. Tyler, L. G. Schultz, J. R. Middleton, B. J. Steevens, J. N. Spain. 2008. Effect of colostrum administration by use of oroesophageal intubation on serum IgG concentrations in Holstein bull calves. AJVR. 69:1158-1163.

Chigerwe M., J. W. Tyler, M. K. Summers, J. R. Middleton, L. G. Schultz, D. W. Nagy. 2009. Evaluation of factors affecting serum IgG concentrations in bottle-fed calves. J Am Vet Med Assoc. 234:785-9.

Corley L. D., T. E. Staley, L. J. Bush, E. W. Jones. 1977. Influence of colostrum on transepithelial movement of Escherichia coli 055. J Dairy Sci. 60:1416-21.

Cottrell E. C., S. E. Ozanne. 2008. Early life programming of obesity and metabolic disease. Physiol Behav. 94:17-28

Crichton J. A., J. N. Aitken and A. W. Boyne. 1959. The effect of plane of nutrition during rearing on growth, production, reproduction and health of dairy cattle. I. Growth to 24 months. Animal Production, 1: 145-162.

Crichton J. A., J. N. Aitken and A. W. Boyne. 1960a. The effect of plane of nutrition during rearing on growth, production, reproduction and health of dairy cattle. II. Growth to maturity. Animal Production, 2:45-57.

Crichton J. A., J. N. Aitken and A. W. Boyne. 1960b. The effect of plane of nutrition during rearing on growth, production, reproduction and health of dairy cattle. III. Milk production during the first three lactations. Animal Production, 2:159-168.

Daniels K. M., A. V. Capuco, M. L. McGilliard, R. E. James, and R. M. Akers. 2009. effects of milk replacer formulation on measures of mammary growth and composition in Holstein heifers. J. Dairy Sci. 92:5937–5950.

Davis Rincker L. E., M. J. VandeHaar, C. A. Wolf, J. S. Liesman, L. T. Chapin, M. S. Weber Nielsen. 2011. Effect of intensified feeding of heifer calves on growth, pubertal age, calving age, milk yield, and economics. J. Dairy Sci. 94:3554-3567.

DeNise S. K., J. D. Robison, G. H. Stott, D. V. Armstrong. 1989. Effects of passive immunity on subsequent production in dairy heifers. J. Dairy Sci. 72:552-554.

Diaz, M. C., M. E. Van Amburgh, J. M. Smith, J. M. Kelsey and E. L. Hutten. 2001. Composition of growth of Holstein calves fed milk replacer from birth to 105 kilogram body weight. J. Dairy Sci. 84:830-842.

Donovan, S.M. and J. Odle. 1994. Growth factors in milk as mediators of infant development. Ann. Review Nutr. 14:147-167.

Dubos R., D. Savage, R. Schaedler, 1966. Biological Freudianism: lasting effects of early environmental influences. Pediatrics 38:789-800.

Elfstrand L., H. Lindmark-Månsson, M. Paulsson, L. Nyberg, B. Åkesson. 2002. Immunoglobulins, growth factors and growth hormone in bovine colostrum and the effects of processing. Int. Dairy J. 12:879-887.

Faber S. N., N. E. Faber, T. C. Mccauley, R. L. Ax. 2005. Case study: Effects of colostrum ingestion on lactational performance. The Prof. An. Sc. 21:420-425.

Fiorotto M. L., T. A. Davis, P. J. Reeds, D. G. Burrin. 2000. Nonnutritive Factors in Colostrum Enhance Myofibrillar Protein Synthesis in the Newborn Pig. Pediatric Res. 48:511-517.

Foldager J., C. C. Krohn, L. Mogensen. 1997. Level of milk for female calves affects their milk production in first lactation. European Ass. Anim. Prod.

Fox D.G., Van Amburgh M.E., Tylutki T.P. 1999. Predicting requirements for growth, maturity, and body reserves in dairy cattle. J Dairy Sci. 82:1968-77.

Godden S. M. 2008. Colostrum management for dairy calves. Vet. Clin. Food Anim. 24:19-39.

Godden S. M., D. M. Haines, K. Konkol, J. Peterson. 2009. Improving passive transfer of immunoglobulins in calves. II: interaction between feeding method and volume of colostrum fed. J. Dairy Sci. 92:1758-1764.

Grusenmeyer, D. J., C. M. Ryan, D. M. Galton, and T. R. Overton. 2006. Shortening the dry period from 60 to 40 days does not affect colostrum quality but decreases colostrum yield by Holstein cows. J. Dairy Sci. 89(Suppl. 1):336.

Hadorn U., H. Hammon, R. M. Bruckmaier, J. W. Blum. 1997. Delaying Colostrum Intake by One Day Has Important Effects on Metabolic Traits and on Gastrointestinal and Metabolic Hormones in Neonatal Calves. J. Nutr. 127:2011-2023.

Hammon H., J. W. Blum. 1997. Prolonged colostrum feeding enhances xylose absorption in neonatal calves. J. Anim. Sci. 75:2915-2919.

Hill, T.M, H.G. Bateman, J. M. Aldrich, and R. L. Schlotterbeck. 2009 Effects of changing the essential and functional fatty acid intake of dairy calves. J. Dairy Sci. 92:670-676.

Hoffman P. C., N. M. Brehm, S. G. Price, A. Prill-Adams. 1996. Effect of accelerated postpubertal growth and early calving on lactation performance of primiparous Holstein heifers. J. Dairy Sci. 79:2024-2031.

Hohenboken W. D., J. Foldager, J. Jensen, P. Madsen, B. B. Andersen. 1995. Breed and nutritional effects and interactions on energy intake, production and efficiency of nutrient utilization in young bulls heifers and lactating cows. Acta Agric. Scand. Sect. A Anim. Sci. 45:92.

Jaster, E. H., G. C. McCoy, and R. L. Fernando. 1990. Dietary fat in milk or milk replacers for calves raised in hutches during the winter. J. Dairy Sci. 73:1843–1850.

Jaster, E. H., G. C. McCoy, N. Spanski, and T. Tomkins. 1992. Effect of extra energy as fat or milk replacer solids in diets of young dairy calves on growth during cold weather. J. Dairy Sci. 75:2524–2531.

Jirtle R. L., M. K. Skinner. 2007. Environmental epigenomics and disease susceptibility. Nat Rev Genet.8(4):253-62.

Johnsson I. D. 1988. The effect of pre-pubertal nutrition on lactation performance by dairy cows. Lactation in the Dairy Cow. p. 171-192.

Joost J., G. C. Van Den Borne, G. E. Lobley, M. W. A. Verstegen, J. Muijlaert, S. J. J. Alferink, W. J. J. Gerrits. 2007. Body fat deposition does not originate from carbohydrates in milk-fed calves. J. Nutr.137: 2234–2241

Keusch G. T. 1977. The consequences of fever. Am. J. Clin. Nutr. 30:1211-1214.

Mäntysaari P., K. L. Ingvartsen, V. Toivonen, K. Sejrsen. 1995. The effect of feeding level and nitrogen source of the diet on mammary development and plasma hormon concentrations fo pre-pubertal heifers. Acta. Agric. Scand. Sect. A 45:236-244.

Meyer M. J. 2005. Developmental, nutritional, and hormonal regulation of mammary growth, steroid receptor gene expression and chemical composition of retained tissues in prepubertal bovine. Ph.D. Diss. Cornell University, Ithaca, NY.

Meyer M. J., A. V. Capuco, D. A. Ross, L. M. Lintault, and M. E. Van Amburgh. 2006a. Developmental and nutritional regulation of the prepubertal heifer mammary gland: I. Parenchyma and fat pad mass and composition. J. Dairy Sci. 89:4289-4297

Meyer M. J., A. V. Capuco, D. A. Ross, L. M. Lintault, and M. E. Van Amburgh. 2006b. Developmental and nutritional regulation of the prepubertal bovine mammary gland: II. Epithelial cell proliferation, parenchymal accretion rate, and allometric growth. J. Dairy Sci. 89:4298-4304

Mills, J. K. 2009. The effect of feeding two sources of medium chain triglycerides on the body composition and metabolism of Holstein calves from birth to 85 kg. M.Sc. thesis. Cornell University, Ithaca, NY.

Moallem U., D. Werner, H. Lehrer, M. Zachut, L. Livshitz, S. Yakoby, A. Shamay. 2010. Long-term effects of ad libitum whole milk prior to weaning and prepubertal protein supplementation on skeletal growth rate and first lactation milk production. J. Dairy Sci. 93:2639-2650.

Moore M., J. W. Tyler, M. Chigerwe, M. E. Dawes, J. R. Middleton. 2005. Effect of delayed colostrum collection on colostral IgG concentration in dairy cows. J. Am. Vet. Med. Assoc. 226:1375-1377.

Nardone A., N. Lacetera, U. Bernabucci, B. Ronchi. 1997. Composition of colostrum from dairy heifers exposed to high air temperatures during late pregnancy and the early postpartum period. J. Dairy Sci 80:838-844.

National Research Council. 2001. Nutrient requirements of Dairy Cattle. Seventh Revised Edition. National Academy Press, Washington, DC.

Nusser, K. D. and S. Frawley. 1997. Depriving neonatal rats of milk from early lactation has long-term consequences on mamotrope development. Endocr. 7:319-323.

Petitclerc D., Chapin L. T., Tucker H. A. 1984. Carcass composition and mammary development responses to photoperiod and plane of nutrition in Holstein heifers. J Anim Sci. 58:913-9.

Pinotti L., F. Rosi. 2006. Leptin in colostrum and milk. Horm. Metab. Res. 38:89-93.

Plagemann A. 2005. Perinatal programming and functional teratogenesis: impact on body weight regulation and obesity. Physiol Behav. 86(5):661-8.

Pritchard D. E., Hafs H. D., Tucker H. A., Boyd L. J., Purchas R. W., Huber J. T. 1972. Growth, mammary, reproductive, and pituitary hormone characteristics of Holstein heifers fed extra grain and melengestrol acetate. J Dairy Sci. 55:995-1004.

Pritchett, L. C., C. C. Gay, D. D. Hancock, T. E. Besser, 1994. Evaluation of the hydrometer for testing immunoglobulin G1 concentrations in Holstein colostrum. J. Dairy Sci., 77:1761–1767.

Pritchett, L. C., C. C. Gay, T. E. Besser, D. D. Hancock. 1991. Management and production factors influencing immunoglobulin G1 concentration in colostrum from Holstein cows. J. Dairy Sci. 74:2336-2341.

Radcliff R. P., M. J. VandeHaar, A. L. Skidmore, L. T. Chapin, B. R. Radke, J. W. Lloyd, E. P. Stanisiewski, H. A. Tucker. 1997. Effect of diet and bovine somatotropin on heifer growth and mammary development. J. Dairy Sci. 80:1996-2003.

Radcliff R. P., M. J. Vandehaar, L. T. Chapin, T. E. Pilbeam, D. K. Beede, E. P. Stanisiewski, H. A. Tucker. 2000. Effects of diet and injection of bovine somatotropin on pre-pubertal growth and first-lactation milk yield of Holstein cows. J. Dairy Sci. 83:23-29.

Raeth-Knight M., H. Chester-Jones, S. Hayes, J. Linn, R. Larson, D. Ziegler, B. Ziegler, N. Broadwater. 2009. Impact of conventional or intensive milk replacer programs on Holstein heifer performance through six months of age and during first lactation. J. Dairy Sci. 92:799-809.

Rauprich, A. B., H. M. Hammon, J. W. Blum. 2000. Influence of feeding different amounts of first colostrum on metabolic, endocrine, and health status and on growth performance of neonatal calves. J. Anim. Sci. 78:896-908.

Reid J. T., J. K. Loosli, K. L. Turk, S. A. Asdell, S. E. Smith. 1957. Progress report on a study of the effect of plane of plane of early nutrition upon reproductive and productive performance of Holstein cattle. J. Dairy Sci. 40:610-611.

Robison J. D., G. H. Stott, S. K. DeNise. 1988. Effects of passive immunity on growth and survival in the dairy heifer. J. Dairy Sci. 71:1283-1287.

Romanyukha A. A., S. G. Rudnev, I. A. Sidorov. 2006. Energy cost of infection burden: An approach to understanding the dynamics of host-pathogen interactions. J. Theoretical Bio. 241:1-13.

Sejrsen K., Huber J. T., Tucker H. A. 1983. Influence of amount fed on hormone concentrations and their relationship to mammary growth in heifers. J Dairy Sci. 66:845-55.

Sejrsen K., J. T. Huber, H. A. Tucker, R. M. Akers. 1982. Influence of nutrition on mammary development in pre- and postpubertal heifers. J. Dairy Sci. 65:793-800.

Shamay A., D. Werner, U. Moallem, H. Barash, I. Bruckental. 2005. Effect of nursing management and skeletal size at weaning on puberty, skeletal growth rate, and milk production during first lactation of dairy heifers. J. Dairy Sci. 88:1460-1469.

Sinha Y. N., Tucker H. A. 1969. Mammary development and pituitary prolactin level of heifers from birth through puberty and during the estrous cycle. J Dairy Sci. 52:507-12.

Smith J. M. 2002. Nutritional modulation of growth and development of Holstein heifers. Ph.D. Diss. Cornell University, Ithaca, NY.

Soberon F. and M. E. Van Amburgh. 2011. Integrating concepts of pre-pubertal mammary development and rates of body growth to describe differences in first lactation milk yield. Proceedings of the Cornell Nutrition Conference for Feed Manufacturers. 73:75-85

Stelwagen K., E. Carpenter, B. Haigh, A. Hodgkinson, T. T. Wheeler. 2009. Immune components of bovine colostrum and milk. J. Anim. Sci. 87(Suppl. 1):3-9.

Swanson E. W. 1960. Effect of rapid growth with fattening of dairy heifers on their lactational ability. J. Dairy Sci. 43:377-387.

Swanson E. W., S. A. Hinton. 1964. Effect of seriously restricted growth upon lactation. J. Dairy Sci. 47:267-272.

Terré M., C. Tejero, A. Bach. Long-term effects on heifer performance of an enhanced-growth feeding programme applied during the preweaning period. J. Dairy Res. 76:331-339.

Tikofsky, J. N., M. E. Van Amburgh and D. A. Ross. 2001. Effect of varying carbohydrate and fat levels on body composition of milk replacer-fed calves. J. Animal Sci. 79:2260-2267.

Tyler J. W., B. J. Steevens, D. E. Hostetler, J. M. Holle, J. L. Jr. Denbigh. 1999. Colostral immunoglobulin concentrations in Holstein and Guernsey cows. Am. J. Vet. Res. 60:1136-9.

Tyler J. W., D. D. Hancock, S. E. Wiksie, S. L. Holler, J. M. Gay, C. C. Gay. 1998. Use of serum protein concentration to predict mortality in mixed-source dairy replacement heifers. J Vet Intern Med. 12:79-83.

Van Amburgh M. E. and M. Meyer. 2005. Target growth and nutrient requirements of post-weaned dairy heifers. Dairy calves and heifers integrating biology and management. NRAES 175:54-65

Van Amburgh M. E., D. M. Galton, D. E. Bauman, R. W. Everett, D. G. Fox, L. E. Chase, H. N. Erb. 1998. Effects of three prepubertal body growth rates on performance of Holstein heifers during first lactation. J. Dairy Sci. 81:527-538.

Van Amburgh, M. E. and J. K. Drackley. 2005 Current perspectives on the energy and protein requirements of the pre-weaned calf. Chap. 5 in ”Calf and heifer rearing: Principles of

rearing the modern dairy heifer from calf to calving". Nottingham Univ. Press. P.C. Garnsworthy, ed. Pp.67-82.

Virtala A. M., Y. T. Gröhn, G. D. Mechor, H. N. Erb. 1999. The effect of maternally derived immunoglobulin G on the risk of respiratory disease in heifers during the first 3 months of life. Prev Vet Med. 39:25-37

Waddington C. H. 1940. Organizers and Genes. Cambridge University Press, Cambridge, UK.

Waldo D. R., Capuco A. V., Rexroad C. E. Jr. 1998. Milk production of Holstein heifers fed either alfalfa or corn silage diets at two rates of daily gain. J Dairy Sci. 81:756-64.

Weaver D. M., J. W. Tyler, D. C. VanMetre, D. E. Hostetler, G. M. Barrington. 2000. Passive transfer of colostral immunoglobulins in calves. J. Vet. Intern. Med. 14:569-577.

Weaver I. C. G., N. Cervoni, F. A. Champagne, A. C. D'Alessio, S. Sharma, J. R. Seckl, S. Dymov, M. Szyf, M. J. Meaney. 2004. Epigenetic programming by maternal behavior. Nature Neuroscience 7:847-854.

CHAPTER II

PRE-WEANING MILK REPLACER INTAKE AND EFFECTS ON LONG TERM PRODUCTIVITY OF DAIRY CALVES

F. Soberon, E. Raffrenato, R. W. Everett and M. E. Van Amburgh

J. Dairy Sci. In press

ABSTRACT

The pre-weaning management of dairy calves over the last 30 years has focused on mortality, early weaning and rumen development. Recent studies suggest that nutrient intake from milk or milk replacer during the pre-weaning period alters the phenotypic expression for milk yield. The objective of this study was to investigate the relationship between nutrient intake from milk replacer and pre and post-weaning growth rate with lactation performance in the Cornell Dairy Herd and a commercial dairy farm. The analysis was done using traditional 305 d first lactation milk yield and residual lactation yield estimates from a Test Day Model (TDM) to analyze the lactation records over multiple lactations. The overall objective of the calf nutrition program in both herds was to double the birth weight of calves by weaning through increased milk replacer and starter intake. First lactation 305d milk yield and residuals from the TDM were generated from 1,244 and 624 heifers from the Cornell Herd and from the commercial farm, respectively. The TDM was utilized to generate lactation residuals after accounting for the effects of test day, calving season, days in milk, days pregnant, lactation number, and year. In addition, lactation residuals were generated for cattle with multiple lactations to determine if the effect of pre-weaning nutrition could be associated with lifetime milk yield. Factors such as pre-weaning average daily gain (ADG), energy intake from milk replacer as a multiple of maintenance and other growth outcomes and management variables were regressed on TDM milk yield data. In the Cornell herd, pre-weaning ADG, ranged from 0.10 kg to 1.58 kg, and was significantly correlated with first lactation yield; for every 1 kg of pre-weaning ADG, heifers on average

produced 850 kg more milk during their first lactation and 235 kg more milk for every Mcal of ME intake above maintenance. In the commercial herd, for every 1 kg of pre-weaning ADG, milk yield increased by 1,113 kg in the first lactation and further, every 1 kg of pre-pubertal ADG was associated with a 3,281 kg increase in first lactation milk yield. Among the two herds, pre-weaning ADG accounted for 22 percent of the variation in first lactation milk yield as analyzed with the TDM. These results suggest that increased growth rate prior to weaning results in some form of developmental programming that is yet to be understood, but has positive effects on lactation milk yield. This analysis identifies nutrition and management of the pre-weaned calf as major environmental factors influencing the expression of the genetic capacity of the animal for milk yield.

Key Words: Calf nutrition, milk production, test day model, epigenetics

INTRODUCTION

Calf management programs have traditionally focused on strategies that restrict the amount of milk or milk replacer offered to the calf, to encourage grain intake in an effort to accelerate weaning, reduce the potential for scours and other illness and reduce the cost of feeding and management (Kertz et al. 1979; Otterby and Linn, 1981; Anderson et al. 1987). However, evaluation of calf management data suggest that strategies that reduce liquid feed intake to enhance starter intake and promote rumen development have not significantly reduced any of those variables (Davis and Drackley, 1998; NAHMS, 2002, 2007).

More recent studies comparing the effects of suckling, controlled intakes and *ad-libitum* feeding of calves from birth up to 56 days of life have found that increasing the nutrient intake prior to 56 days of life from milk resulted in increased milk yield during first lactation ranging from 450 to 1,300 kg compared to the milk yield of restricted fed calves during the same period (Foldager and Krohn, 1994; Bar-Peled et al, 1997; Shamay et al., 2005; Terré et al., 2009;

Moallem et al. 2010). Two other studies that utilized milk replacer showed no significant effect of level of nutrient intake pre-weaning on first lactation milk yield (Raeth-Knight et al. 2009; Morrison et al. 2011). However, the study of Raeth-Knight et al. (2009) potentially lacks sensitivity in determining milk yield differences because it is difficult to separate effects of herd, season, days in milk, days pregnant and other environmental factors among farms given that calves were returned to several farms for measurement of milk yield differences. Overall, the sum of this data suggests programming events occur during the pre-weaning period that alter the milk yield potential of the calf and these events are associated with enhanced nutrient intake from milk or milk replacer prior to weaning.

The concept of a "lactocrine hypothesis" has been recently introduced and describes the effect of milk-born factors, including colostrum in this definition, on the epigenetic development of specific tissues or physiological functions (Bartol et al., 2008). Conceptually this topic is not new but the terminology is useful and the ability of several groups to make a direct connection from a milk-born factor to a developmental function at the tissue or organ level is significant (Nusser and Frawley, 1997; Bagnell et al., 2005). Data relating to this topic has been described and discussed by others in neonatal pigs (Donovan and Odle, 1994; Burrin et al., 1997) and calves (Baumrucker and Blum, 1993; Blum and Hammon, 2000; Rauprich et al. 2000). The implication of this hypothesis and these observations are that the neonate can be programmed maternally and post-natally to alter development of a particular process, and based on the observations in calves it is not well understood if the lactation response is a function of total nutrient intake or if there are factors in whole milk that are responsible for the enhanced milk yield. In a recent study (Moallem et al., 2010), the effects of pre-weaning nutrition on first lactation milk yield were associated with the type and quality of nutrients fed. Moallem et al. (2010) observed 10.3% higher milk yields during first lactation from heifers fed whole milk ad-libitum compared to heifers fed milk replacer ad-libitum during the same period and suggested that milk replacer did not contain the same biologically active factors as milk and thus did not impart any lactocrine effects on the calves. Since a large percentage of calves in the U.S. are fed

milk replacer (NAHMS, 2007), the question remains whether enhancing growth through increased intake of milk replacer can also support these changes in lactation milk yield. If so, then factors like protein and energy intake are more important for the neonate than growth factors or peptides found in whole milk (Grosvenor et al. 1993; Meisel, 2005).

The analysis of first lactation and subsequent lactation performance of large numbers of calves and heifers in prospective studies starting in early life is difficult and few studies have been able to capture enough data to make reasonable conclusions (Reid et al., 1964; Van Amburgh et al, 1998). Further, analyses of herd level data over time and among herds to evaluate early life nutrition and management effects might require more rigorous mathematical approaches similar to those used to generate heritability and predicted transmitting ability of genetic traits. An approach using a test day model (TDM) (Cornell Research Foundation and R.W. Everett, 1994) has been used to evaluate extended lactations (Van Amburgh et al. 1997), first lactation milk response to pre-pubertal ADG (Van Amburgh et al., 1998) and bST responses in commercial herds (Bauman et al., 1999). Extending this approach to the effects of calf growth and management on lactation milk yield seems logical. This approach should result in the least biased evaluation of the data since variation in performance over time could obscure the changes in lactation yield due to early life management for the same reasons that unadjusted mixed model analyses of 305d milk yield over time does not generate accurate predicted transmitting ability and heritability values for traits. Further, this approach allows for this evaluation without a controlled experiment.

The objective of this study was to investigate the relationship between pre-weaning nutrient intake from milk replacer and future milk yield using standard 305d milk yields and with a mathematical approach used to conduct genetic evaluations within and among herds. Another objective was to determine what factors during the early rearing period have an impact on lifetime performance of dairy calves.

MATERIALS AND METHODS

Calf growth, nutrition and management data were collected from the records of two New York dairy farms, the Cornell Teaching and Research dairy herd and a family owned commercial dairy farm near Watertown, NY. Both herds utilized similar milk replacers and due to the management strategies on each farm, have useful, but different growth measurements for calves and heifers that allowed us to partition the effects of nutrition, management and stage of growth on milk yield differently. In this study, all calves on both farms were managed in a similar manner without a traditional control and treatment. Accordingly, the mathematical approach for generating data to evaluate for milk yield effects was similar to that used to estimate heritability and predicted transmitting ability where there are generally no controls and treatments, but rather within herd comparisons of production among contemporaries and the variability among individuals within the herd observed.

The pre-weaning growth objective in the Cornell herd was to double the birth weight by sixty days of age and to achieve that, milk replacer solids were fed at 1.5% of birth weight for the first 7 d of life and then 2 to 2.5% of birth weight from day 8 to 42 on a DM basis, diluted to 15% DM for feeding. Within the Cornell herd, birth weight, birth height, weaning weight, weaning height, age at first calving (AFC), and monthly average ambient temperatures were collected; average daily gain (ADG) pre-weaning was calculated from birth and weaning weights. Further, in the Cornell herd, milk replacer intake was monitored and recorded, and that information was available and used in this analysis. Weaning from milk replacer in the Cornell herd was achieved by approximately 49 days by restricting milk replacer to 50% of prior intake for 7 d and feeding once per day in the evening. Calves were held in hutches or a calf barn for another 7 to 10 days and fed starter grain only until they were moved to group housing. The milk replacers used during the period of these observations were both commercially available 28% crude protein and were either 15% or 20% fat (Excelerate, 28% CP, 15% fat, 4.65 Mcals ME/kg, Milk Specialties Inc, Carpentersville, IL or Cows Match, 28% CP, 20% fat, 4.87 Mcals ME/kg, Land O'Lakes

Animal Milk Products, Inc., Shoreview, MN), respectively. Calves were offered the same total of milk replacer solids throughout the year and no adjustments were made to accommodate the change in maintenance requirements due to changes in ambient temperature. Over the pre-weaning period, the calves consumed between 4.5 and 5.3 Mcal ME/d from the milk replacers as estimated by the NRC (2001). The starter grain was a commercially available starter that was 23% CP, 1.84 Mcals/kg (Cargill Animal Nutrition, Minneapolis, MN) and was offered free choice starting at approximately day 5 of life. Starter grain intakes were not recorded and the implications of this will be discussed. Water was offered free choice with some exceptions during winter months, when it was offered for 4 to 5 h per day due to freezing conditions. Monthly average ambient temperature data was used with the calf body weight information to estimate the daily maintenance requirements of calves (NRC, 2001) and this data was further used to estimate intake energy above maintenance from milk replacer prior to weaning for all of the calves in the data set. This allowed us to generate correlations between energy intake above maintenance from milk replacer with milk yield of the same animal once they completed a lactation and this provided an additional parameter to evaluate the effect of early life milk replacer nutrient intake on future milk yield. Growth data were collected starting in 1998 and the lactation data collection was through 2008.

The records from the Cornell herd were reviewed and any calf that was recorded as having diarrhea or treated with antibiotics was documented and categorical variables were designated for statistical evaluation of the effect of either or both of these outcomes on milk yield. Based on the available records and farm protocols, it was assumed that the majority of antibiotic treatment was primarily given for signs of respiratory illness.

On the commercial farm, birth weights, weaning weights, breeding weights, and AFC data were collected; pre-weaning ADG and ADG until breeding were calculated from the data. A standard calf feeding protocol existed on the dairy and the average milk replacer feeding rate was 0.9 kg per day from day 7 to weaning at approximately 49 days. The milk replacer used during the period of these observations was 28% crude protein and 15% fat (Excelerate, Milk

Specialties Inc, Carpentersville, IL, 4.65 Mcals ME/kg). Given the feeding rates and milk replacer composition, the calves were offered approximately 4.2 Mcal ME/d over the pre-weaning period (NRC, 2001). The starter grain was a commercially available 20% CP starter (DM basis). Health data were not available from the commercial dairy herd.

Milk production and milk composition records were collected for both farms through Dairy Herd Improvement (Dairy Records Management System, Raleigh, NC) and analyzed either as actual 305d milk yield or with the TDM. For the Cornell herd, 1,244 completed first lactation records were available for analyses that covered seven years of lactation data from 2001 to 2008. The lactation data from the commercial dairy covered the calendar years 1999 to 2004 and after editing for missing data resulted in 623 completed first lactation records. In addition, due to the length of time the data covered, we were able to collect lactation records of animals that had completed up to three lactations, allowing us to evaluate the effect of pre-weaning nutrient intake on lifetime milk yield. For the Cornell herd there were 826 and 450 cattle with completed second and third lactations, respectively. For the commercial dairy, 484 and 271 second and third completed lactations were analyzed respectively. Reasons for culling and other management factors were not analyzed.

Test Day Model

Due to potential effects of calving year, season, management and environment on lactation performance, a linear model that describes the biology of those influences was applied to the production data (Everett and Schmitz, 1994; Van Amburgh et al, 1997; Bauman et al., 1999). The TDM assumes that it is inappropriate to compare animals among farms without considering the management conditions within herd over time. Residuals from the TDM are simultaneously adjusted for herd test day, age, days in milk, calendar month fresh, pregnancy and management effects. Test day residuals include the random genetic cow effects and treatment effects. The model accommodates the fact that conditions vary from herd to herd as well as from environment to environment and conditions change over time within herd. The TDM estimates

within herd, biological effects such as age, days in milk, and stage of pregnancy. The model used to describe milk, fat, or protein on test day was:

$$Y_{ijklmno} = t_i + a_{jk} + d_{jl} + f_{jm} + c_{jn} + e_{ijklmno}$$

Where:

$Y_{ijklmno}$ = the dependent, continuous variable,

t_i = the i^{th} test day observed for a herd,

a_{jk} = the k^{th} age in months for the o^{th} observation where j = 1,2 divides the herd data into halves, the oldest data having separate fixed effects solutions from the newest data,

d_{jl} = the l^{th} day in milk for the test day of the o^{th} observation where l describes the 10 day intervals and ranges from 1 to 45 for the 1^{st} and 2^{nd}+ lactations,

f_{jm} = the m^{th} month of freshening associated with the o^{th} observation,

c_{jn} = the n^{th} day pregnant where n = 1 is the 1^{st} 5 months of pregnancy, n = 2 is the 6^{th} month,…, and n = 5 for the 9^{th}+ months of pregnancy for 1^{st} and 2^{nd} lactations, and

$e_{ijklmno}$ = the residual for the o^{th} observation of a cow in the n^{th} period of pregnant, the m^{th} month fresh, the l^{th} day in milk, the k^{th} age in months in the j^{th} period and tested on the i^{th} herd test day.

The observation y is a vector of all milk, fat, or protein observations for a cow and the elements of y are assumed to be correlated. The fixed effects equations for the linear model are:

$$x'R^{-1}\, x\beta = x'R^{-1}y,$$

where R describes the relationships among the test day observations in the y vector on a cow. If a cow is tested n times, R is an n x n matrix describing the residual variance-covariance structure of the n test days for the cow. R is assumed to have an autocorrelation structure such that $R/\sigma^2 = \rho$ is an autocorrelation matrix, where σ_2 is the variance of the associated test day data and ρ_{ij} is the correlation between tests i and j and equals:

$$\rho^q \text{ and } q = |(t_i - t_j)/30|,$$

where t_i and t_j are days in milk for test i and j, respectively, and ρ = 0.73, 0.58, and 0.69 for milk yield, fat yield, and protein yield, respectively. The model was fit to the data and residuals were obtained as $e = y - x'\beta$. The residuals were standardized to a common variance for all test days.

Test day residuals were combined to produce TDM residual lactation records which were used in the analysis to determine the effect of pre-weaning and pre-pubertal nutrition of calves and heifers on subsequent lactation production. Since the residuals are from a grand mean, the values will be both positive and negative and the difference between residuals is the variable of interest. We are making the assumption that a majority of the test day residuals are associated with either unexplained variation after the adjustments described above or to genetic relationships among the variable of interest, which was also evaluated.

Statistical Analysis

Each farm was analyzed separately due to the environmental conditions specific to the farm that would influence both growth and the lactation milk yields. After the TDM residual lactation data were generated, the residuals were regressed on the measured growth and intake variables from each herd. Factors analyzed on both farms included pre-weaning ADG, birth weight, weaning weight, AFC, birth year, birth month and season of birth. For the Cornell herd, 305 d milk yield and TDM lactation residuals were also regressed on calculated metabolizable energy (ME) intake from milk replacer above maintenance from milk replacer. Linear, cubic and quadratic relationships among the measured intake and growth variables and TDM residuals were conducted using a generalized linear model procedures as well as mixed model procedures within SAS 9.2 (SAS Institute, Cary, NC, 2004). For all data, cubic and quadratic relationships were non-significant and were dropped from the analyses.

To analyze the 305 d milk yield the statistical models differed due to the calf and heifer growth data available from each farm. For the Cornell herd, the model used was:

$$Y_{ij} = S_i + G_j + E_{ij}$$

Where,

Y_{ij} is the dependent, continuous variable,

S_i = the effect of year of calving,

G_j = the average daily gain of the calf prior to weaning, and

E_{ij} = the residual error of the i^{th} year of calving and the j^{th} average daily gain.

A separate model was used to evaluate energy intake above maintenance:

$$Y_{ij} = IE_i + E_{ij}$$

Where,

Y_{ij} is the dependent, continuous variable,

IE_i = the intake energy above maintenance from milk replacer of the calf prior to weaning, and

E_j = the residual error of the j^{th} intake energy above maintenance.

For the commercial dairy, the model used was:

$$Y_{ijk} = S_i + G_j + PW_k + E_{ijk}$$

Y_{ijk} is the dependent, continuous variable,

S_i = the effect of year of calving,

G_j = the ADG of the calf prior to weaning,

PW_k = the post-weaning ADG, and

E_{ijk} = the residual error of the i^{th} year of calving and the j^{th} pre-weaning average daily gain and k^{th} post-weaning ADG.

In addition, data from the Cornell herd for calf diarrhea and antibiotic treatment was analyzed for proportion of calves treated and then a mixed model analyses was conducted to determine the impact of either of these observations on the first lactation milk yield and the interaction between ADG and milk yield. Also, the relationship between energy intake above maintenance and diarrhea and antibiotic treatment for first lactation milk yield was also analyzed by mixed model approaches.

Due to the availability of subsequent lactation data, for the Cornell herd, regressions of pre-weaning ADG and intake over maintenance on TDM residual milk was also conducted for second and third lactation and cumulative milk from first through third lactation. In the commercial herd, pre-weaning ADG, birth to breeding ADG and weaning to breeding ADG per animal was regressed on TDM residual milk yield for second and third lactations and cumulative milk yield from first through third lactation. Significance for all analyses was declared for $P < 0.05$. Trends were declared at $P < 0.10$.

RESULTS AND DISCUSSION

Data on birth weight, growth rate, age at first calving, milk yield and other performance parameters for each herd are found in Table 2.1. The mean pre-weaning growth rates from the Cornell Herd were 0.82±0.18 kg/d with a range from 0.10 to 1.58 kg/d, and this was surprising given the amount of milk replacer consumed by the calves. We believe this range in pre-weaning growth data reflects several factors. First, it demonstrates that although the calves are offered adequate nutrients above maintenance from milk replacer, the effects of cold and heat stress are present in the data set. Monthly average temperatures at the Cornell herd averaged 8.6 °C and ranged from -9.0 to 21.9°C. Further, there was no characterization of colostrum status available and this has been shown to impact pre-pubertal ADG (Faber et al. 2005).

In the Cornell herd, the effect of diarrhea or antibiotic treatment on pre-weaning ADG was not significant and ADG differed by approximately 30 g/d for calves that had either event in their records ($P > 0.1$). However, for calves that had both events recorded, pre-weaning ADG was lower by approximately 50 g/d ($P < 0.01$). Over the eight year period, approximately 59% of all of the calves had at least one of the recorded events.

Table 2.1. Growth, performance and yield parameters from each farm used in the analyses of lactation performance[1].

	Cornell Herd[2]	s.d.	Commercial Herd	s.d.
First lactation records (n)	1,244		623	
Birth weight, kg	41.68	5.09	42.55	5.10
Birth height, cm	80.87	5.71	n/a	n/a
Average monthly temperature, °C	8.57	9.22	9.41	9.33
Average Mcals above maintenance from milk replacer, Mcals/d	2.81	0.61	NA	NA
Weaning weight, kg	82.08	10.25	84.13	10.81
Weaning height, cm	93.79	9.90	n/a	n/a
Pre-weaning ADG, kg/d	0.82	0.18	0.66	0.11
Post-weaning ADG, kg/d	NA	NA	0.91	0.10
Average age at first calving, d	691	54	687	64
First lactation 305d milk yield, kg	10,899	1,781	13,583	1,285

[1] Data are averages over the time period studied.
[2] In Harford, NY
[3] NA – not applicable

On the commercial dairy, the observed pre-weaning ADG was similar in range and the mean was 0.66 ± 0.11 kg with a range from 0.32 to 1.27 kg. This data most likely represents the reality of growth rates observed on most farms assuming that environmental conditions and calf health challenges are reflected in the range.

On both farms, first lactation milk yield was positively correlated with pre-weaning ADG, and weaning weight ($P < 0.03$), and in the Cornell herd energy intake above maintenance from milk replacer and ambient temperature ($P < 0.001$) (Table 2.2). Further, on the commercial farm, milk production was positively correlated with ADG from birth to breeding ($P < 0.01$) and from weaning to breeding ($P < 0.1$) (Table 2.2). On the commercial farm ADG from birth to breeding and ADG from weaning to breeding were highly correlated with each other (Correlation coefficient of 0.94; $P < 0.01$). On both farms first lactation milk yield was significantly affected by season of birth and year of birth. Among both herds age at first calving was also evaluated as a factor affecting first lactation milk yield and found to be non-significant (data not shown) ($P = 0.59$).

Also, since culling is highly associated with low milk production, there was a concern that as multiple lactations were analyzed some bias could be introduced by culling out lower producing animals, thus influencing the multiple lactation information. The Cornell data set was analyzed to examine potential bias of the data from the cattle with second or third lactations since those animals were also within the first lactation analyses. The TDM residuals for first lactation cattle (n= 1,244; mean = -128.1 kg) were compared to the first lactation residuals for the cattle that completed second (n= 826; mean = -143.5 kg) and third (n= 450; mean = -171.4 kg) lactations (Table 2.3). The TDM residuals among the first lactation for all parity groups were not significantly different, thus we concluded there was no bias due to preferential culling in the analysis of multiparous cattle within this data set.

Table 2.2. Equations developed from linear regression of the following pre-weaning and management parameters with first lactation Test Day Model residuals from each farm.

Cornell Dairy (Harford, NY)		
Dependent variables	**Derivation**	**Significance**
Mcals above maintenance from milk replacer prior to weaning	y = -783.18 + 235.42x	($P < 0.001$)
Pre-weaning ADG (kg)	y = -816.63 + 849.63x	($P < 0.001$)
Birth weight (kg)	y = -246.34 – 2.78x	($P = 0.724$)
Weaning weight (kg)	y = -1,354.38 + 15.05x	($P < 0.001$)
Temperature at birth (°C)	y = -331.76 + 23.80x	($P < 0.001$)

Commercial Dairy (NY)		
Dependent variables	**Derivation**	**Significance**
Pre-weaning ADG (kg)	y = -682.01 + 1,112.71x	($P = 0.03$)
Birth weight (kg)	y = -794.84 – 19.63x	($P = 0.07$)
Weaning weight (kg)	y = -1,237.60 + 15.32x	($P < 0.01$)
ADG From birth to breeding (kg)	y= -2,985.00 + 3,280.55x	($P < 0.01$)
ADG From weaning to breeding (kg)	y = -1,061.46 + 1,168.48x	($P = 0.10$)

Table 2.3. Test Day Model milk residuals in kg for cattle with one, two or three completed lactations in the Cornell herd (Harford, NY) and the effect of pre-weaning ADG on each lactation milk yield.

Lactation	First			Second			Third		
	n	Mean TDM	Extra milk kg/kg ADG	n	Mean TDM	Extra milk kg/kg ADG	n	Mean TDM	Extra milk kg/kg ADG
1^{st}	1244	128.1	849.6						
1^{st} - 2^{nd}	826	143.5	941.3	826	611.5	888.1			
1^{st} – 3^{rd}	450	171.4	1100.2	450	892.8	1131.1	450	757.7	48.3

To make a direct comparison with other studies, the 305d first lactation milk yields from the Cornell herd were regressed on the pre-weaning growth rates of the calves and in this analyses for every kg of ADG prior to weaning, the 305d milk yield increased by 704 kg in the first lactation ($P < 0.01$). This effect was linear within the data set and implies that under the conditions of this analysis the greater the ADG of the calf prior to weaning, the greater their potential first lactation milk response, consistent with other studies (Shamay et al., 2005, Moallem et al., 2010).

Although the analyses of the 305d first lactation milk yield resulted in identifying pre-weaning ADG as a factor affecting first lactation milk yield, another approach was to use the TDM lactation milk yield to evaluate the response. The use of the TDM analyses should be a less biased solution for milk yield. Accordingly, the TDM lactation yields were analyzed by regressing the residuals on pre-weaning ADG and for every kg of ADG pre-weaning, heifers produced 850 kg more milk during their first lactation ($P < 0.01$) (Table 2.4). To ensure there was no bias in the response due to some genetic component, the milk response identified by the TDM was analyzed with the sire, dam and individual predicted transmitting ability for milk and the relationships were non-significant, implying the increase in milk yield was environmental in nature and equally affected individuals of high and low genetic merit. Since this appears to be an environmental response, calves and cattle on each farm could have different milk yield responses due to nutrition, housing, and other environmental factors. Therefore, analyzing animals on individual farms should demonstrate similar relationships if the effect is consistent, but the magnitude of the response might be different.

The same relationship between TDM milk yield and pre-weaning ADG was analyzed on the commercial farm and for every kg of ADG pre-weaning, heifers produced 1,114 kg more milk during their first lactation ($P = 0.03$) (Table 2.5). Thus, on the commercial farm, the response was approximately 31% greater than the Cornell dairy despite lower average pre-weaning ADG, and was consistent with the difference in first lactation milk yield differences between the two farms (Table 2.1). The average milk yield on the commercial herd was approximately 25%

Table 2.4. Differences in Test Day Model residual milk (kg) for first, second and third lactation as well as cumulative milk from first through third lactation by ADG before weaning and the energy intake over predicted maintenance for calves in the Cornell herd (Harford, NY)[1].

		Cornell Herd			
Lactation	**n**	**Predicted difference in milk per kg of pre-weaning ADG**	***P* value**	**Predicted difference in milk by each additional Mcal over maintenance pre-weaning[2]**	***P* value**
1st	1244	849.63	< 0.01	235.42	< 0.01
2nd	826	888.08	< 0.01	108.39	0.26
3rd	450	48.32	0.91	351.39	< 0.01
1st through 3nd	450	2,279.53	0.01	902.76	< 0.01

[1]Monthly average ambient temperatures were used to calculate the maintenance requirements during the pre-weaning period for each calf.

[2]Mcal over maintenance were calculated using NRC, 2001 equations.

Table 2.5. Differences in Test Day Model residual milk (kg) for first, second and third lactation as well as cumulative milk from first through third lactation by average daily gain prior to weaning and from weaning to breeding for the commercial herd.

Commercial Herd					
Lactation	**n**	**Predicted difference in milk per kg of pre-weaning ADG**	***P* value**	**Predicted difference in milk per kg of ADG from weaning to breeding[1]**	***P* value**
1st	623	1,113.97	0.03	1,168.48	0.10
2nd	484	-526.44	0.49	2,719.87	0.01
3rd	271	1,293.47	0.18	2,874.88	0.05
1st through 3nd	271	1,286.18	0.51	8,199.80	< 0.01

[1]ADG from weaning to breeding had a coefficient of correlation of 0.94 with ADG from birth to breeding ($P < 0.01$).

greater than the Cornell herd over the period studied, thus the calf response to early life nutrient intake is most likely associated with the overall management level applied to the lactating cattle in the dairy. When combining both farms and using a model that included farm and season of birth as class variables and birth weight as a covariate, for every additional kg of ADG pre-weaning, heifers produced 970 kg more milk during their first lactation ($P < 0.01$). The uniformity of this relationship over 1,867 lactations provides positive evidence that the pre-weaning period represents a time of opportunity to alter the set points of calves for potential life time milk production and is consistent with other data (Shamay et al., 2005; Moallem et al., 2010).

Moallem et al. (2010) fed both milk and milk replacer, and the calves fed milk replacer did not show a significant milk response, and they suggested that the effects they observed were possibly due to bioactive factors in milk that did not exist in milk replacer. However, the response observed in the current study suggests that nutrient supply from high quality milk replacer is effective at stimulating the milk yield response. Comparing the data from Moallem et al. with the current data would indicate that the digestibility, protein quality and protein level of the milk replacer along with total energy intake appears to be critical to generate the milk response.

In the data from Cornell, first lactation milk yield was not significantly affected by reported cases of diarrhea. However, calves receiving antibiotics had significantly reduced milk yield and produced 493 kg less milk in the first lactation ($P > 0.01$) than calves with no record of being treated. Regardless of antibiotic treatment, the effect of ADG on first lactation milk yield was significant in all calves ($P < 0.05$). Calves treated with antibiotics produced 623 kg more milk per kg of pre-weaning ADG while calves that did not receive antibiotics produced 1,407 kg more milk per kg of pre-weaning ADG. The effect of increased nutrient intake from milk replacer was still apparent in the calves that were treated, but the milk yield response was most likely attenuated due to factors associated with sickness and nutrient partitioning away from growth functions (Johnson, 1998; Dantzer, 2006).

In this data set, we had the opportunity to evaluate the effect of pre-weaning ADG on milk production in the second and third lactations. Data from both herds demonstrated positive relationships between pre-weaning ADG and milk yield in subsequent lactations (Tables 2.4 and 2.5). In the Cornell herd, the effect was significant for the second lactation, positive but non-significant for the third lactation, and when analyzed over the three lactations, highly significant and substantial in the amount of milk represented by pre-weaning ADG. In the commercial herd, the data were only significant for the first lactation and the negative residuals in the second lactation could not be explained by any variables there were available in the data set. Overall the data were positive over the three lactations in the commercial herd, although not significant. This demonstrates that the effect of early life nutrient intake can be variable by herd and the variation within herd is difficult to identify in measuring this response over multiple lactations. However, the data strongly suggest that the effect of early life nutrition and management previously attributed only to the first lactation can now be discussed in terms of lifetime productivity.

The ADG of the calves were responsive to the environment, especially under conditions of cold stress and maintenance requirements of the calves increased during periods of cold stress. Thus, at constant feeding rates, despite being higher than industry averages, growth rate and first lactation milk yield was reduced by the average ambient temperature at birth representing changes in maintenance requirements (Figures 2.1 and 2.2).

Among these two farms, calves born during the winter produced on average 556 kg less milk during their first lactation than calves born during the summer ($P < 0.01$) (data not shown). Thus, the season in which a calf was born reflected the effects of environmental temperature on the maintenance requirements of the calves. However, specific local environments at each farm interacted differently; at the Cornell farm, calves born during the summer produced more milk than calves born during any other season ($P < 0.01$) while at the commercial farm, calves born during the fall produced more milk than calves born during any other season ($P < 0.01$). We assume these observations are related to energy intake above maintenance, however, we cannot

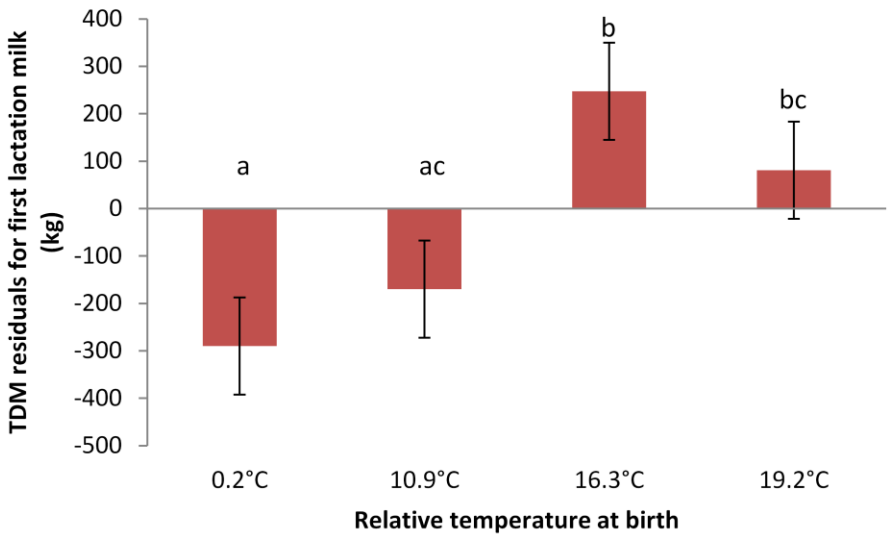

Figure 2.1. Test Day Model lactation residuals in kilograms of milk (±SD), averaged by temperature at the time of birth in the Cornell herd (Harford, NY). Columns with different superscripts differ $P < 0.05$

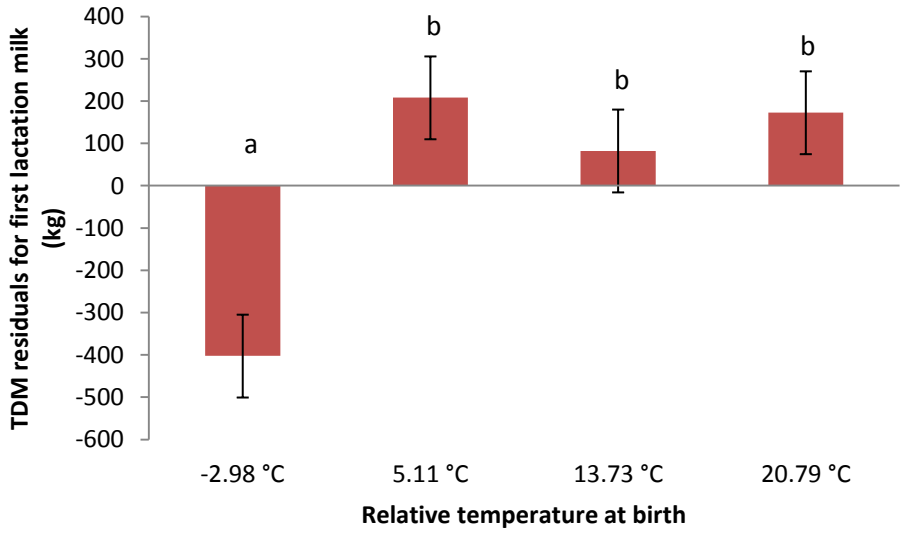

Figure 2.2. Test Day Model lactation residuals in kilograms of milk (±SD), averaged by temperature at the time of birth in the commercial herd. Columns with different superscripts differ $P < 0.05$.

rule out the effects of photoperiod or differences in colostrum status of calves in explaining some of the milk response (Rius and Dahl, 2006).

To explore this in a different manner and make the data more quantifiable, the growth data and milk yield were analyzed using temperature at birth. Within the Cornell dairy, calves born during the colder months (0.2°C) produced 532.2 kg less milk than calves born in thermoneutral conditions (16.3°C) ($P < 0.01$, Figure 2.1). Since all calves were fed the same amount of milk replacer throughout the year, we concluded that the effect of ADG on first lactation milk production was an indirect effect of nutrient intake above maintenance during the first 49 days of life. To quantify this relationship, information regarding the milk replacer offered to each calf during the first 49 days of life and mean monthly temperatures for the time they were born were recorded. To evaluate the effect of intake over maintenance as another variable affecting first lactation milk yield, the 2001 Dairy NRC equation for maintenance requirements for calves was used to calculate the Mcals of energy required for each calf and then the Mcals of energy consumed above maintenance were estimated. Using the calculated information on Mcals of energy consumed above maintenance, the TDM residuals were regressed on the Mcals of energy above maintenance. Within the Cornell herd, positive correlations with first, second and third lactation milk yields were observed for Mcal of energy consumed above the maintenance requirements (Table 2.4). In the Cornell herd, for every Mcal of additional energy consumed from milk replacer during the pre-weaning period, calves produced 235 kg more milk ($P < 0.01$) during first lactation or a total of 903 kg more milk per Mcal when analyzed over three lactations ($P < 0.01$). Calves born during the colder months (0.2°C) consumed on average 1.43 Mcals/d less energy above maintenance than calves born during the warmer months (19.2°C). Based on NRC calculations, this difference in energy above maintenance is equivalent to 0.3 kg of ADG and provides some indication of the sensitivity of calf to this input relative to the signals to enhance milk producing ability. To evaluate the possible limits of these correlations, we analyzed the cubic and quadratic effects of Mcals above maintenance with milk yield. Within our data set, a linear equation best described this relationship, indicating that within the range of data

analyzed (ADG ranging from 0.10 kg to 1.58 kg and for Mcals above maintenance from 0.92 Mcals to 4.13 Mcals) there was no plateau in the milk yield response. Also, when analyzed with the data for antibiotic treatment, the effect of intake over maintenance was not different between calves that were treated or not ($P > 0.1$).

Most of the data in the literature related to pre-pubertal ADG and future milk yield has demonstrated negative effects of increasing the level of nutrient intake in pre-pubertal heifers (Foldager and Sejrsen, 1987; Radcliff et al. 2000). However, in the current study data from the commercial farm demonstrates that if the calves received a higher level of nutrient intake during the pre-weaning phase, higher levels of nutrient intake post-weaning will have a positive effect on first lactation milk yield. In this data, for every additional kg of ADG from birth to breeding, heifers produced 3,281 kg of milk during their first lactation ($P < 0.01$; Table 2.5) and this effect was observed over the three lacatations. If only the post-weaning period is considered, for every additional kg of ADG from weaning to breeding, heifers produced 8,200 kg of milk during three lactations ($P < 0.01$; Table 2.5). The primary difference between this data set and data generated previously is pre-weaning nutrition. In previous studies (Radcliff et al, 2000; Van Amburgh et al. 1998) heifers were started on treatment diets post-weaning and no attempt was made to modify pre-weaning nutrition or management. The calves in this data set were fed a higher level of nutrient intake prior to weaning and this appears to have altered the pre-pubertal growth response allowing heifers to be able to respond to higher levels of nutrient intake post-weaning. Similar observations were described by Moallem et al. (2010) and data from Meyer et al. (2006) demonstrated that calves fed greater amounts of higher protein milk replacer prior to weaning had significantly greater bromodeoxyuridine incorporation and thus greater mammary parenchyma proliferation prior to weaning. Although mammary DNA is not a good indicator of future milk yield, the observation that there are cells in the neonatal mammary gland that are responsive to nutrient supply might provide a possible mechanism and area of study for these long-term responses.

These observations reinforce the role that management plays in phenotypic expression of genetic capacity. The observation that pre-weaning growth rate accounted for approximately 22% of the variation in first lactation milk yield further indicates that there is greater milk producing capacity that can be modified in these calves, once we more fully understand the signals that are being triggered by this nutrient intake response. Based on the relationship between pre-weaning growth and milk yield, it might appear that any factors that enhance protein accretion and thus growth, will enhance the milk yield capacity of the calf. Therefore, to potentially stimulate this long-term response energy intake above maintenance and protein status of the calf become critical factors, but these effects were not directly analyzed within this study. This was implied in this study by the composition of milk replacer fed to the calves. This observation would be consistent with the requirements of calf, especially the more contemporary requirements determined from serial harvest studies on young dairy calves where protein requirements for higher gain would be between 26 and 28% CP (Van Amburgh and Drackley, 2005; Bartlett et al., 2006). The relationship between the protein status of the calf and future milk yield was suggested in the data of Moallem et al. (2010) where the calves fed the lower protein quality milk replacer did not demonstrate a significant milk yield response despite being offered the milk replacer on an ad libitum basis.

Calf starter intake was not measured in this study and relative to energy intake is a shortcoming of the data with regards to the possible impact of this additional energy on milk yield. The role of starter intake could be important however, it is hard to establish dry matter intakes from starter grain that provide adequate energy supply for optimum growth rates prior to 49 days of age, the period in which the calf appears to be sensitive to this effect, especially if consideration is given to absorbed energy substrates and not simply rumen development and gut fill (Stobo et al. 1966). Studies in calves fed restricted levels of milk replacer (10% initial BW at 12.5% solids) evaluating starter grain formulation, DM intakes and rumen development demonstrate growth rates over the first 5 weeks of 0.18 to 0.35 kg/d (Coverdale et al., 2004; Lesmeister and Heinrichs, 2004). Based on the data presented in this paper, the growth rates

observed in those studies would not stimulate the factors responsible for the enhanced milk yield response and therefore the absence of pre-weaning grain intake is most likely not confounding the relationships observed in this study.

These observations imply that the nutritional or metabolic programming that occurs during the first two months of life has lifelong implications on milk production. Lifelong implications of a stimulus shortly after birth were traced to epigenetic changes in rats (Weaver et al., 2004). They demonstrated that the behavior of the mother affected the way her pups responded to stress throughout their life; these effects were mediated by epigenetic regulations. The observation that nutrition can impart long-lasting changes has gained considerable attention over the last few years in the area of human development and great progress has been made (Hanley et al., 2010). It appears that similar gains can be made in milk production through programming effects that are modified through a lactocrine type mechanism in the first two months of life in the neonatal calf.

CONCLUSIONS

This analysis presents data that reinforces the observation that lifetime performance is influenced by early life development and dairy producers have the ability to manipulate this early life programming via nutrition. The length of time that heifer calves are responsive to the effects of nutrition warrants further investigation. However, we now know that this manipulation must start immediately after birth and continue for at least five weeks and must be in the form of liquid feed in order to have a positive influence on lifetime performance.

ACKNOWLEDGMENTS

We gratefully acknowledge Milk Specialties Global and Land O'Lakes, Inc. for financial and other support of this work, the New York dairy farm that provided us with access to the farm's

data and the staff of the Teaching and Research Farm for their help and support of this work. We also thank Dr. John Ferry for his help in securing a portion of this data.

REFERENCES

Anderson, K. L., T. G. Nagaraja, and J. L. Morrill. 1987. Rumen metabolic development in calves weaned conventionally or early. J. Dairy Sci. 70:1000-1005.

Bagnell, C.A., W. Yan, A.A. Wiley, F.F. Bartol. 2005. Effects of relaxin on neonatal porcine uterine growth and development. Ann. NY Acad. Sci. 1041:248-255.

Bar-Peled, U., B. Robinzon, E. Maltz, H. Tagari, Y. Folman, I. Bruckental, H. Voet, H. Gacitua, and A. R. Lehrer. 1997. Increased weight gain and effects on production parameters of Holstein heifer calves that were allowed to suckle from birth to six weeks of age. J Dairy Sci. 80:2523-2528.

Bartlett, K.S., F. K. McKeith, M. J. VandeHaar, G. E. Dahl, and J. K. Drackley. 2006. Growth and body composition of dairy calves fed milk replacers containing different amounts of protein at two feeding rates. J Anim Sci. 84:1454-1467.

Bartol, F.F., A.A. Wiley, and C.A. Bagnell. 2008. Epigenetic programming of procine endometrial function and the lactocrine hypothesis. Reprod. Dom. Anim. 43:273-279.

Bauman, D.E., R.W. Everett, W.H. Weiland, R.J. Collier. 1999. Production responses to bovine somatotropin in Northeast dairy herds. J. Dairy Sci. 82:2564-2573.

Baumrucker, C. R. and J.W. Blum 1993. Secretion of insulin-like growth factors in milk and their effect on the neonate. Livestock Prod. Sci. 35:49-72.

Blum, J.W. and H. Hammon. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. Livestock Prod. Sci. 66:151-159.

Burrin, D. G., T. A. Davis, S. Ebner, P.A. Schoknecht, M.L. Fiorotto, P.J. Reeds. 1997. Colostrum enhances the nutritional stimulation of vital organ protein synthesis in neonatal pigs. J. Nutr. 127:1284-1289.

Cornell Research Foundation and Everett, R.W. 1994. Method of bovine management. U.S. Patent Number 5,351,644.

Coverdale, J. A., H. D. Tyler, J. D. Quigley, III, and J. A. Brumm. 2004. Effect of various levels of forage and form of diet on rumen development and growth in calves. J. Dairy Sci. 87:2554–2562.

Dantzer, R. 2006. Cytokine, sickness behavior, and depression. Neurol Clin. 24:441-460.

Davis, C. L., and J. K. Drackley. 1998. The Development, Nutrition,and Management of the Young Calf. Iowa State Univ. Press, Ames.

Donovan, S.M. and J. Odle. 1994. Growth factors in milk as mediators of infant development. Ann. Review Nutr. 14:147-167.

Everett, R. W. and F. Schmitz. 1994. Dairy genetics in 1994 and beyond. Cow and sire evaluation using test-day records, DairyGene, and DairyView for farm management. Pp 4-39. Mimeo Ser. No. 170, Cornell Coop. Ext., Cornell Univ., Ithaca, NY.

Faber, S. N., N.E. Faber, T.C. McCauley, and R.L. Ax. 2005. Case study: effects of colostrum ingestion on lactational performance. Prof Anim. Scientist. 21:420-425.

Foldager, J. and C.C. Krohn. 1994. Heifer calves reared on very high or normal levels of whole milk from birth to 6-8 weeks of age and their subsequent milk production. Proc. Soc. Nutr. Physiol. 3:301

Foldager, J., and K. Sejrsen. 1987. Mammary gland development and milk production in dairy cows in relation to feeding and hormone manipulation during rearing. In: Research in Cattle Production, Danish Status and Perspectives. p 102. Landhusholdningsselskabet, Frederiksberg, Denmark.

Grosvenor, C. E., M. F. Picciano, and C. R. Baumrucker. 1993. Hormones and growth factors in milk. Endocrinology Rev. 14:710-728.

Hanley, B., J. Dijane, M. Fewtrell, A. Grynberg, S. Hummel, C. Junien, B. Koletzko, S. Lewis, H. Renz, M. Symonds, M. Gros, L. Harthoorn, K. Mace, F. Samuels and E. M. van Der Beek. 2010. Metabolic imprinting, programming and epigenetics - a review of present priorities and future opportunities. Br J. Nutr. 104 Suppl 1:S1-25.

Johnson, R.W. 1998. Immune and endocrine regulation of food intake in sick animals. Domest Anim Endo. 15:309–319.

Kertz, A. F., L.R. Prewitt, J.P. Everett 1979. An early weaning calf program: summarization and review. J. Dairy Sci. 62:1835-1843.

Lesmeister, K.E. and A. J. Heinrichs. 2004. Effects of corn processing on growth characteristics, rumen development, and rumen parameters in neonatal dairy calves. J. Dairy Sci. 87:3439-3450.

Meisel, H. 2005. Biochemical properties of peptides encrypted in bovine milk proteins. Curr. Med. Chem. 12:1905-1919.

Meyer, M. J., A. V. Capuco, D. A. Ross, L. M. Lintault, and M. E. Van Amburgh. 2006. Developmental and nutritional regulation of the prepubertal bovinemammary gland: II. epithelial cell proliferation, parenchymal accretion rate, and allometric growth. J. Dairy Sci. 89:4298–4304.

Moallem, U., D. Werner, H. Lehrer, M. Zachut, L. Livshitz, S. Yakoby, and A. Shamay. 2010. Long-term effects of ad libitum whole milk prior to weaning and prepubertal protein supplementation on skeletal growth rate and first-lactation milk production. J. Dairy Sci. 93:2639-2650

Morrison S. J., H. C. F. Wicks, A. F. Carson, R. J. Fallon, J. Twigge, D. J. Kilpatrick, S. Watson. 2011. The effect of calf nutrition on the performance of dairy herd replacements. Animal, Available on CJO doi:10.1017/S1751731111002163.

National Animal Health Monitoring Service. 2002. Dairy 2002. Part I: Reference of dairy health and management in the United States. USDA-APHIS-VS. http://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy02/Dairy02_dr_PartI.pdf. Accessed 3.23.2011.

National Animal Health Monitoring Service. 2007. Dairy 2007. Heifer calf health and management practices on U.S. dairy operations. USDA-APHIS-VS.

http://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy07/Dairy07_ir_CalfHealth.pdf. Accessed 3.23.2011.

National Research Council (NRC). 2001. Nutrient Requirements of Dairy Cattle. 7th Rev. Ed. National Academy Press, Washington, DC.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. Ed., Natl. Acad. Sci., Washington, D. C.

Nusser, K. D. and S. Frawley. 1997. Depriving neonatal rats of milk from early lactation has long-term consequences on mamotrope development. Endocr. 7:319-323.

Otterby, D.E. and J. G. Linn. 1981. Advances in nutrition and management of calves and heifers. J Dairy Sci. 64:1365-1377.

Radcliff R. P., M. J. Vandehaar, L. T. Chapin, T. E. Pilbeam, D. K. Beede, E. P. Stanisiewski, and H. A. Tucker. 2000. Effects of diet and injection of bovine somatotropin on prepubertal growth and first-lactation milk yields of Holstein cows. J Dairy Sci 83:23–29.

Raeth-Knight, M., H. Chester-Jones, S. Hayes, J. Linn, R. Larson, D. Ziegler, B. Ziegler and N. Broadwater. 2009. Impact of conventional or intensive milk replacer programs on Holstein heifer performance through six months of age and during first lactation. J Dairy Sci. 92:799-809.

Rauprich, A. B., H. M. Hammon, J. W. Blum. 2000. Influence of feeding different amounts of first colostrum on metabolic, endocrine, and health status and on growth performance of neonatal calves. J. Anim. Sci. 78:896-908.

Reid, J. T., J. K. Loosli, G. W. Trimberger, K. L. Turk, S. A. Asdell and S. E. Smith. 1964. IV. Effect of plane of nutrition during early life on growth, reproduction, production, health and longevity of Holstein cows. Birth to fifth calving. Causes and prevention of reproductive failure in dairy cattle. Cornell Research Bulletin 987. Ithaca, NY

Rius, A. G. and G. E. Dahl. 2006. Exposure to long-day photoperiod prepubertally may increase milk yield in first-lactation cows. J. Dairy Sci. 89:2080–2083

Shamay, A., D. Werner, U. Moallem, H. Barash, and I. Bruckental. 2005. Effect of nursing management and skeletal size at weaning on puberty, skeletal growth rate, and milk production during first lactation of dairy heifers. J. Dairy Sci. 88:1460–1469.

Stobo, I.J.F., J.H.B. Roy, H. J. Gaston. 1966. Rumen development in the calf. 1. The effect of diets containing different proportions of concentrates to hay on rumen development. Br. J. Nutr. 20:171-188.

Terré, M., C. Tejero, and A. Bach. 2009. Long-term effects on heifer performance of an enhanced growth feeding programme applied during the pre-weaning period. J. Dairy Res. 76:331-339.

Van Amburgh, M. E. and J. K. Drackley. 2005 Current perspectives on the energy and protein requirements of the pre-weaned calf. Chap. 5 in ”Calf and heifer rearing: Principles of rearing the modern dairy heifer from calf to calving”. Nottingham Univ. Press. P.C. Garnsworthy, ed. Pp.67-82.

Van Amburgh, M. E., D. M. Galton, D. E. Bauman, and R. W. Everett. 1997. Management and economics of extended calving intervals with use of bovine somatotropin. Livestock Prod. Sci. 50:15-28.

Van Amburgh, M.E., D. M. Galton, D. E. Bauman, R. W. Everett, D. G. Fox, L. E. Chase and H. N. Erb. 1998. Effects of three prepubertal body growth rates on performance of Holstein heifers during first lactation. J Dairy Sci. 81(2):527-38

Weaver, I. C. G., N. Cervoni, F. A. Champagne, A. C. D’Alessio, S. Sharma, J. R. Seckl, S. Dymov, M. Szyf and M. J. Meaney. 2004. Epigenetic programming by maternal behavior. Nat. Neurosci 7:847-54.

CHAPTER III

SHORT COMMUNICATION: GENE EXPRESSION CHANGES DURING MAMMARY GLAND DEVELOPMENT

F. Soberon, M. Foote, M. J. Meyer and M. E. Van Amburgh

ABSTRACT

Calves that received greater nutrients prior to weaning from milk replacer from birth to 100 kg BW had significantly increased mammary epithelial cell proliferation compared to calves fed a more restricted diet. Additional work has demonstrated the presence of a putative stem cell population that has been considered nutritionally responsive and might account for the observed mammary cell proliferation. Furthermore, changes in mammary growth rate occur from birth to puberty; an example is the change from allometric to isometric growth during the peri-pubertal period.The first objective of this study was to evaluate genes that might be responsible for signaling this change in mammary growth from an allometric to an isometric rate. Thus, the second objective of this study was to identify genetic marker of mammary stem cells associated with proliferation.. Heifers (n = 72) were reared on one of two dietary treatments: restricted (R) to 650 g/d gain or elevated (E) 950 g/d gain. Heifers were harvested at 100, 150, 200, 250, 300, or 350 kg BW. Mammary samples (n = 5 to 8) were excised from the mid parenchyma tissue (MPT) and extra parenchyma tissue (EPT), snap-frozen and stored at -80°C. Gene expression in mammary tissue was determined by RT-PCR. Data were analyzed using a mixed model with 18S RNA as a covariate. Telomerase transcriptase was used as a marker for stem cells. In addition, SERPINB5 (maspin) was analyzed as a marker of myoepithelial cell development and a possible signal for changes in growth rate of the mammary gland. Telomerase expression in EPT was lowest at 100 kg BW for both treatments and tended to decrease in R heifers at 350 kg BW. Telomerase expression did not differ in MPT but was lower at 100 and 150 kg BW for both treatments. Expression of SERPINB5 was significantly increased in EPT at 250 kg BW for both

treatments whereas SERPINB5 expression in MPT remained constant among all weight groups. In the initial mammary growth data, allometric growth was retarded and isometric growth resumed at approximately 250 kg BW. Thus, this suggests an increased in the population of myoepithelial cells in the border of the mammary parenchyma to signal the end of allometric growth. Myoepithelial cells might in turn arrest the effect of estrogen in the mammary cells extending into the fat pad.

Key words: Mammary development; gene expression

Short communication

High energy intakes and the associated growth rates during the rearing period have long been associated with a decrease in mammary gland development (Sejrsen et al., 1982; Mäntysaari et al., 1995; Capuco et al., 1995). However, Meyer et al. (2006a) showed that mammary development was not associated with energy intake, and growth rate but rather with age, and if age was considered, the development of the mammary gland was not affected by energy intake. Furthermore, it has been demonstrated that increasing the ADG of pre-weaned calves has positive effects on milk production (Soberon et al., 2012).

Little is known about the mechanism through which the mammary gland is responsive to early life nutrient intake, or about the signaling pathways that promote the changes between allometric and isometric growth of the mammary gland. Ballagh et al. (2008) suggested the possible involvement of myoepithelial cells in the arrest of allometric growth by comparing pre-pubertal ovariectomized heifers with intact control heifers. They suggested that the ovaries influence the development of the mammary gland by limiting the proliferation and activity of myoepithelial cells. Ballagh et al. (2008) also hypothesized that myoepithelial cells might be responsible for limiting growth in the developing gland. Myoepithelial cells are known to express maspin, a protease inhibitor classified as a tumor suppressor due to its ability to inhibit

the growth and proliferation of breast cancer tissue through the down regulation of estrogen signaling (Bailey et al., 2006).

Another possible effect of nutrition on the developing mammary gland could result from the proliferation of stem cells (Ellis and Capuco, 2002). However, no conclusive evidence has been reported about the dynamics of these cell populations in the neonatal or pre-pubertal mammary gland (Daniels et al., 2008). Additionally in the murine model, prostaglandin E stimulates the proliferation of mammary epithelium in the presence of epithelial growth factor (Bandyopadhyayl et al., 1987) thus making it a potential candidate for the bovine mammary epithelium.

The primary objectives of this study were to investigate the presence of myoepithelial cells in the mammary gland at different stages of development as well as their role in the development of the mammary gland of heifers fed at two different levels of nutrient intake using maspin as a marker for those cells. The secondary objectives were to determine possible differences in stem cell abundance in the mammary gland and to better understand possible signaling pathways involving prostaglandins in the mammary gland at various developmental stages under different levels of nutrient intake and growth rate.

The experimental design is described in detail in Meyer et al., (2006a). All protocols involving the use of animals were reviewed and approved by the Cornell University Institutional Animal Care and Use Committee. Briefly, 72 Holstein heifer calves (44.2 kg of BW, 9.9 d of age) were assigned to one of two planes of nutrition. The elevated (E) treatment was designed to achieve a gain of 950 g/d of BW while the restricted (R) treatment was designed to achieve a gain of 650 g/d of BW. Prior to weaning, all calves were fed milk replacer (MR) two times per day. E calves received a MR with 29% CP, 19% fat fed at 0.32 Mcal intake energy/kg of $BW^{0.75}$, whereas R calves received a MR with 22% CP, 21% fat fed at 0.20 Mcal intake energy/kg of $BW^{0.75}$. Weaning was initiated after 6 wk and lasted for 7 d. A textured calf pellet starter (22% CP, Cargill, Inc.) was offered from wk 3 through wk 10. After wk 10 all calves were fed a TMR.

Heifers were weighed weekly and the amount of MR or TMR was adjusted accordingly to obtain the desired weight gains.

Six calves per treatment were harvested at the following live weights: 100, 150, 200, 250, 300 or 350 kg. At harvest, the udder was removed and separated at the medial suspensory ligament. The left half was immediately dissected, and tissue from the mid-parenchyma (MPT), the extra-parenchyma (defined as the border between parenchyma and fat pad; EPT) or fat pad were snap frozen in liquid nitrogen for RNA isolation.

Total RNA was isolated from two regions of the mammary gland using the RNeasy Lipid Tissue Mini kit with on-column DNase digestion (Qiagen Inc.) Quality and quantity of RNA was determined using the Agilent 2100 Bioanalyzer. One microgram of DNAse treated RNA was reversed transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Transcript abundance was determined by SYBR Green quantitative RT-PCR (Applied Biosystems). Melt curve analysis was performed at the end of the amplification to verify the presence of a single product. At least four different primer combinations were tested for each gene of interest and the best combination was used for tissue analysis (Table 3.1). Data were analyzed using the mixed procedures of SAS 9.2 (SAS Inst. Inc., Cary, NC). Terms in the model included treatment, BW, expression of 18s as a covariate and the interaction of treatment and BW. Significance was declared at $P < 0.05$.

Maspin expression in the MPT increased in restricted animals at 150 kg, but no differences were detected at any other harvest weight. Mammary tissue from both treatments displayed increased expression of maspin in the extra-parenchyma region at 250 kg, coinciding with the onset of puberty and the shift from allometric to isometric growth (Figure 3.1). Since maspin is a marker for the presence of myoepithelial cells (Ballagh et al., 2008), the increased expression of maspin at the onset of puberty might indicate the proliferation of myoepithelial cells at this stage of mammary development. Maspin is classified as a class II tumor suppressor in breast cancer as well other cancers (Bailey et al., 2006) and it is associated with the down regulation of estrogen receptor-α as well as the progesterone receptor (Lockett et al., 2005).

Table 3.1. Primers use for RT-PCR

Gene	Forward primer	Reverse primer
18 s	GATCCATTGGAGGGCAAGTCT	GCAGCAACTTTAATATACGCTATTGG
EP2	CGGACCATCTTATTCTCCTG	AAATCGTGAAAGGCAAGGAG
PTGES	GGAACGACCCAGATGTG	GAAAGAGTAGACAAAGCCCA
TERT	CGCTCTACTTCGTCAAGG	CTCTGCCAGCTTATCCTG
SERPINB5	ATGCCAAAGTCAAACTCTCCAT	GACATCCCAGAGAAATCAGAGG

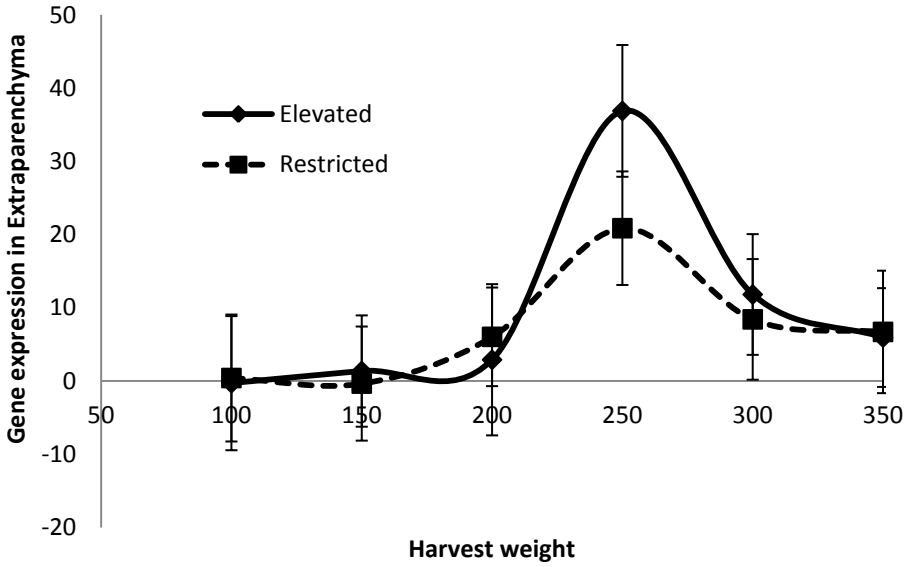

Figure 3.1. Relative expression of SERPINB5 (maspin) in the extra-parenchyma region of the mammary gland of heifer calves at different body weights for calves raised on an elevated (950g/d) or restricted (650 g/d) level of nutrition. Means and Std error shown.

Exogenous estrogen was observed to have significant impact in the mammary fat pad and parenchyma tissue of pre-pubertal calves by increasing the abundance of IGF-1, progesterone receptor as well as proliferating cell nuclear antigen transcripts. In the mammary parenchyma, exogenous estrogen also reduced the expression of estrogen receptor α, but this was not the case in the mammary fat pad (Meyer et al., 2006). Mammary gland development has been described as changing from an allometric to an isometric rate during the peri-puberty period (Sinha and Tucker, 1969; Meyer et al., 2006b). It is possible that this change comes about through the effects of maspin and myoepithelial cells acting indirectly on the mammary tissue through estrogen.

Telomerase expression in EPT was lowest ($P < 0.05$) at 100 kg BW for both treatments and tended ($P < 0.10$) to decrease in R at 350 kg BW. Telomerase expression did not differ between TRT in MPT but was lower at 100 and 150 kg BW ($P < 0.05$). Telomerase expression tended to increase with weight in MPT up to 200 kg, after which its expression plateaued. Telomerase expression in the EPT did not show any clear patterns.

Prostaglandin E syntheses in the EPT of the mammary gland did not differ between treatments but tended to increase with BW after 200 kg. The expression of prostaglandin E receptor 2 was higher in the elevated group at 350 kg but was not different between treatments at any other harvest weight.

The development of the mammary gland in the bovine is regulated by multiple signaling systems. The data from this study suggest the regulation of pre-pubertal mammary growth from an allometric rate to an isometric rate in the peri-pubertal period is in part regulated by the presence of myoepithelial cells. The signaling pathway by which myoepithelial cells could arrest the allometric growth rate might be through the expression of maspin. The direct or indirect mechanism through which maspin regulates mammary epithelium growth is not fully understood but if similar to the data from breast cancer, it is most likely by altering estrogen signaling from the ovary through estrogen receptor binding or down-regulation of the receptor. No differences were detected among treatments in stem cell proliferation using telomerase expression as a

marker for stem cells, but the increase in telomerase expression in mammary tissues as heifers matured suggests that this population of cells is active. Finally, prostaglandin E synthesis and receptors did not differ among treatments but increased with maturity of the heifer indicating a role for prostaglandins in mammary development.

REFERENCES

Bailey, C. M., Z. Khalkhali-Ellis, E. A. Seftor, and M. J.C. Hendrix. 2006. Biological functions of maspin. J. Cell. Physiol. 209:617-24.

Ballagh, K., N. Korn, L. Riggs, S. L. Pratt, F. Dessauge, R. M. Akers, and S. Ellis. 2008. Hot Topic: Prepubertal ovariectomy alters the development of myoepithelial cells in the bovine mammary gland. J. Dairy Sci. 91:2992-95

Bandyopadhyayl, G. K., W. Imagawa, D. Wallace, and S. Nandi. 1987. Linoleate metabolites enhance the in vitro proliferative response of mouse mammary epithelial cells to epidermal growth factor. J. of Bio Chem., 262:2750-56.

Capuco A. V., J. J. Smith, D. R. Waldo, and C. E. Rexroad. 1995. Influence of prepubertal dietary regimen on mammary growth of Holstein heifers. J. Dairy Sci. 78:2709-2725.

Daniels, K. M., A. V. Capuco, R. E. James, M. L. McGilliard, and R. M. Akers. 2008. Diet does not affect putative mammary epithelial stem cells in pre-weaned Holstein heifers. J. Anim. Sci. 86 Suppl:121 (abstract).

Ellis, S., and A. V. Capuco. 2002. Cell proliferation in bovine mammary epithelium: Identification of the primary proliferative cell population. Tissue & Cell 34: 155–163.

Lockett, J., S. Yin, X. Li, Y. Meng, and S. Sheng. 2005. Tumor suppressive maspin and epithelial homeostasis. J. Cell. Biochem. 97: 651-60.

Mäntysaari P., K. L. Ingvartsen, V. Toivonen, K. Sejrsen. 1995. The effect of feeding level and nitrogen source of the diet on mammary development and plasma hormone concentrations of pre-pubertal heifers. Acta. Agric. Scand. Sect. A 45:236-244.

Meyer M. J., A. V. Capuco, D. A. Ross, L. M. Lintault, and M. E. Van Amburgh. 2006b. Developmental and nutritional regulation of the prepubertal bovine mammary gland: II. Epithelial cell proliferation, parenchymal accretion rate, and allometric growth. J. Dairy Sci. 89:4298-4304

Meyer M. J., A. V. Capuco, D. A. Ross, L. M. Lintault, and M. E. Van Amburgh. 2006a. Developmental and nutritional regulation of the prepubertal heifer mammary gland: I. Parenchyma and fat pad mass and composition. J. Dairy Sci. 89:4289-97.

Meyer M. J., A. V. Capuco, Y. R. Boisclair, M. E. Van Amburgh. 2006. Estrogen-dependent responses of the mammary fat pad in prepubertal dairy heifers. J. Endocrinol. 190:819-27.

Sejrsen K., Huber J. T., Tucker H. A., Akers R. M. 1982. Influence of nutrition of mammary development in pre- and postpubertal heifers. J Dairy Sci. 65:793-800.

Sinha Y. N., Tucker H. A. 1969. Mammary development and pituitary prolactin level of heifers from birth through puberty and during the estrous cycle. J Dairy Sci. 52:507-12.

Soberon, F., E. Raffrenato, R. W. Everett, M. E. Van Amburgh. 2012. Pre-weaning milk replacer intake and effects on long term productivity of Dairy calves. J. Dairy Sci. (In press).

CHAPTER IV

EFFECTS OF PRE-WEANING NUTRIENT INTAKE IN THE DEVELOPING MAMMARY PARENCHYMAL TISSUE AND FAT PAD

F. Soberon and M. E. Van Amburgh

ABSTRACT

Historically, the mammary gland has been considered to grow at an isometric rate during the first 2 mo of life followed by an allometric rate until peri-puberty. The objectives of this study were to describe the effects of nutrient intake pre-weaning and mammary gland development and to investigate cell specific activity during this phase of development. Twelve dairy heifer calves were fed either a constant amount of a 28% CP, 15% fat milk replacer (MR) per day that was equivalent to 2.8 Mcal ME Intake per d (Control, n=6) or 0.3 Mcal ME Intake /per kg $BW^{0.75}$ (from 4.2 to 8.4 Mcals ME Intake per d) (Enhanced, n=6). All calves had full access to water and a 22% CP commercial calf starter. Calves were harvested at 54 ± 2 d. Control calves consumed 32.6 ± 2.4 kg of MR and 6.7 ± 0.5 kg of calf starter per calf while the Enhanced calves consumed 69.5 ± 2.4 kg of MR and 1.9 ± 0.5 kg of calf starter per calf over the 54 d period. Further, to evaluate putative stem cell proliferation, BrdU (5 mg/kg) was injected intramuscularly from 12 to 15 and 24 to 27 days of life. Initial and final BW for the Control and Enhanced treatments were 39.2, 61.0, 39.7, and 83.2 kg, respectively. At harvest, weights of liver, kidneys, pancreas, whole skinned mammary gland and mammary parenchyma were measured. Growth rate of each organ was estimated from differences in organ weight as a percentage of BW. The mammary glands of the calves fed the Enhanced treatment were heavier at harvest; furthermore, when parenchymal weight was analyzed, Enhanced calves had 5.9 times greater parenchymal mass ($P < 0.01$). Thus, mammary gland development was responsive to nutrient intake prior to weaning. Based upon differences in nutrient supply, allometric growth of the Enhanced treatment calves' mammary gland was initiated pre-weaning. Further

characterization of mammary stem cells as percent of epithelial cells revealed no significant differences between treatments, however given the significant expansion of the parenchyma tissue of the calves fed greater nutrients there are more stem cells present in the mammary gland of Enhanced calves as a function of the final cell number and tissue mass.
Key Words: mammary gland, pre-weaning nutrition, growth rate

INTRODUCTION

Pre-pubertal mammary development has historically been described as having two distinct phases, isometric and allometric growth (Sinha and Tucker, 1969). Allometry was a termed coined by Huxley in 1936 that describes the ratio between the growth of two parts. This ratio can be either positive or negative but when the ratio is one then the growth of the two parts is considered isometric whereas, any ratio greater than one implies a growth rate faster than the part of comparison or allometric (Huxley, 1950). Prior to approximately 3 mo of age, the mammary gland has been shown to grow at a rate similar to the body (isometric) and then from approximately 3 months of age to the peri-puberty period positive allometric growth was observed, (Sinha and Tucker, 1969; Meyer et al. 2006b). However, Brown et al. (2005) and Meyer et al. (2006b) reported that mammary epithelium proliferation could be influenced by diet during the pre-weaning period, but not post-weaning. In the data of Meyer et al. (2006b), mammary cell proliferation assessed by brdU labeling as a marker for DNA proliferation appeared to be allometric from birth in calves fed higher levels of nutrient intake, suggesting that the previously observed phases of growth in the data of Sinha and Tucker (1969) might have been due to nutrient intake relative to maintenance, at least prior to weaning. Furthermore, Meyer et al. (2006b) suggest that there are cells within the mammary gland that are nutritionally responsive in the early neonatal period. Given the emerging data describing the effects of early nutrition on long-term productivity (Moallem et al. 2010; Soberon et al. 2012), research to describe the milk yield response must focus on understanding the factors responsible for this

long-term enhancement of productivity. One hypothesis is that mammary cells are nutrient responsive during this phase of development so proliferation can be altered during this pre-weaning phase and this is an outcome of specific cells types. Thus, the objectives of this study were to determine the effects of pre-weaning liquid feed intake on organ size, mammary gland development and putative stem cell proliferation in dairy calves.

MATERIALS AND METHODS

All protocols involving the use of animals were reviewed and approved by the Cornell University Institutional Animal Care and Use Committee. Twelve Holstein heifer calves from the Cornell Research Farm (Harford, NY) were randomly assigned at birth to one of two treatments (TRT). All calves received four L of colostrum within one h of birth and two L 12 h later. Calves assigned to the Enhanced TRT were fed 0.3 Mcal ME Intake per kg $BW^{0.75}$ in three daily feedings, and amount of milk replacer was adjusted weekly according to changes in BW, daily amounts of energy received by each calves increased from 4.2 to 8.4 Mcals Intake Energy per d. Calves in the Control group were fed 2.8 Mcal ME per day in two daily feedings throughout the study and this TRT was designed to reflect the previous industry standard pre-weaning milk replacer feeding rates. All calves were fed a milk replacer containing 28% protein and 15% fat (Excelerate, Milk Specialties Inc, Carpentersville, IL; Table 4.1). Milk replacer refusals were recorded at each feeding. Starting on d 18, calves were offered a commercially available starter grain (22% CP, Cargill, Inc.; Table 4.1); starter grain consumption was recorded daily. Fresh water was available at all times.

All calves were housed in individual hutches and bedded with sawdust throughout the study. Weights and hip heights of each calf were measured weekly one h after the morning feeding. Calves received milk replacer up to the day they were harvested at 54 d. All calves received four daily intramuscular injections of 5-bromo, 2-deoxyuridine (BrdU; Sigma-Aldrich, Saint Louis,

Table 4.1. Milk replacer and starter grain chemical composition as reported by manufacturer.

Analysis on dry matter basis	***Milk replacer[1]***	***Starter grain[2]***
Crude protein, %	28.5	22.0
Crude fat, %	15.0	4.3
Neutral detergent fiber, %	0.2	33.1
Calcium, %	1.0	1.6
Phosphorus, %	> 0.6	1.0
Vitamin A, IU/g	> 16.5	43.1
Vitamin D3, IU/g	> 5.5	NA
Vitamin E, IU/kg	> 110.3	197.0
Gross energy, Mcals/kg	5.1	1.8

[1]Excelerate, Milk Specialties Inc, Carpentersville, IL
[2]Cargill, Inc.
NA = not available

MO) at a concentration of five mg/kg of body weight to label DNA in putative stem cells (Smith, 2005; Huderson et al., 2011); injections were given from d 12 to 15 and from d 24 to 27.

On the day of harvest, calves were fed at 0700 h, weighed and loaded for transport to the Cornell University abattoir (Ithaca, NY). Calves were harvested by stunning with a captive bolt, followed directly by exsanguination. Immediately after exsanguination, the mammary gland was removed from the body and skinned; the skinned mammary gland was weighed and the mammary gland was then sectioned at the mid-line and each half weighed independently. The parenchymal tissue was dissected by visual identification of tissue structure, and the parenchymal mass of each quarter was individually weighed. After weighing, a representative sample of epithelium from each quarter was fixed over night in 10% paraformaldehyde in phosphate buffered saline pH 7.2 (Fisher Scientific, Pittsburg, PA). After 24 h tissue samples were moved to 70 % ethanol until they were embedded for immunohistochemistry. The ovaries in each calf were visually inspected to confirm their presence and identify any activity. The liver, pancreas and kidneys were also removed and weights were recorded for each calf. All proliferation calculations were made on fresh tissue measurements.

Immunohistochemistry

Immunohistochemistry procedures were performed according to Capuco et al. (2001). In brief slides were deparaffinized and rehydrated to 70 % ethanol, and then tissue was subjected to microwave antigen retrieval in citrate buffer pH 6.0. After cooling, endogenous peroxides were blocked with a 0.5 % solution of H_2O_2. Protein was block using a 10 % solution of goat serum and casein. Primary antibody (Anti-Bromodeoxyuridine, monoclonal antibody from goat) was diluted 1:250 and added at a concentration of 4 μg/ml and incubated at 37 °C for 1.5 h. Second antibody (fruting goat) was added and incubated at room temperature for 30 min. Signal amplification was using the advidin-biotin complex system (SA-AP) and incubated for 15 min. Colorization was performed using the AEC chromogen substrate solution (Invitrogen corp,

Camarillo, CA) for 30 min. Slides were then conterstained in hematoxylin and blotted for mounting.

Slides were observed under light microscope, areas of epithelial cells were identified using a 10x lens, and then a 40x lens was used to photograph the area. Pictures were taken from random areas within the mammary epithelium and all recorded images were used for the analysis. Number of images per slide varied depending on tissue size and abundance of epithelium within the tissue, but averaged 5.5 images per calf. Each image was classified using a 4 point scale where "0" was given to images without any BrdU positive epithelial cells, "1" described images with one or two BrdU positive epithelial cells, "2" described images with 3 to 6 BrdU positive epithelial cells and "3" described images with more than 6 BrdU positive cells (Figure 4.1).

Statistical analysis

Individual growth rates were determined by regression of individual calf body weights throughout the 54 d period. Mammary and other organ growth data were analyzed by comparison of the tissue weight at harvest by treatment and the model used was: $Y_i = S_i + E_i$ Where: Y_i is the dependent, continuous variable; S_i = the effect of treatment, and E_i = the residual error of the the i^{th} average daily gain.

Mammary BrdU labeling was analyzed using the scoring approach described earlier. Each slide was classified and every slide was used in the analysis the model used was: $Y_i = S_i + E_i$ Where: Y_i is the dependent, variable; S_i = the effect of treatment, and E_i = the residual error of the i^{th} treatment.

Data were analyzed using the mixed procedures of SAS 9.2 (SAS Inst. Inc., Cary, NC). Significance was declared at $P < 0.05$.

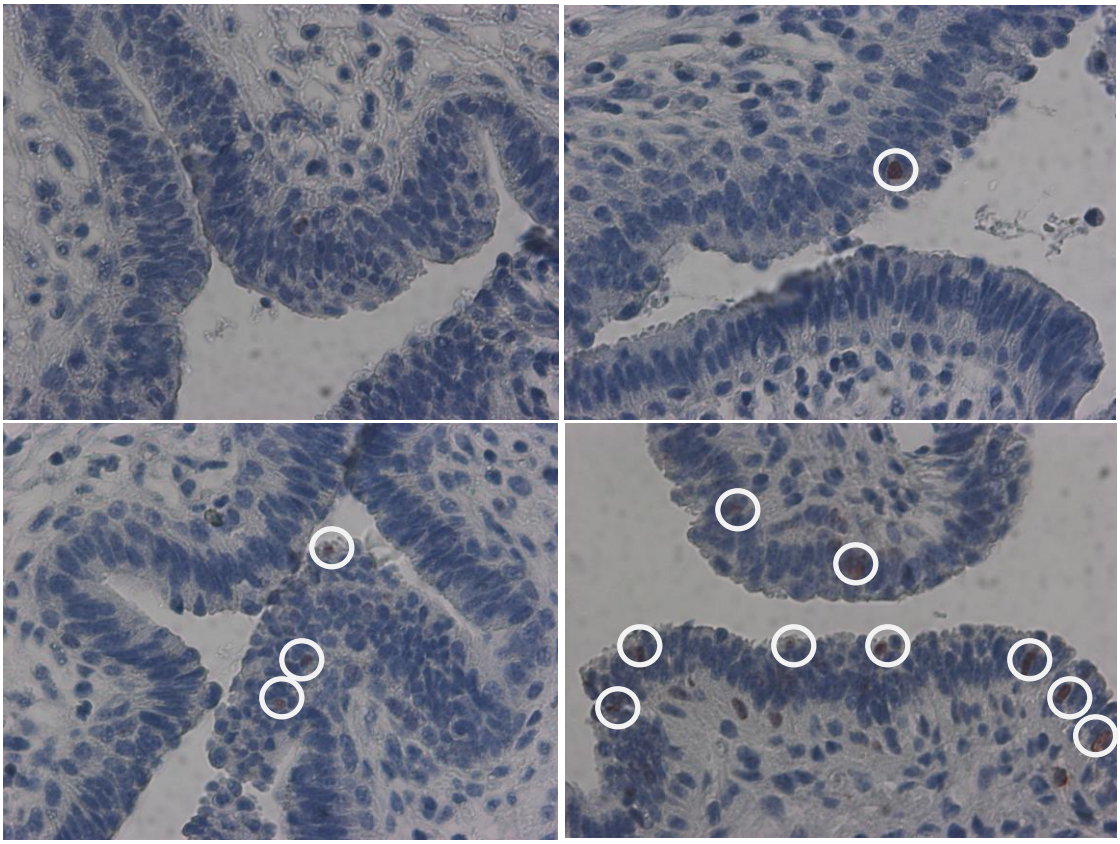

Figure 4.1. Scale used to classified images for the presence of BrdU positive cells in mammary epithelium from dairy calves. Starting in the top left with "0", top right "1", bottom left "2" and bottom right "3". On average 5.5 images/slides were counted per calf.

RESULTS AND DISCUSSION

Growth performance of both Enhanced and Control calves is presented in Table 4.2. Calves in the Enhanced treatment had higher milk replacer intakes but lower starter grain intakes than control calves (69.5 and 1.9 vs. 32.6 and 6.7 kg of DMI from MR and starter grain for Enhanced and Control respectively; $P < 0.01$). Similarly, calves in the Enhanced group exhibited higher ADG than Control calves throughout the study (0.82 vs. 0.39 kg for Enhanced and Control; $P < 0.01$); Enhanced calves also had higher gains in hip height throughout the study (0.31 vs. 0.18 cm/d for Enhanced and Control; $P < 0.01$). At the time of harvest, calves were 54 ± 2 d of age and the Enhanced calves were 22.2 kg or 36.4% heavier than control calves ($P < 0.01$; Table 4.2). Calves in the Enhanced group tended to be 5.2 cm or 6% taller than Control calves ($P = 0.08$). The current growth recommendation for dairy calves is to double the birth weight by weaning (Van Amburgh et al., 2008); the calves in the Enhanced group achieved this target at 2.1 times their birth weight, while the Control calves were only 1.6 times their birth weight at the time of weaning.

A previous study demonstrated strong correlations between energy intake above maintenance during the pre-weaning period and future milk production during lactation (Soberon et al., 2012). In this study, the Enhanced calves received on average 3.1 Mcal more energy per d than Control calves (5.9 vs 2.8 Mcal/d for Enhanced and Control; Table 4.2); energy requirements were calculated using body weight and ambient temperatures (NRC, 2001) and this value was subtracted from the corresponding energy intakes. This resulted in 3.75 Mcal of intake above maintenance for the Enhanced TRT calves and only 0.89 Mcal of intake above maintenance for Control calves. According to Soberon et al. (2012), this difference in intake would correspond to 670 kg more milk during first lactation for calves fed the enhanced diet.

At the time of harvest, Enhanced calves were 36.4% heavier than Control calves (Table 4.2). The weight of the pancreas from all calves was similar at 54 d of age (Table 4.3), suggesting

Table 4.2. Body weight, hip height at birth and at harvest, ADG, average change in hip height and intake data for calves fed 2.8 Mcal of Energy Intake/d (Control; n = 6) or calves fed 0.3 Mcal of Energy intake/kg $BW^{0.75}$ updated weekly as body weight increased (Enhanced; n = 6).

	Control	Enhanced	S.E.	P value
Birth weight (kg)	39.2	39.7	2.5	0.90
Hip height at birth (cm)	78.3	76.3	2.4	0.56
Harvest weight (kg)	61.0	83.2	3.9	< 0.01
Hip Height at harvest (cm)	87.3	92.5	1.9	0.08
Age at harvest (days)	54.3	54.0	0.9	0.80
ADG (kg/d)	0.39	0.82	0.03	< 0.01
Average change in hip height (cm/d)	0.18	0.31	0.02	< 0.01
Total milk replacer intake (kg)	32.6	69.5	2.4	< 0.01
Average milk replacer intake (kg/d)	0.6	1.3	0.04	< 0.01
Starter grain intake (kg)	6.7	1.9	0.5	< 0.01
Total energy intake from milk/d (Mcals, IE)	2.8	5.9	0.17	< 0.01
Maintenance requirements (Mcals/d)[1]	1.9	2.2	0.08	0.02
Mcals above maintenance (Mcals/d)	0.9	3.8	0.11	< 0.01

[1]Energy requirements were calculated using body weight and ambient temperature according with equations from the NRC (2001).

Table 4.3. Organ weights (grams) and as a percent of the body weight for calves fed 2.8 Mcal of Energy Intake/d (Control; n = 6) or calves fed 0.3 Mcal of Energy intake/kg $BW^{0.75}$ (Enhanced; n = 6) from birth until harvest at 54 d.

	Control	Enhanced	S.E.	P value
Pancreas (g)	32.90	29.47	4.39	0.61
Pancreas as % of BW	0.06%	0.04%	0.01%	0.11
Liver (kg)	1.35	2.35	0.82	< 0.01
Liver as % of BW	2.23%	2.84%	0.09%	< 0.01
Kidneys (g)	183.60	319.72	33.29	0.02
Kidney as % of BW	0.30%	0.38%	0.03%	0.09
Whole mammary (g)	75.48	337.58	29.14	< 0.01
Mammary gland as % of BW	0.12%	0.41%	0.03%	< 0.01
Parenchyma (g)	1.10	6.48	1.00	< 0.01
Parenchyma as % of Mammary gland	1.35%	1.90%	0.37%	0.30
Parenchyma as % of BW	0.002%	0.008%	0.001%	< 0.01

that organs such as the pancreas grow at a steady rate independent of nutrient intake during the first 54 d of life.

In contrast, the kidneys of Enhanced calves were heavier than the kidneys of Control calves (Table 4.3). However, when analyzed as a percentage of body weight, there were no differences between Enhanced and Control, suggesting that the kidneys grow at a similar rate irrespective of nutrition.

The livers of Enhanced calves were heavier than the livers of Control calves (Table 4.3). This difference in liver weight was expected (2.8 vs. 2.2% of body weight for Enhanced and Control; $P < 0.01$; Table 4.3), given that the liver size is dynamic and responsive to nutrient intake, even when analyzed as a percentage of body weight. Similar results were observed by Diaz et al. (2001) where the livers of the calves fed higher nutrient intake were 27% larger than restricted calves at similar body weights and reflected growth rates similar to calves on this study.

Similarly, the mammary glands of calves in the Enhanced group were also heavier than the mammary glands of control calves (Figure 4.2). When analyzed as a percentage of body weight, the mammary glands of Enhanced calves were 3.4 times heavier than those of control calves ($P < 0.01$). This was similar to the data from Meyer et al. (2006a) were the mammary gland of calves fed for higher nutrient intake weighed 2.3 times more than that of calves fed restricted nutrients when comparing calves of similar age. Further, Brown et al. (2005) compared the mammary tissue of eight wk old heifer calves grown at two different rates of gain and observed that the parenchyma tissue of heifers fed more nutrients was 3.75 times heavier than that of calves fed a restricted diet. When compared as g of parenchyma per 100 kg of BW, the parenchyma tissue was 3.3 times heavier than that of the restricted calves (Brown et al. 2005).

When comparing calves grown at different rates of gain and evaluating parenchymal tissue at similar BW, heifers offered diets restricted in nutrient intake have heavier parenchyma tissue; mainly due to a constant daily growth of the mammary parenchyma tissue (Meyer et al., 2006a). However, this pattern of growth appears to be modified due to nutrient intake in calves over the first 2 months of life (Brown et al., 2005; Meyer et al., 2006a)

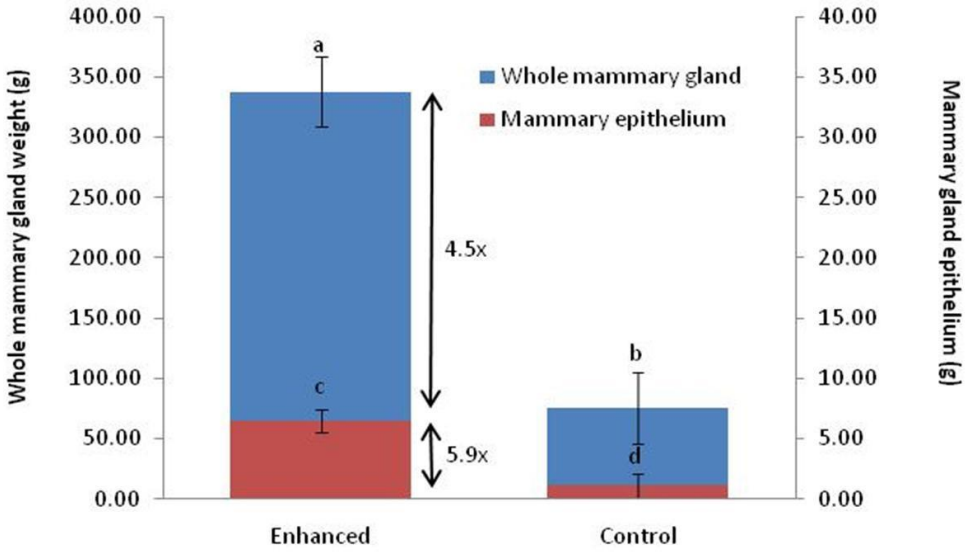

Figure 4.2. Weights of the whole skinned mammary gland and weight of isolated parenchymal mass for calves fed 2.8 Mcal of Energy Intake/d (Control; n = 6) or calves fed 0.3 Mcal of Energy intake/kg $BW^{0.75}$ (Enhanced; n = 6) from birth until harvest at 54 d. Means and Std dev shown.

Previous studies in pre-pubertal calves have reported that increase ADG from 3 to 4 mo until puberty result in increase accumulation of adipose tissue in the mammary gland; however, mammary parenchymal tissue growth appeared to be impaired by higher energy intake and the associated accelerated rates of BW gain (Sejrsen et al., 1982; Mäntysaari et al., 1995; Capuco et al., 1995; Meyer et al., 2006a). However, Meyer et al. (2006a) reported that for calves between 150 and 350 kg of body weight, the mammary epithelium had a consistent growth rate regardless of nutrient intake or ADG and grew at approximately 5.9 mg DNA per day among treatments. In contrast to those reports in pre-pubertal calves, the opposite effect of nutrients intake and ADG was observed in pre-weaning calves where the parenchymal mass of the mammary glands of Enhanced calves weighed 5.9 times more than the parenchymal mass of Control calves (6.48 vs 1.1 g for Enhanced and control; $P < 0.01$; Table 4.3 and Figure 4.2). Meyer et al. (2006) observed that the parenchymal mass of calves fed higher nutrient intake was 2.1 times heavier than restricted calves at similar age. The parenchymal mass as well as the liver and the whole mammary gland grew at an allometric rate when compared to the rest of the body during the pre-weaning period.

Furthermore, when the parenchymal mass was analyzed as a percent of the whole mammary gland, there were no differences among Enhanced and Control; this would suggest that during the pre-weaning stage, both the parenchymal mass as well as the fat pad of the mammary glands are equally responsive to nutrient intake.

Within the mammary parenchyma sampled for immunohistochemistry, no differences were apparent between TRT for BrdU positive cells when using a four point scale (0.58 vs. 0.64 for the Enhanced and the control TRT; $P = 0.86$). However, if the parenchymal tissue mass differences are considered, the total number of positive cells within the mammary gland would be expected to be higher for the Enhanced TRT. Future work should attempt to collect whole mammary parenchyma for immunohistochemistry analysis to better determine differences among nutritional TRT. The amount of tissue at this stage of development is relatively low making multiple method comparisons difficult. Daniels et al. (2009) followed a similar approach

to that described in the present study, but administered the BrdU at 32 d of life and observed no differences in BrdU label retaining cells. In contrast to the current study, Daniels et al. did not observe differences in parenchymal mass at the time of harvest at 64 d. The composition of the milk replacers offered by Daniels et al. (2009) was different for each TRT and could have contributed to the differences in responsiveness of the parenchymal mass.

In response to an increased nutrient intake above maintenance during pre-weaning, the mammary parenchyma appears to shift to an allometric phase of growth immediately after birth. In this study, the mammary parenchyma demonstrated the greatest response to increased energy intake of all the major organs measured. These findings aligned with other studies that observed increased proliferation of mammary epithelium prior to weaning of calves fed higher levels of nutrient intake (Brown et al 2005; Meyer et al, 2006b).

We hypothesized that previous studies reporting isometric growth prior to weaning did not provide the calf with sufficient nutrient intake from milk or milk replacer to stimulate this type of growth response in the mammary gland. It appears that in the neonatal period, the mammary parenchyma, and most likely very specific cells (Ellis and Capuco, 2002) are nutritionally responsive. Previous work, attempted to identify these cells using a similar protocol to the one used in the current study but gave the BrdU injections at 32 d of life for four days; they observed differences in labeling by region of the mammary gland, with the highest degree of labeling towards the gland cistern, but no differences among dietary treatments were observed (Daniels et al. 2009); The timing of nutritional sensitivity of such populations of cells has not been determined and therefore this study attempt to determine the effect at an earlier age by starting BrdU labeling on d 12, and due to a difference in the sampling technique, the current study did not evaluate differences in labeling by region of the parenchyma. However, the developmental response is significant and a nutritionally sensitive cell type may be responsible. This proliferation might be part of the mechanism stimulating greater lactation yield of the calf once they reach lactation (Moallem et al. 2010; Soberon et al., 2012). Identification of the specific

cells and the factors stimulating this proliferation is warranted and is a focus of continuing research.

CONCLUSIONS

The mammary gland of dairy calves is responsive to nutrient intake during the pre-weaning stage and this differs from post-weaning mammary gland development. The allometric stage of growth in the mammary gland appears to begin at birth if sufficient nutrients from milk or milk replacer are provided to the neonatal calf. It is still to be determined if the responsiveness of the mammary gland is due to a specific group of cells, or to a generalized response to nutrients. However, a better understanding of the effect of early life nutrient intake on mammary development and overall development on future milk production deserves further investigation.

ACKNOWLEDGMENTS

We gratefully acknowledge Milk Specialties Global and Land O'Lakes support of this work and Debbie Ross for technical support and the staff of the Teaching and Research Farm as well as Melanie Soberon, Ryan Higgs, Cao Zhijun, Samuel Fessenden and Brianna Pomeroy for their help and support of this study.

REFERENCES

Brown E. G., M. J. VandeHaar, K. M. Daniels, J. S. Liesman, L. T. Chapin, J. W. Forrest, R. M. Akers, R. E. Pearson, M. S. Weber Nielsen. 2005. Effect of increasing energy and protein intake on mammary development in heifer calves. J. Dairy Sci. 88:595-603.

Capuco A. V., D. L. Wood, R. Baldwin, K. Mcleod, M. J. Paape. 2001. Mammary cell number, proliferation, and apoptosis during a bovine lactation: relation to milk production and effect of bST. J. Dairy Sci. 84:2177-2187.

Capuco A. V., J. J. Smith, D. R. Waldo, and C. E. Rexroad. 1995. Influence of prepubertal dietary regimen on mammary growth of Holstein heifers. J. Dairy Sci. 78:2709-2725.

Daniels K. M., A. V. Capuco, M. L. McGilliard, R. E. James, and R. M. Akers. 2009. effects of milk replacer formulation on measures of mammary growth and composition in Holstein heifers. J. Dairy Sci. 92:5937–5950.

Diaz, M. C., M. E. Van Amburgh, J. M. Smith, J. M. Kelsey and E. L. Hutten. 2001. Composition of growth of Holstein calves fed milk replacer from birth to 105 kilogram body weight. J. Dairy Sci. 84:830-842.

Ellis, S. and A. V. Capuco. 2002. Cell proliferation in bovine mammary epithelium: identification of the primary proliferative cell population. Tissue & Cell, 2002 34:155–163.

Huderson, B. P., B. T. Velayudhan, R. E. Pearson, S. E. Ellis, R. M. Akers. 2011. Effect of exogenous somatotropin in Holstein calves on mammary gland composition and proliferation. J. Dairy Sci. 94:5005–5016.

Huxley J. S. 1950. Growth and form transformation. Proc. Of the Royal Society of London. Biological Sci. 137:465-469.

Mäntysaari P., K. L. Ingvartsen, V. Toivonen, K. Sejrsen. 1995. The effect of feeding level and nitrogen source of the diet on mammary development and plasma hormon concentrations fo pre-pubertal heifers. Acta. Agric. Scand. Sect. A 45:236-244.

Meyer, M. J., A. V. Capuco, D. A. Ross, L. M. Lintault, and M. E. Van Amburgh. 2006a. Developmental and nutritional regulation of the prepubertal heifer mammary gland: I. Parenchyma and fat pad mass and composition. J. Dairy Sci. 89:4289-4297.

Meyer, M. J., A. V. Capuco, D. A. Ross, L. M. Lintault, and M. E. Van Amburgh. 2006b. Developmental and nutritional regulation of the prepubertal bovine mammary gland: II. Epithelial cell proliferation, parenchymal accretion rate, and allometric growth. J. Dairy Sci. 89:4298-4304.

Moallem U., D. Werner, H. Lehrer, M. Zachut, L. Livshitz, S. Yakoby, and A. Shamay. 2010. Long-term effects of ad libitum whole milk prior to weaning and prepubertal protein supplementation on skeletal growth rate and first-lactation milk production. J. Dairy Sci. 93:2639–2650.

Sejrsen K., Huber J. T., Tucker H. A., Akers R. M. 1982. Influence of nutrition of mammary development in pre- and postpubertal heifers. J Dairy Sci. 65:793-800.

Sinha Y. N., and H. A. Tucker. 1969. Mammary development and pituitary prolactin level of heifers from birth through puberty and during the estrous cycle. J. Dairy Sci. 52:507-512.

Smith G. H. 2005. Label-retaining epithelial cells in mouse mammary gland divide asymmetrically and retain their template DNA strand. Development 132:681-687.

Soberon, F., E. Raffrenato, R. W. Everett, M. E. Van Amburgh. 2012. Pre-weaning milk replacer intake and effects on long term productivity of Dairy calves. J. Dairy Sci. (In press).

Van Amburgh M. E., E. Raffrenato, F. Soberon. 2008. Early life management and long-term productivity of dairy calves. Proc. Cornell Nutr. Conf.. Syracuse, NY. 70:185-192.

CHAPTER V

EFFECTS OF COLOSTRUM INTAKE AND PRE-WEANING NUTRIENT INTAKE ON POST-WEANING FEED EFFICIENCY AND VOLUNTARY FEED INTAKE

F. Soberon and M. E. Van Amburgh

ABSTRACT

Non nutritional factors in colostrum have long been recognized as valuable for the development of the newborn calf, however, the benefits of colostrum intake surpass those related to the immune system. The objectives of this study were to evaluate if the non-nutritional factors in colostrum as well as nutrient availability during the pre-weaning period have permanent effects on feeding behavior or efficiency of nutrient utilization. Calves were fed either 2 L (n = 53) or 4 L (n = 72) of pooled colostrum, within 1 h of birth. Calves receiving 4 L of colostrum were fed an additional 2 L of colostrum 12 h after the first feeding while calves fed 2 L were fed 2 L of milk replacer (MR, 28% CP, 15% fat, Excelerate, MSG, Carpentersville, IL). Plasma IgG content was determined for all calves 24 to 48 h after the first colostrum feeding. After the second feeding, all calves were fed MR by an automated feeder (Förster-Technik, Engen, Germany). Half the calves on each colostrum treatment were allowed to consume 4 L per d of MR while the other half were allowed to consume up to 12 L per d and intake was recorded by the feeders. Calves had access to a calf starter starting on d 3. All calves were weaned at 52 days and offered the same ration; DMI was recorded daily for one month post-weaning. Only 14% of the calves on the Low colostrum treatment had plasma IgG concentrations below 10 mg/ml; all calves that received 4 L of colostrum had plasma IgG concentrations above 15 mg/ml. Calves fed 4 L MR had similar ADG pre-weaning regardless of colostrum (0.35 $\pm$ 0.04 kg/d). However, calves offered 12 L MR per d demonstrated greater ADG when they had 4 L of colostrum at birth (0.78 kg/d vs. 0.55 kg/d). Also, during the post-weaning period, calves fed 4 L colostrum had greater DMI than calves receiving 2 L of colostrum independent of previous MR intake (2.8

vs. 2.2 kg/d). The data suggests that some non-nutritional components of colostrum are altering metabolic programming responsible for regulating appetite or nutrient utilization in calves.

Key Words: Colostrum, feed efficiency, appetite

INTRODUCTION

Non-nutritional factors in colostrum have long been recognized as valuable for the development of the newborn calf, however, the benefits of colostrum intake might exceed those related to the immune system. Data from previous studies indicated that different levels of colostrum ingestion altered growth, metabolism and endocrine status in calves (Hadorn et al., 1997; Kühne et al., 2000; Rauprich et al., 2000) but those studies were generally intensive, short-term observations. Jones et al. (2004) demonstrated that in calves fed a serum based colostrum replacer compared to dam's colostrum, feed efficiency was significantly lower during the first 29 d of life even though plasma IgG content of both groups of calves were not significantly different, indicating that factors other than immunoglobulins are important for altering nutrient utilization.

Longer term studies evaluating colostrum status as well as nutritional status indicate that factors in colostrum impact the animal even as they mature and reach lactation in support of their offspring. DeNise et al. (1989) demonstrated that for every unit of serum IgG content above 12 mg/ml, there was an 8.5 kg increase in mature equivalent milk yield. Further, Brown Swiss calves fed either four vs. two L of colostrum just after birth demonstrated a 30% increase in pre-pubertal growth rate and a 16% increase in survival through the end of the second lactation and the cattle reaching the end of the second lactation produced 1,026 kg more milk (Faber et al., 2005). In the studies of Robison et al. (1988) and Faber et al. (2005), the data are not clear if the growth rate effects are due to increased appetite and thus dry matter intake or if there is a feed efficiency effect beyond any difference in dry matter intake above maintenance. The data

suggests there are effects of colostrum status on either nutrient intake or on the ability of the calf to partition nutrients to growth and lactation away from what would be considered maintenance requirements but this could not be determined by the data presented.

We hypothesized that some of the non-nutritional and non-immunoglobulin factors in colostrum as well as the nutrient availability during the pre-weaning period have effects on feeding behavior or efficiency of nutrient utilization.

The objectives of this study were to determine the effects of colostrum intake and milk replacer offered on voluntary feed intake and feed efficiency in pre and post weaned Holstein calves.

MATERIALS AND METHODS

All protocols involving the use of animals were reviewed and approved by the Cornell University Institutional Animal Care and Use Committee. One hundred and fifty-one Holstein calves were randomly assigned to one of four treatments (TRT) at birth in two blocks. The first block (n=66) was filled between January 15th, 2010 and April 10th, 2010 and the second block (n=85) was filled between November 24th, 2010 and March 3rd, 2011. All calves were fed pooled colostrum from cows that were evaluated for bacterial concentration and IgG content using the single radial immunodiffusion kit (VMRD, Inc. Pullman, WA). Colostrum samples were sent to Dairy One (Ithaca, NY) for bacteria counts. Any colostrum exceeding 80,000 CFU/ml was not used in the pool and any colostrum less than 50 mg/dl IgG was not included in the pool. Calves were housed in individual pens for the first two days of life and then housed in a comingled pen and fed milk replacer (MR, 28% CP, 15% fat, Excelerate, MSG, Carpentersville, IL) through an automatic feeder (Förster-Technik, Engen, Germany). The milk replacer was reconstituted to 15% solids when mixed with water in the feeder. The DM content of the reconstituted MR was calibrated at the beginning of the study and checked every two weeks.

The treatments were: TRT 1, High High (HH; n = 43): fed four L of colostrum at birth and two L 12 h after, starting on day two they were offered increasing amounts of MR from 5 up to 12 L/d in a 10 d period through the automatic feeder. The calves on TRT 2, High Low (HL; n = 40) were fed four L of colostrum at birth and two L 12 h after, starting on day two they were limited to no more than 5 L per day to achieve an ADG of 0.35 kg per day at the automatic feeder. The calves on TRT 3, Low High (LH; n = 29) were fed two L of colostrum at birth and two L of MR 12 h after, starting on day two they were offered increasing amounts of MR from 5 to 12 L/d in a 10 d period on the automatic feeder. Finally, the calves on TRT 4, Low Low (LL; n= 39) were fed two L of colostrum at birth and two L of MR 12 h after, and starting on day two they were fed 5 L per day to achieve an ADG of 0.35 kg .

Weight and hip height was recorded at birth and once a week until 35 d post-weaning. A blood sample was taken between 24 and 48 h of life via jugular venipuncture into Vacutainer containing Sodium Heparin (BD Franklin Lakes, NJ) and centrifuged for plasma harvest and frozen at -20 °C until further analysis of plasma IgG concentrations (Single Radial immunodufusion Kit, VMRD, Inc Pullman, WA). All calves had access to water from a common, frost-free waterer starting on d two and a pelleted calf starter (22% CP, Cargill, Inc.) through an automatic feeder starting at 20 d (Förster-Technik, Engen, Germany). All calves were weaned by 48 d, and five to eight d after weaning calves were moved to smaller pens and housed in pairs by TRT group. Calves were fed exclusively the pelleted calf starter during this period. Feed intake and body weights were monitored for 28 d after moving to these pens. Water was offered at free choice during the entire study.

Statistical analysis

Individual growth rates were determined by regression of each calf's body weights over the period analyzed and the slopes were considered the ADG. Data were analyzed using the mixed procedures of SAS 9.2 (SAS Inst. Inc., Cary, NC). Terms in the model included block, and birth wt when applicable. For DMI and feed efficiency post-weaning, pen instead of calf was use as

experimental unit. Interactions among block were analyzed and found to be non-significant. The model used was: $Y_{ij} = S_{ij} + G_j + E_{ij}$ Where: Y_{ij} = is the dependent, continuous variable; S_i = the effect of block; G_j = the effect of treatment, and E_{ij} = the residual error of the the i^{th} block and the j^{th} treatment. Significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Colostrum administration has consistently been a component of a successful calf raising program (Davis and Drackley, 1998; Godden, 2008). Most reports in the literature have focused on the effects of colostrum administration and plasma IgG levels, and the general consensus is that calves with plasma IgG level above 10 g/L at 24 h post colostrum administration will perform better than calves with plasma IgG levels below this cut off point (Robison et al., 1988; DeNise et al., 1989; Furman-Fratczak et al., 2011). Therefore, this study was designed to provide enough colostrum to all calves to avoid failure of passive transfer and meet the 10 mg/dl threshold considered adequate on commercial dairy farms (Beam et al., 2009). Calves in the Low colostrum TRT had plasma IgG concentrations of 14.4 ± 0.98 g/L. The calves in the High colostrum TRT had plasma IgG concentrations of 26.1 ± 0.98 g/L which were significantly higher than those fed the Low colostrum TRT. However, the plasma IgG levels for both TRT are similar to or higher than values reported by others (DeNise et al., 1989; Jones et al., 2004).

Birth weights and birth hip height of calves were not different among TRT. Pre-weaning growth rates were influenced by colostrum TRT differently according to the allowance of MR intake. For calves assigned to the restricted feeding of milk replacer, there was no difference in pre-weaning ADG (0.42 vs. 0.39 kg) and therefore no difference in weaning weight or weaning hip height for calves in either colostrum TRT (63.5 vs. 62.4 kg. and 88.6 vs. 89.6 cm. for HL and LL respectively; Table 5.1). However, calves assigned to the high allowance of milk replacer had higher ADG and higher weaning weights when they had consumed more colostrum at birth (0.79 and 78.2 vs. 0.67 and 72.2 kg for pre-weaning ADG and weaning weight of HH and LH calves

Table 5.1. Weights, heights, average daily gains and post-weaning dry matter intakes for calves (n = 125) fed either 4L of colostrum and up to 12 L of MR (HH), 4L of colostrum and 4 L of MR (HL), 2 L of colostrum and up to 12 L of MR (LH), or 2 L of colostrum and 4 L of MR (LL). Means and standard deviations shown.

Treatment	***HH***	***HL***	***LH***	***LL***	
	Mean	Mean	Mean	Mean	Std dev
N	34	38	26	27	
Days on treatment	84.3	83.3	82.8	82.8	0.69
Birth wt, kg	44.02	43.43	41.81	43.32	0.95
Birth hip height, cm	80.48	80.27	80.03	80.94	0.56
IgG concentration, mg/dl*	2,746[a]	2,480[b]	1,466[c]	1,417[c]	98
Weaning wt, kg	78.19[a]	63.48[b]	72.17[c]	62.43[b]	1.89
Weaning hip height, cm	93.00[a]	88.62[b]	91.53[a]	89.58[b]	0.60
ADG pre-weaning (0 to 52 d), kg	0.79[a]	0.42[b]	0.67[c]	0.39[b]	0.03
ADG birth to 80 d, kg	0.78[a]	0.59[bc]	0.66[b]	0.53[c]	0.03
Total milk replacer intake, kg DM[1]*	44.4[a]	20.5[b]	40.9[c]	20.0[b]	1.2
Grain intake pre-weaning, kg[1]*	2.5[a]	12.0[b]	2.1[a]	9.7[b]	1.5
Feed efficiency pre-weaning[2]*	0.61	0.61	0.65	0.61	0.04
Hip height gain, pre-weaning, cm/d	0.25[a]	0.16[b]	0.23[a]	0.16[b]	0.01
Hip height gain, birth to 80 d, cm/d	0.21[a]	0.16[b]	0.18[c]	0.15[b]	0.01
ADG post-weaning[3], kg	1.07[a]	0.97[ab]	0.88[b]	0.92[b]	0.06
DMI post-weaning[3], kg/d	2.89[ab]	2.89[a]	2.58[c]	2.66[bc]	0.10
Feed efficiency post-weaning	0.33	0.34	0.34	0.36	0.01

[1]Data from 5 wk during the pre-weaning period was used in the analysis

[2]DMI includes milk replacer intake and grain intake from birth to weaning

[3]Measured during 3 weeks after a 1 week adaptation period to pens

* Data is only reported for calves in the second block

[abc]Values within the same line with different superscripts differ $P < 0.05$

respectively; $P < 0.05$; Table 5.1). Robison et al. (1988) observed differences in growth performance in restricted fed calves; however, researchers observed the maximum difference in performance among calves with different colostrum status after weaning, when feed was offered at libitum.

A possible mechanism for differences in performance of calves receiving different amounts of colostrum is an increased glucose absorption capacity. Hammon and Blum (1997) observed improved capacity to absorbed both glucose and xylose in calves fed colostrum vs. calves fed MR on their first meal and this was recently reinforced by Steinhoff-Wagner et al., (2011) where calves fed colostrum had greater glucose absorption at 4 d of age compared to calves fed milk replacer.

Differences in growth rates were still present during the post-weaning period when all calves were receiving the same diet such that calves in the HH TRT had higher ADG than calves that received low colostrum; calves in the HL TRT were intermediate. These findings suggest that the benefits of additional amounts of colostrum at birth are enhanced by proper nutrition during the pre-weaning period. Other studies that tested the effects of nutrition during two different periods of heifer rearing concluded that the effects of pre-weaning nutrition on future performance are enhanced by proper nutrition during subsequent stages of development. Moallem et al. (2010) observed that calves feed high volumes of whole milk pre-weaning and fed a diet supplemented with additional protein pre-puberty, produced 3 kg/d more milk than calves that received lower quality nutrition during the pre-weaning period or that were not supplemented with additional protein. Shamay et al. (2005) observed that calves fed ad libitum amounts of milk produced 981 kg more milk during their first lactation than calves fed restricted amounts of milk replacer. Moreover, if the calves that were fed at libitum milk were supplemented with additional protein during the pre-pubertal stage, the produced 550 kg more milk than calves that were not supplemented. Supplementation of protein during the pre-pubertal period had no effect on milk production of calves that were restricted during the pre-weaning period, this findings are

equivalent to the current study were colostrum administration did not have an effect on ADG of restricted calves.

Milk replacer intake was not different between the two restricted groups (20.5 vs. 20.0 ±1.2 kg DM for HL and LL TRT respectively; Table 5.1). However, for calves that were allowed to consume up to 12 L/d, calves in the High colostrum TRT consumed more DM from milk replacer than calves fed the Low colostrum TRT (44.4 vs 40.9 ±1.2 kg DM for HH vs. LH TRT respectively; $P < 0.05$; Table 5.1). This suggests subtle differences in either health status or appetite regulation existed in the calves fed the 4 L of colostrum at birth. It is still unclear whether this is due to IgG status or other factors in colostrum imparting effects on appetite however the difference in feed intake is significant especially if it persisted into the post-weaning phase.

During the pre-weaning period, grain intake did not differed among colostrum TRT but was 4.7 times higher for calves fed restricted amounts of milk replacer (Table 5.1). Calf starter grain consumption post-weaning was higher for calves in the High colostrum TRT but was not affected by milk replacer TRT.

There were no TRT effects on feed efficiency when grain intake was included in the analysis (Table 5.1), also there were no differences in feed efficiency of any TRT when analyzed for the first 4 wk of life (data not shown); these findings differs from those of Jones et al. (2004) were feed efficiency was reported to be doubled for calves fed fresh colostrum in comparison with colostrum replacer. Differences among studies' findings may be explained by differences in TRT. In Jones et al., (2004) calves in the colostrum replacer TRT were most likely completely deprived from some of the growth factors present in fresh colostrum where as all calves in the present study received the same colostrum varying only in the amount offered. Other source of variation among studies might be due to the amount of milk replacer fed during the pre-weaning period in each of the studies.

Skeletal growth, as measured by hip height was enhanced during the pre-weaning period for HH and LH TRT (Table 5.1). When four wks post weaning are included in the analysis, calves in

the HH TRT had higher gains in hip height than all other TRT, followed by LH calves, calves in the Low milk replacer groups (HL and LL) were not different from each other on hip height at any stage.

CONCLUSIONS

In this study, colostrum status impacted growth rates of calves provided adequate nutrients above maintenance. Post-weaning feed intake was modestly reduced in this study in calves fed less colostrum although IgG status was positive and above industry standards. This suggests that non-nutritive and non-immune factors in colostrum have a subtle effect on appetite regulation in the calf. Longer term measurements are needed to identify whether feed intake regulation or feed efficiency are significantly altered for longer periods of time post-weaning. If nutrition and management during the pre-weaning period are designed to maximize the potential of the calves, colostrum management will have a greater impact on performance than if nutrition and management are restricted during the pre-weaning period. Similar observations were made by Jones et al. (2004). In the same manner performance during the post-weaning stage is linked to management during the pre-weaning period.

ACKNOWLEDGMENTS

We gratefully acknowledge Milk Specialties Co. (Carpentersville, IL), Milk Products, Inc. (Chilton, WI) and Cargill for support of this work and the staff of the Teaching and Research Farm for their help and support of this study.

REFERENCES

Bar-Peled, U., B. Robinzon, E. Maltz, H. Tagari, Y. Folman, I. Bruckental, H. Voet, H. Gacitura, and A. R. Lehrer. 1997. Increased weight gain and effects on production parameters of Holstein heifer calves that were allowed to suckle from birth to six weeks of age. J. Dairy Sci. 80:2523-2528.

Beam A. L., J. E. Lombard, C. A. Kopral, L. P. Garber, A. L. Winter, J. A. Hicks, J. L. Schlater. 2009. Prevalence of failure of passive transfer of immunity in newborn heifer calves and associated management practices on US dairy operations. J. Dairy Sci. 92:3973-3980.

DeNise, S. K., J. D. Robison, G. H. Stott, and D. V. Armstrong. 1989. Effects of passive immunity on subsequent production in dairy heifers. J. Dairy Sci. 72:552-554.

Faber, S. N., N. E. Faber, T. C. McCauley, and R. L. Ax. 2005. Case Study: Effects of colostrum ingestion on lactational performance. Prof. Anim. Scientist 21:420.

Godden S. Colostrum management for dairy calves. 2008. Vet. Clin. Food Anim. 24:19-39.

Hadorn U., H. Hammon, R. M. Bruckmaier and J. W. Blum. 1997. Delaying colostrum intake by one day has important effects on metabolic traits and on gastrointestinal and metabolic hormones in neonatal calves. J. Nutr. 127: 2011–2023

Jones C. M., R. E. James, D. D 3rd Quigley, M. L. McGilliard. 2004. Influence of pooled colostrum or colostrum replacement on IgG and evaluation of animal plasma in milk replacer. J Dairy Sci. 87:1806-14.

Kühne S., H. M. Hammon, R. M. Bruckmaier, C. Morel, Y. Zbinden, and J. W. Blum. 2000. Growth performance, metabolic and endocrine traits, and absorptive capacity in neonatal calves fed either colostrum or milk replacer at two levels. J. Anim. Sci. 78:609–620

Rauprich A. B., H. M. Hammon and J. W. Blum. 2000. Influence of feeding different amounts of first colostrum on metabolic, endocrine, and health status and on growth performance in neonatal calves. J. Anim. Sci. 78:896–908

Soberon, F., E. Raffrenato, R. W. Everett, M. E. Van Amburgh. 2011. Pre-weaning milk replacer intake and effects on long term productivity of Dairy calves. J. Dairy Sci. (In press).

Robison J. D., G. H. Stott, S. K. DeNise. 1988. Effects of Passive Immunity on Growth and Survival in the Dairy Heifer. J Dairy Sci 71:1283-1287.

Davis, C. L., J. K. Drackley. 1998. The Development, Nutrition, and Management of the Young Calf. Iowa State Univ. Press, Ames.

Furman-Fratczak K., A. Rzasa, T. Stefaniak. 2011. The influence of colostral immunoglobulin concentration in heifer calves' serum on their health and growth. J. Dairy Sci. 94 :5536–5543.

Hammon H., J. W. Blum. 1997. Prolonged colostrum feeding enhances xylose absorption in neonatal calves. J. Anim. Sci. 75:2915-2919.

Moallem U., D. Werner, H. Lehrer, M. Zachut, L. Livshitz, S. Yakoby, A. Shamay. 2010. Long-term effects of ad libitum whole milk prior to weaning and prepubertal protein supplementation on skeletal growth rate and first lactation milk production. J. Dairy Sci. 93:2639-2650.

Shamay A., D. Werner, U. Moallem, H. Barash, I. Bruckental. 2005. Effect of nursing management and skeletal size at weaning on puberty, skeletal growth rate, and milk production during first lactation of dairy heifers. J. Dairy Sci. 88:1460-1469.

Steinhoff-Wagner, J., Gors, S., Junghans, P., Bruckmaier, R. M., Kanitz, E., Metges, C. C., Hammon, H.M. 2011. Intestinal glucose absorption but not endogenous glucose production differs between colostrum- and formula-fed neonatal calves. J.Nutr. 141:48-55.

CHAPTER VI

COLOSTRUM CONCENTRATIONS AND ABSORPTION OF VARIOUS GROWTH FACTORS IN NEWBORN DAIRY CALVES

F. Soberon, S. R. Brown, D. A. Ross, S. H. Pelton and M. E. Van Amburgh

ABSTRACT

Colostrum status has been linked with increased performance in dairy and beef cattle. The increase in performance has been associated with immunoglobulin status, but additional data suggests it is not solely explained by changes in the immune system or by caloric intake, but rather involves metabolic changes initiated by signals transmitted from the cow to her offspring through the colostrum in what has been called the Lactocrine hypothesis. Colostrum is rich in many factors that could contribute to these effects. This study was designed to provide an initial assessment of absorption of glucose, insulin, IGF-1, growth hormone and leptin from colostrum into plasma. Calves were fed either 2 or 4 L of pooled colostrum at birth and blood plasma was frequently sampled during the first 4 h post feeding, and again 12 and 13 h post feeding. Plasma glucose concentrations were similar for both TRT. Plasma insulin concentration increased significantly 2.5 h after feeding. Plasma concentrations of IGF-1, growth hormone and leptin tended to increase between 2.5 and 3 h post feeding. A similar pattern for increased plasma concentration of all protein hormones suggests that colostrum hormones appear to be directly absorbed by the calf into blood circulation through the same mechanism used to absorb immunoglobulins.

INTRODUCTION

The first days of life have a critical impact on the future performance of dairy calves, and require careful and precise management practices from dairy producers. These management strategies focus primarily on the calf's undeveloped immune system, which place it at an immunological disadvantage.

Colostrum, the first mammary secretion produced by mammalian mothers just prior to the birth of their young, plays a vital role in the development of numerous species. The importance of colostrum administration goes beyond the immunological benefits in that it can best be explained by its role in extending the maternal influence beyond the uterus in what has been called the "Lactocrine effect" (Bartol et al., 2009).

Colostrum has the potential to influence metabolic programming. Metabolic programming refers to the long-lasting effects on gene expression of nutritional status and various environmental conditions that occur during early development (Kaske et al., 2010). Metabolic programming has been described in multiple species including humans, pigs, dogs, mice, and rats. In the case of dairy cattle, colostrum status and non-immunoglobulin components of colostrum have been shown to affect feed efficiency and future milk yield (DeNise et al., 1989; Jones et al., 2004; Faber et al., 2005).

Steinhoff-Wagner et al. (2011) examined the impact of colostrum feeding on oral glucose absorption, first-pass uptake, and endogenous glucose production; at four days of age, they found improved glucose absorption in calves fed colostrum compared to calves devoid of colostrum at birth. These were similar findings to those reported by Hammon and Blum (1997), who reported improved xylose absorption at 5 d of life in calves fed colostrum vs. milk replacer. Further, Hardon et al. (1997) observed higher plasma concentrations of glucose, albumin, insulin and insulin-like growth factor-1 (IGF-1) in calves fed colostrum during their first day of life in comparison to delaying colostrum for one d. Lepine et al. (1991) found that colostrum intake improved the capacity for glucose synthesis and oxidation. In every case, glucose was suggested

as a trigger of metabolic programming. In young, growing animals, IGF-1 has anabolic effects and can be decreased by malnutrition. It is also known that dietary IGF-1 can increase intestinal growth, while altering the action of IGF-1 intestinal receptors (Baumrucker et al., 1994) which might impact rate of maturation of the gastrointestinal tract and absorptive capacity.

The purpose of this study was to evaluate which components in colostrum might be responsible for the observed effects of colostrum feeding on the performance of dairy calves. In light of previous research, glucose, insulin, IGF-1, growth hormone (GH), and leptin, were selected as factors that could be responsible for part of the observed effects.

MATERIALS AND METHODS

All protocols involving the use of animals were reviewed and approved by the Cornell University Institutional Animal Care and Use Committee. Twenty Holstein calves born at the Cornell Teaching and Research Center were randomly assigned to one of two treatments (TRT) at birth. High TRT were administered four L of pooled colostrum at birth via esophageal tube, followed by two L of pooled colostrum 12 h later. Calves on Low TRT were administered two L of pooled colostrum at birth via esophageal tube, followed by two L of milk replacer (28% CP 15% fat, Excelerate Milk Replacer, MSG, Carpentersville, IL) 12 h later.

The pooled colostrum consisted of colostrum from the first milking of cows at the Cornell University Teaching and Research facility (Harford, NY), that were analyzed for IgG content and bacterial count. Only colostrum samples with less than 75,000 colony forming units per ml (CFU/ml) and with more than 50 mg/ml of IgG were used in the pool. Colostrum was mixed and then allocated into two-liter plastic bags and frozen until administration.

Calves received their first feeding by esophageal tube to ensure that the total volume of colostrum was administered. Calves were then offered a bottle at their second feeding.

After the calf was adequately dried, a five cm square of hair was trimmed from the neck area and cleaned using 70% ethanol. A Medicut intravenous cannula was used to insert

approximately 70 cm of a sterile catheter filled with sterile saline solution containing 100 IU of heparin into the jugular vein. The catheter was taped to the calf's neck and the neck was wrapped with Co-FlexTM bandage wrap to keep the catheter in place and the neck area clean. Once the catheter was functional, a 4 ml baseline blood sample was taken and calves were fed according to treatment. All calves were fed within two h of life.

Blood samples, 4 ml in volume, were taken after feeding at 15 min intervals for an hour, 30 min intervals for the second hour, every hour for the next two hours and again at 12 h coinciding with their second feeding, and at 13 h post first feeding. After each blood sample was taken, 2 ml of a sterile saline solution containing 100 IU of heparin was used to flush the line. During the sampling phase, calves were placed in a hutch with sawdust for bedding and a small heater for warmth. Following the final blood sample, the catheter was removed and calves were removed from the study.

At every blood sampling, 3.5 ml out of the total amount sampled were placed in a Vacutainer containing sodium fluoride (15 mg) and potassium oxalate (12 mg) (Becton Dickson, Franklin Lakes, NJ) and 0.5 ml were placed in a Vacutainer containing sodium heparin (95 USP), (Becton Dickson, Franklin Lakes, NJ). All samples were immediately placed on ice. The sodium fluoride/potassium oxalate samples were centrifuged for 10 min at 3,000 rpm and 4˚ C; supernatant was removed and placed in 1.5 ml micro-centrifuge tubes and frozen at -10°C. The heparinized samples were used for hematocrit analysis using micro-capillary tubes, which were centrifuged at room temperature for 2 min.

Laboratory Analysis

Colostrum protein, fat, and lactose concentrations, as well as percent total solids and somatic cell count (SCC) of pooled samples were measured using mid-infrared spectroscopy according to AOAC (2000) methods (Dairy One Cooperative, Ithaca, NY). Colostrum samples were freeze-dried (48 h with shelf temperature at 20˚C; VirTis 20 SRC-X, The VirTis Co., Inc., Gardiner,

NY) for dry matter (DM) measurement. Dry matter was determined at 106˚C in a forced-air oven for 48 h.

Plasma glucose concentrations were measured by a PGO enzyme quantitative, enzymatic determination in aqueous solutions of serum (Sigma, Saint Louis, MO) as per manufacturer recommendations. In short, samples were diluted to 50% concentration prior to combination with the PGO enzyme reaction solution. Reactions proceeded to completion in approximately 30 min at 37˚C incubation in a water bath. Final sample absorbencies were measured by a spectrometer at 450 nm and glucose concentrations based were calculated using a standard curve.

Plasma insulin concentration was determined by a double antibody radioimmunoassay (RIA) as described by McGuire et al. (1995). Millipore porcine insulin radioimmunoassay kits were employed, utilizing ^{125}I-labeled insulin and porcine insulin antiserum. The basic buffer was a mixture of 0.05M phosphosaline (pH 7.4) containing 0.025 M EDTA, 0.08% sodium azide, and 1% RIA grade bovine serum albumin. Samples were diluted with assay buffer before adding the ^{125}I-labeled insulin and then the primary antibody (guinea pig anti-porcine insulin serum, Millipore, St. Charles, MO). Samples were shaken, covered, and incubated for 20 h at 4˚C. On day two, a precipitating agent (goat anti guinea pig IgG serum: 3% PEG and 0.05% Triton X-100 in 0.05M phosphosaline, 0.025M EDTA, and 0.08% sodium azide) was added; samples were again shaken and incubated for an additional 20 min at 4˚C. Following incubation, samples were centrifuged for 20 min at 4˚C and 1,700 x g. Finally, supernatant was decanted and radioactivity in the remaining pellet quantified (Macromedic 4/200 Automatic Gamma Counter, Macromedic Systems, Seattle, WA).

IGF-1 concentrations were also assessed utilizing a double antibody RIA as described by McGuire et al. (1995). Recombinant bovine IGF-1 was used for the initial iodination and all standards. Prior to measuring IGF-1 concentrations via the RIA, it was necessary to remove all IGF-1 binding proteins from both the plasma and colostrum samples. Plasma samples underwent a two-day dissociation, according to the extraction procedure developed by Plaut, et al. (1991). Plasma samples (91 µl) were mixed with 109 µl of glycyl-glycine HCl and 100 µl of double-

distilled water. The acidified samples were then incubated in a water bath at 37˚C for 48 h. When samples were removed from the water bath, 600 µl of RIA buffer were added; samples were shaken, and finally placed on ice. Aliquots (30 µl) of the incubated plasma were then treated with a primary antibody (rabbit antiserum to hIGF-1 from NIDDK-NIH) and a second antibody (goat anti-rabbit IgG from Pel-Freez 12190-4 in a 1:20 dilution with RIA buffer) 24 h later. Following addition of the second antibody, samples were shaken and incubated for 30 min at 4˚C. Then, 1 ml of 6% polyethylene glycol (PEG) in saline was added to each sample, followed by centrifugation at 2,750 x g for 20 min at 4˚C. Samples were then decanted and blotted dry before a second addition of PEG, this time 1 ml at 4% PEG. A second centrifugation at 2,750 x g for 20 min at 4˚C was followed by decanting, drying, and counting of samples on a γ-counter (1277 Gammamaster, LKB Wallac, Mt. Waverley, Victoria, Australia).

Growth hormone concentrations in calf serum were measured using a growth hormone RIA described by Rosenberg et al. (1989), with the exception of the type of bovine growth hormone used for iodination and standards, which was obtained from Pharmacia Animal Health (Kalamazoo, MI). Samples were first thawed and then centrifuged at 4˚C and 16,110 x g for 10 min; these samples were then pipetted in duplicate (100 µl). To the samples, 200 µl of the first antibody (rabbit anti-growth hormone, diluted from stock of 1:400 in 0.05M EDTA-PBS to 1:35,000 with 1:400 NRS in 0.05M EDTA-PBS) were added, followed by 100 µl of trace (^{125}I-bovine GH, diluted with 1% BSA-PBS to about 200,000 cpm per 100 µl). Samples were then shaken, covered in plastic wrap and incubated for 48 h at 4˚C. Following incubation, the second antibody (anti-rabbit gamma globulin, diluted 1:25 in PBS) was added; samples were again shaken and incubated for 48 h at 4˚C. Finally, in a cold room (4˚C), two ml of cold PBS was added and samples were centrifuged for 30 min at 4˚C and 1,700 x g. Tubes were decanted and kept inverted to drain onto absorbent paper; the remaining pellet in each tube was counted by a γ-counter (1277 Gammamaster, LKB Wallac, Mt. Waverley, Victoria, Australia) for one minute per tube.

Leptin levels in blood plasma were evaluated as described by Ehrhardt et al. (2000). In brief, 100 µl of plasma were diluted to 400 µl with assay buffer and pre-incubated for 2 h at 20 °C. This was followed by the addition of 100 µl of primary antiserum (diluted 1:300). After 16 h incubation at 4 °C, ^{125}I-labeled recombinant bovine leptin (20,000 c.p.m. in 100 µl assay buffer) was added and allowed to equilibrate for 8 h at 4 °C. Separation of bound and free ligands were done by the addition of 200 µl 50 mM phosphate buffer, ph 8, containing 5% ovine anti-rabbit γ-globulin. After 16 h incubation at 4 °C, 1 ml ice-cold 50 mM phosphate buffer, pH 8, containing 3% polyethylene glycol was added. The tubes were centrifuged immediately (1670 x g, 30 min at 4 °C) and the supernatant decanted. Precipitated radioactivity was quantified by γ-counting (Macromedic 4/200 Automatic Gamma Counter, Macromedic Systems, Seattle, WA).

Statistical Analysis

All data were analyzed using the MIXED procedures of SAS 9.2 (2002). Birth weight, time between birth and first feeding, sex and colostrum pool used were analyzed to determine effects on treatment from these variables. Blood metabolites were analyzed as repeated measures using the sample drawn before first feeding as a covariate. Due to the differences in the volume of colostrum fed to calves by treatment, all blood parameters were adjusted to a constant hematocrit value to account for any variation in hydration status. Four different covariant structures were tested and the one with the lowest AIC was chosen in most cases it was compound symmetry. The model used was: $Y_{ijk} = S_i + G_j + D_k + F_{jk} + E_{ijk}$ Where; Y_{ijk} = is the dependent, continuous variable; S_i = the effect of basal concentrations; G_j = the effect of treatment; D_k = the effect of time of sample; F_{jk} = the effect of the interaction of treatment and time; E_{ikj} = the residual error of the the ith basal concentration, the jth treatment and the kth of time of sample. Significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

In total, 10 calves were fed according to the High colostrum TRT and 10 calves were fed the Low colostrum TRT. Calves fed the High TRT had an average birth body weight (BW) of 45.7 kg, which differed ($P < 0.05$) from the average BW of 41.5 kg for the Low TRT calves (Table 6.1). Given the study design, differences among the High and Low colostrum TRT were assumed to be random since calves were assigned to treatment prior to birth. Calves on both TRT were fed pooled colostrum that had to be thawed prior to feeding. The lag time between birth and first feeding was dependent on the time it took for colostrum to thaw, resulting in the High TRT being fed 20 min later than the Low colostrum TRT ($P < 0.05$; Table 6.1).

Colostrum had an average IgG concentration of 166 g/L, 29 mg/ml of lactose, 927 uU/ml of insulin, 40.7 ng/ml of IGF-1, 3.6 ng/ml of GH and 23.4 ng/ml of leptin. This resulted in calves on the High TRT consuming 664 g of IgG, 116 g of lactose, 3.7 million uU of insulin, 0.16 mg of IGF-1, 0.014 mg of GH and 0.09 mg of leptin. Consequently, the calves on the Low TRT received 334 g IgG, 58 g of lactose, 1.9 million uU insulin, 0.08 mg IGF-1, 0.01 mg GH, and 0.05 mg of leptin.

The hematocrit values were significantly different between treatments and most likely reflect the change in plasma volume associated with the difference in colostrum intake between treatments (Table 6.1). This also justifies the need to adjust the hormone concentrations for the change in plasma volume.

Plasma glucose concentrations were similar in both TRT throughout the sampling period (112.1 versus 113.5 mg/dL; $P = 0.93$; Table 6.2). Plasma glucose concentrations were higher for both TRT 12 h post first feeding and one h post second feeding when compared to plasma concentrations during the first 4 h post first feeding (Figure 6.1). Hammon and Blum (1997) observed similar mean glucose concentrations (110.5 and 98.2 mg/dL) for calves fed either 3 L or 1.5 L of colostrum at birth. Kühne et al. (2000) did not observed differences in glucose

Table 6.1. Initial measurements for calves fed 4 L of colostrum (high) or 2 L of colostrum (low) after birth. Mean and standard error are shown.

Treatment	High (n= 10)	Low (n= 10)	Std error
BW at birth (kg)	45.8[a]	41.6[b]	0.49
1st Colostrum feeding as % of BW	8.99[a]	4.79[b]	0.08
Time fed after birth (min)	81.4[a]	61.9[b]	2.19
Average hematocrit	29.7[a]	33.1[b]	0.37

[ab] Values among rows with different subscripts differ by $P<0.05$

Table 6.2. Average plasma concentrations of glucose, insulin, insulin-like growth factor-1 (IGF-1), growth hormone (GH) and leptin adjusted for hydration status for calves fed 4 L of colostrum (high), or 2 L of colostrum (low) after birth over a 13 hour sampling period; means and standard errors shown.

Component	Units	High (n= 10)	Low (n= 10)	Std error
Glucose	mg/dl	112.11	113.51	10.83
Insulin	uU/ml	76.35	59.43	14.35
IGF-1	ng/ml	40.90	39.55	3.60
GH	ng/ml	8.18	7.81	0.96
Leptin	ng/ml	5.39	5.07	0.27

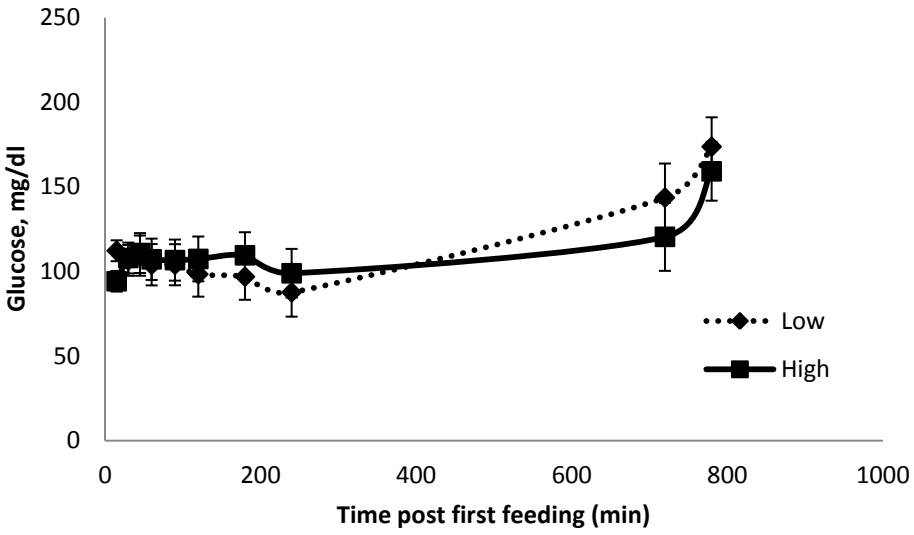

Figure 6.1. Glucose concentration in plasma of calves that received either 4 L (High, n=10) or 2 L (Low, n=10) of colostrum at birth adjusted by hydration status and using a blood sample before first feeding as a covariate. Means and standard errors are shown. (Base $P < 0.01$; TRT $P = 0.93$; time $P < 0.01$; TRT by time $P = 0.35$)

concentrations of calves fed different amounts of colostrum during the first 3 d, however when calves were offered milk replacer instead of colostrum for their first feeding, glucose concentrations increased over the seven h post first feeding, but no other difference was observed (Kühne et al., 2000).

Plasma insulin concentrations in calves fed 2 L of colostrum decreased consistently from 15 min post first feeding until 12 h post feeding (Figure 6.2). Plasma Insulin concentration in calves fed 4 L of colostrum increased steadily from 15 min post feeding until three h post feeding, when it reached a maximum concentration and then began to decline. Insulin concentrations in the Low TRT calves were similar to those reported by Hadorn et al. (1997) who reported a maximum insulin concentrations at 0.5 h post feeding and then a steady decline during the next 7 h on calves fed 3 L of colostrum. Hammon and Blum (1998) observed similar insulin concentrations in calves fed 1.5 L of colostrum for their first feeding. In contrast, Kühne et al. (2000) observed significant insulin concentration increases during the first 2 h post-feeding on calves fed 1.25 and 1.75 L of colostrum in their first feeding (Kühne et al., 2000). Results from the current study suggest that the amount of colostrum offered in relation to body weight has an effect on insulin concentration post feeding. Whether this difference is achieved by normal insulin responses to nutrient supply or through absorption is still not clear. By 12 h post feeding, all calves had similar insulin concentrations in plasma and both TRT had a similar insulin concentration one h after the second feeding. After the second feeding, insulin concentrations followed glucose concentrations; however, this was not the case during the first 4 h post colostrum feeding. This observation is interesting in the current study because comparison of the glucose and insulin levels suggest insulin resistance in the calves fed the High TRT. Insulin levels at around 200 min post-feeding were significantly different but the glucose levels were unchanged. These insulin levels might simply reflect the amount of insulin required to dispose of the absorbed glucose, which in itself might be important. The significant difference could also reflect a certain level of absorption, but this is not well documented and should be explored more mechanistically.

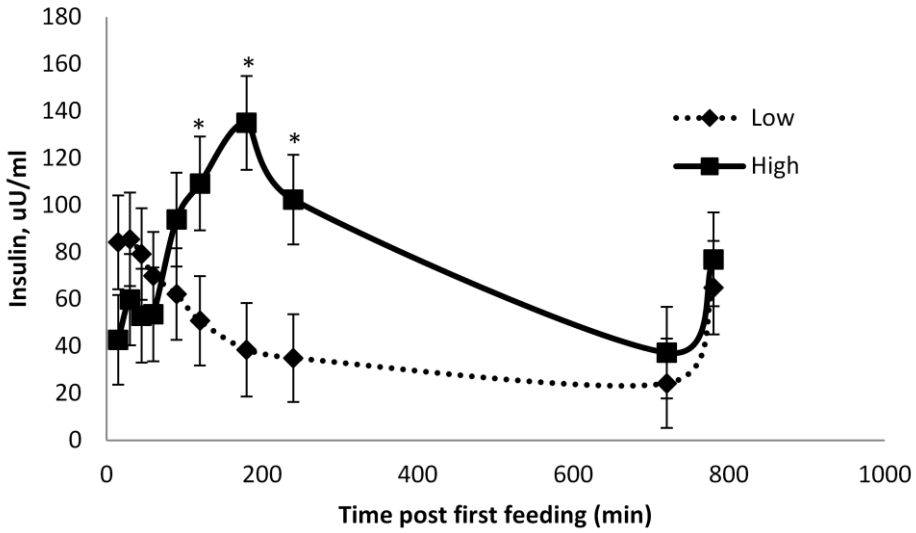

Figure 6.2. Insulin concentration in plasma of calves that received either 4 L (High, n=10) or 2 L (Low, n=10) of colostrum at birth adjusted by hydration status and using a blood sample before first feeding as a covariate. Means and standard errors are shown. Time points mark with an asterisk differ P <0.05. (Base *P* = 0.32; TRT *P* = 0.42; time *P* = 0.01; TRT by time *P* < 0.01)

Insulin growth like factor-1 (IGF-1) plasma concentrations were similar for the first 2.5 h post feeding. By three h post feeding, IGF-1 concentrations in High TRT calves tended to be higher than for the Low TRT. These elevated IGF-1 concentrations remained up to 12 h post-feeding (Figure 6.3). Mean IGF-1 plasma concentrations of 40 ng/ml were lower than the mean IGF-1 concentrations during the first week of life reported by others of 180 ng/ml (Hadorn et al., 1997; Smith et al., 2002). Hadorn et al. (1997) and Kühne et al. (2000), reported a stable concentration of IGF-1 in calves fed between 1.25 and 3L of colostrum for 7 h after consumption.

In both TRT, GH concentrations declined for the first two h post feeding. Plasma GH concentrations in calves fed the Low TRT remained lower and constant from 2 to 4 h post feeding while GH plasma concentrations of calves fed the High TRT started increasing 3 h post first feeding. Kühne et al. (2000), observed significant increase in GH concentrations during the first 7 h post-feeding for calves fed 1.75 L of colostrum but not for calves fed 1.25 L of colostrum. Growth hormone concentrations in both TRT were similar by 12 h post feeding and responded similarly to the second feeding (Figure 6.4). The basal concentrations of GH were similar to those reported by Hadorn et al. (1997) at approximately 11 ng/ml; however, they reported an increase of up to 22 ng/ml during the following 7 h post feeding and decreasing GH concentrations after feeding on d 2 of life, while in the current study GH concentration declined after the first and second feeding. The disparity between the data of Hadorn et al. and the current study is hard to interpret. The immediate decrease over the first 100 min might reflect utilization of the GH available during fetal development and the appearance of endogenous production as the system begins to regulate itself outside the dam. The GH-IGF-1 axis develops during the first weeks of life. In fetal calves, liver specific GH receptor transcript was not expressed (Lucy et al., 1998). Further, calves were shown not to respond to GH treatment prior to 22 d of life (Smith et al., 2002). However, in this study, differences between TRT were observed at the same sampling times for both hormones at three and four h post feeding, but by 12 h, only IGF-1 concentrations were different between TRT. The tendency for increased concentration at 3 and 4 h post feeding

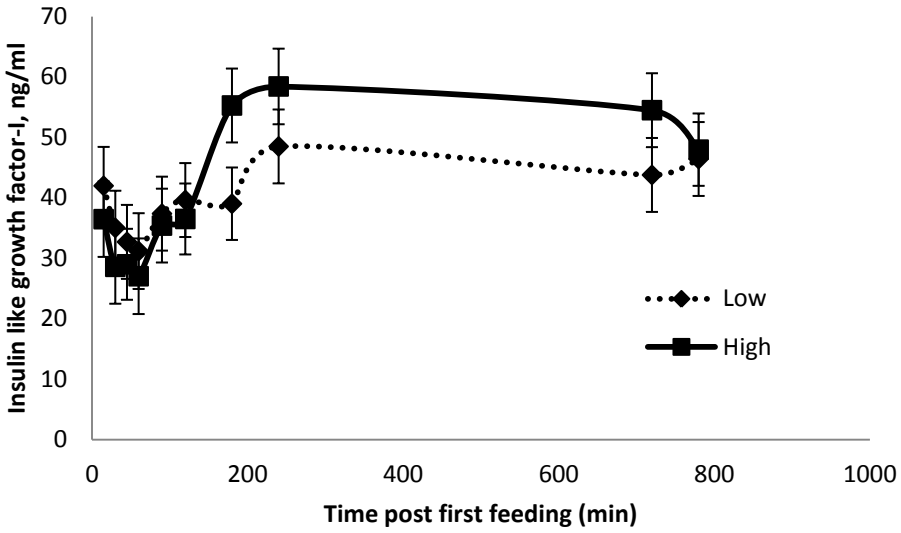

Figure 6.3. Insulin like growth factor-1 (IGF-1) concentration in plasma of calves that received either 4 L (High, n=10) or 2 L (Low, n=10) of colostrum at birth adjusted by hydration status and using a blood sample before first feeding as a covariate. Means and standard errors are shown. (Base $P < 0.01$; TRT $P = 0.79$; time $P < 0.01$; TRT by time $P = 0.31$)

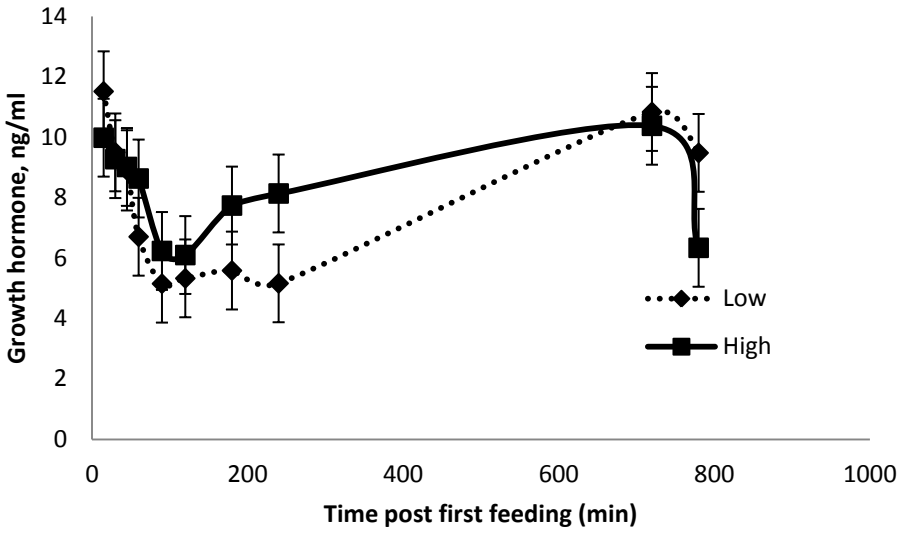

Figure 6.4. Growth hormone concentration in plasma of calves that received either 4 L (High, n=10) or 2 L (Low, n=10) of colostrum at birth adjusted by hydration status and using a blood sample before first feeding as a covariate. Means and standard errors are shown. (Base $P < 0.01$; TRT $P = 0.79$; time $P < 0.01$; TRT by time $P = 0.04$)

could be attributed to hormone absorption from colostrum. Growth hormone concentrations increase in underfed animals, and this was demonstrated in one d old calves fed only water during their first day of life (Hadorn et al., 1997) suggesting that although the somatotropic axis is not fully developed, the mechanisms regulating the system are in place at birth.

Leptin plasma concentrations were similar for both TRT during the first 2 h post feeding (Figure 6.5). Plasma leptin concentration in the Low TRT calves remained constant, while plasma leptin concentrations in the High TRT calves increased by 2.5 h post feeding and remained higher than the Low TRT calves until 12 h post feeding. Plasma leptin concentrations measured in the current study are higher than the average leptin plasma concentrations reported by Block et al. (2003) who reported a nearly constant plasma leptin concentration of 2.3 ng/ml from wk 1 to 250 d. However, they reported increasing plasma leptin concentrations in calves growing at a rate of 1.2 kg/d compared to calves growing at 0.6 kg/d. Leptin plasma concentration was reported to be responsive to nutrient intake starting wk 3 of life (Block et al., 2003) which suggests that in this is possibly due to absorption and not production. The observed average leptin concentration of 5.4 ng/ml is the first report of plasma leptin concentration during the first 12 h of life and is most likely elevated due to maternal influence. With the current data, it is not possible to determine when the drop in leptin plasma concentration occurred nor its metabolic implications. The plasma concentration increase between 2.5 and 3 h is consistent with the increase in plasma concentrations for insulin, IGF-1 and GH, suggesting that direct absorption from colostrum is occurring for all of these hormones between 2 and 3 h post feeding, although this was not directly measured in this study.

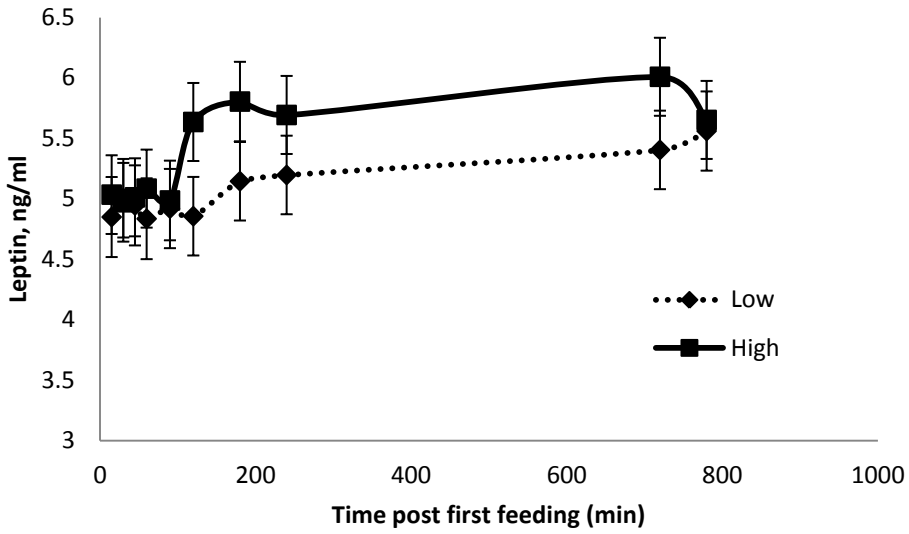

Figure 6.5. Leptin concentration in plasma of calves that received either 4 L (High, n=10) or 2 L (Low, n=10) of colostrum at birth adjusted by hydration status and using a blood sample before first feeding as a covariate. Means and standard errors are shown. (Base $P < 0.01$; TRT $P = 0.44$; time $P < 0.01$ TRT by time $P = 0.28$)

CONCLUSIONS

Although no significant differences were observed between TRT, the consistency of differences between TRT indicates the possibility of direct absorption of different protein hormones from colostrum. While some of these hormones have been shown to have local gut effects, this does not preclude the possibility of absorption into blood through the same mechanisms used to absorbed immunoglobulins from colostrum. The potential metabolic effect of increasing the concentrations of some of these growth factors or maternal hormones was not evaluated in this study and warrants further investigation.

REFERENCES

Bartol, F. F., Wiley, A. A., Bagnell, C. A. 2009. Epigenetic programming of porcine endometrial function and the lactocrine hypothesis. Rep. Domestic Anim. 43:273-279.

Baumrucker, C. R., Hadsell, D. L., Blum, J. W. 1994. Effects of dietary insulin-like growth factor-1i on growth and insulin-like growth-factor receptors in neonatal calf intestine. J. Anim. Sci. 72:428-433.

Block S. S., J. M. Smith, R. A. Ehrhardt, M. C. Diaz, R. P. Rhoads, M.. E. Van Amburgh and Y. R. Boisclair. 2003. Nutritional and developmental regulation of plasma leptin in dairy cattle. J. Dairy Sci. 86:3206-3214.

Calloway, C. D., Tyler, J. W., Tessman, R. K., Hostetler, D., Holle, J. 2002. Comparison of refractometers and test endpoints in the measurement of serum protein concentration to assess passive transfer status in calves. J. Am. Vet. Med. Assoc. 221:1605-1608.

DeNise S. K., J. D. Robison, G. H. Stott, D. V. Armstrong. 1989. Effects of passive immunity on subsequent production in dairy heifers. J. Dairy Sci. 72:552-554.

Ehrhardt R.A., R. M. Slepetis, J. Siegal-Willott, M. E. Van Amburgh, A. W. Bell, Y. R. Boisclair. 2000. Development of a specific radioimmunoassay to measure physiological changes of circulating leptin in cattle and sheep. J. Endo. 166:519-28.

Faber S. N., N. E. Faber, T. C. Mccauley, R. L. Ax. 2005. Case study: Effects of colostrum ingestion on lactational performance. The Prof. An. Sc. 21:420-425.

Hadorn U., H. Hammon, R. M. Bruckmaier, J. W. Blum. 1997. Delaying colostrum intake by one day has important effects on metabolic traits and on gastrointestinal and metabolic hormones in neonatal calves. J. Nutr. 127:2011-2023.

Hammon H., J. W. Blum. 1997. Prolonged colostrum feeding enhances xylose absorption in neonatal calves. J. Anim. Sci. 75:2915-2919.

Hammon H., J. W. Blum. 1998. Metabolic and endocrine traits of neonatal calves are influenced by feeding colostrum for different durations or milk replacer. J. nutrition 128:642-632.

Jones, C. M., R. E. James, J. D. Quigley III, M. L. McGilliard. 2004. Influence of Pooled Colostrum or Colostrum Replacement on IgG and Evaluation of Animal Plasma in Milk Replacer. J. Dairy Sci. 87:1806–1814.

Kaske, M., Wiedemann, S., Kunz, H. 2010. Metabolic programming: Background and potential impact for dairy cattle. Vlaams Diergeneeskundig Tijdschrift, 79:445-451.

Kühne S., H. M. Hammon, R. M. Bruckmaier, C. Morel, Y Zbinden, J. W. Blum. 2000. J. Dairy Sci. 78:609-620.

Lepine, A. J., Boyd, R. D., Whitehead, D. M. 1991. Effect of colostrum intake on hepatic gluconeogenesis and fatty-acid oxidation in the neonatal pig. J. Anim. Sci.69:1966-1974.

Lucy M. C., C. K. Boyd, A. T. Koenigsfeld, C. S. Okamura. 1998. Expression of somatotropin receptor messenger ribonucleic acid in bovine tissues. J. Dairy Sci. 81:1889-1895.

McGuire, M.A., Bauman, D.E., Dwyer, D.A., Cohick, W.S. 1995. Nutritional modulation of the somatotropin/insulin-like growth factor system: Response to feed deprivation in lactating cows. J. Nutr. 8:493-502.

Plaut, K., Cohick, W.S., Bauman, D.E., Baxter, R.C. 1991. Evaluation of interference by insulin-like growth factor 1 (IGF-1) binding proteins in a radioimmunoassay for IGF-1 in serum from dairy cows. Dom. Anim. Endo., 8:393-405.

Rosenberg, E., Thonney, M.L., Butler, W.R. 1989. The effects of bovine growth hormone and thyroxine on growth rate and carcass measurements in lambs. J. Anim. Sci. 67:3300-3312.

Smith J. M., M. E. Van Amburgh, M. C. Diaz, M. C. Lucy, D. E. Bauman. 2002. Effect of nutrient intake on the development of the somatotropic axis and its responsiveness to GH in Holstein bull calves. J. Anim. Sci. 80:1528-1537.

Steinhoff-Wagner, J., Gors, S., Junghans, P., Bruckmaier, R. M., Kanitz, E., Metges, C. C., Hammon, H.M. 2011. Intestinal glucose absorption but not endogenous glucose production differs between colostrum- and formula-fed neonatal calves. J.Nutr. 141:48-55.

CHAPTER VII

A COMPARISON OF WHEY BASED PROTEIN VERSUS MRP (ENZYME MODIFIED PROTEINS) CONTAINING MILK REPLACERS WITH AND WITHOUT A SPECIFIC AMINO ACID PROFILE IN NEONATAL CALVES

F. Soberon, A. Severy and M. E. Van Amburgh

ABSTRACT

The most nutritionally effective milk replacers (MR) are produced from all milk proteins. Supplementing non-milk protein MR with amino acids (AA) might help overcome some of the performance limitations previously observed with the use of plant proteins. The objectives of this study were to compare the performance of calves fed an all-milk containing MR compared to a MR with 50% of the protein replaced by a proprietary formulation of enzymatically modified plant proteins (MRP) as formulated with or without a specific AA profile calculated from body composition and efficiency of use data. Eighty calves were blocked by birth weight and sex by 24 hrs of age and assigned to one of four treatments (TRT): Control: whey based protein MR (Excelerate® milk replacer, Milk Specialties Global (MSG), Carpentersville, IL); TRT 1: Control plus AA supplied by added crystalline AA; TRT 2: MRP containing MR formulated at 28% CP and 15% fat containing proprietary MRP protein (MSG, Carpentersville, IL) representing approx. 50% of the MR protein; TRT 3: MRP containing MR with supplemental AA. All MR were formulated to be isocaloric and isonitrogenous. Calves were fed only MR to day 28, offered grain from day 29 to 42, and then weaned in a step-down manner over 7 d. Growth and starter intakes were measured until day 70. Calves fed the all-milk containing MR had greater ADG up to weaning ($P < 0.05$) whereas post-weaning, increased starter intake in calves fed TRT 2 and 3 negated the growth advantage. Calves fed all-milk containing MR demonstrated greater change in hip height by weaning compared to calves fed MRP containing

MR ($P < 0.05$) and the difference remained until day 70. Prior to weaning performance was greater in calves fed milk protein base MR.

Key Words: Calf nutrition, milk replacer, enzyme modified protein

INTRODUCTION

The most nutritionally effective milk replacers are still produced from all milk proteins consisting of whey proteins and various whey protein fractions (Davis and Drackley, 1998; Tannan, 2005). All milk protein milk replacers are expensive due to the cost of milk proteins. Furthermore, new uses for whey proteins in human foods are spurring competitive demand and increased pricing for these proteins. Opportunities to reduce the cost of manufacturing milk replacers are available through the utilization of alternative protein sources, such as defatted soy flour, soy protein concentrate, soy protein isolate, hydrolyzed proteins, such as hydrolyzed soy, wheat and potato proteins, eggs and other non-animal proteins. However, with increased inclusion rates of these alternative proteins, reductions in animal performance are observed.

The reduction in performance is primarily due to the carbohydrate moieties associated with selected proteins, which have been found to act as anti-nutritive factors (Lalles, 1993; Lalles and Toullec, 1998; Tannan, 2005). Defatted soy flour is the least processed of the soy protein sources and thus contains the greatest amounts of such anti-nutritive factors including trypsin inhibitors, glycinin, β-conglycinin, lectins, and β-mannans. Digestibility of soy protein products ranges from 41 to 91% depending on the degree of extraction, isolation, hydrolysis and the inclusion rate in milk replacers.

Part of the effect of reduced digestion is through increased endogenous protein secretion by the gut after recognition of the plant carbohydrate and proteins. The increased excretion of endogenous proteins most likely contributes to a loss of energy equivalent to up to 15% of the intake energy of the calf and this is one reason why reduced performance is observed in the calf

when high quantities of soy protein are fed (Tannan, 2005). Endogenous proteins are readily re-digested, so it is assumed that protein status of the animal is only partially compromised by the feeding of low quality soy protein sources. When included in milk replacers, hydrolyzed soy proteins show increased digestibility and animal performance (Lalles, et al. 1995; Montagne et al., 2001) demonstrating that hydrolyzing the carbohydrates and reducing the antigenic proteins will enhance feed quality and potential inclusion rate of the protein.

Enzymatically modified plant proteins (MRP) are available with the carbohydrate moieties removed, thus reducing antigenic activity and enhancing protein digestibility. Application of these proteins in milk replacers has resulted in improved calf performance, however, there are data available suggesting that the amino acid balance of the formulations are still not consistent with calf requirements, especially when supplied adequate energy for growth above maintenance.

Recent work by Hill et al. (2008) and Nabte-Solis (2008) indicates the possibility of more effectively balancing milk replacer diets for amino acid profile and quantity which greatly improves the efficiency of use of absorbed protein for growth. This provides the opportunity to potentially improve the effectiveness of alternative proteins. Aside from the change in endogenous protein production, alternative protein sources are not always balanced for amino acids, and the differences in amino acid balance and alternative proteins are not well researched or understood.

The objectives of this study were to compare the performance of calves fed an all-milk containing MR compared to a MR with 50% of the protein replaced by a proprietary formulation of enzymatically modified plant proteins (MRP) as formulated with or without a specific AA profile calculated from body composition and efficiency of use data.

MATERIALS AND METHODS

All protocols involving the use of animals were reviewed and approved by the Cornell University Institutional Animal Care and Use Committee. Eighty Holstein heifer and bull calves from the Cornell Research Dairy where used and they entered the study at birth. All calves received four L of colostrum at birth and another two L 12 h after. Blood samples were taken via jugular venipuncture into Vacutainer containing Sodium Heparin (BD Franklin Lakes, NJ) within 72 h of colostrum feeding, samples were frozen for later analyses of IgG concentration using the single radial immunodiffusion kit (VMRD, Inc. Pullman, WA)

The calves were weighed, blocked by sex of calf and assigned to one of four treatments (TRT) in a restricted randomization based on sex in a balanced design. The TRT groups were: Positive control: (PCTRL; n = 20), whey based protein, non-medicated milk replacer 28% crude protein and 15% fat –accelerated commercial formulation as produced by MSG (Excelerate milk replacer product). Treatment 1: Positive control plus amino acid profile (PCTRL+AA; n = 20), whey based protein, non-medicated milk replacer 28% crude protein and 15% fat –accelerated commercial formulation as produced by MSG with a specific amino acid profile supplied by added crystalline amino acids. Treatment 2: MRP containing milk replacer (MRP; n = 20), non-medicated milk replacer formulated to the same protein and fat specifications as the PCTRL containing proprietary MRP protein source (MSG, Dundee, IL) (representing approximately 50% of the milk replacer protein source) and whey proteins. Treatment 3: MRP containing milk replacer plus amino acid profile (MRP+AA; n = 20), non-medicated milk replacer formulated to the same protein and fat specifications as the PCTRL containing proprietary MRP protein source (MSG, Dundee, IL) (representing approximately 50% of the milk replacer protein source) and whey proteins with supplemental crystalline amino acids.

All treatments in this study were designed to be isocaloric and isonitrogenous, so that the direct effects of varying the sources of protein and carbohydrate were not confounded. Table 7.1 shows the chemical composition of all 4 milk replacers. Milk replacer was fed to deliver 0.30

Table 7.1. Chemical composition of milk replacers as formulated.

Description	Units	PCTRL[1]	PCTRL+AA[2]	MRP[3]	MRP+AA[4]
Crude Protein	%	28.5	28.5	28.5	28.5
Crude Fat	%	15	15	15	15
Crude Fiber	%	-	-	0.04	0.04
Ash	%	6.97	6.96	6.62	6.50
Gross Energy	Kcal/Kg	4616.58	4619.63	4597.45	4588.23
Lactose	%	39.62	39.70	39.14	38.90
Units Pro.-Non-Milk	%	-	1.90	15.61	19.22
Calcium	%	0.90	0.90	0.90	0.90
Phosphorus	%	0.67	0.67	0.67	0.67
Alanine	%	1.49	1.38	1.15	0.95
Arginine	%	0.76	0.71	1.21	1.12
Aspartate	%	3.04	2.84	2.56	2.18
Cystine	%	0.87	0.80	0.43	0.30
Glutamate	%	5.04	4.70	5.82	5.19
Glycine	%	0.54	0.50	0.84	0.78
Histidine	%	0.47	1.01	0.59	1.01
Isoleucine	%	1.76	1.64	1.39	1.24
Leucine	%	2.99	2.90	2.38	2.90
Lysine	%	2.79	2.61	2.78	2.61
Methionine	%	0.58	0.64	0.57	1.14
Phenylalanine	%	0.92	1.81	1.17	1.82
Proline	%	1.90	1.78	1.88	1.64
Serine	%	1.52	1.42	1.24	1.05
Threonine	%	1.96	1.82	1.26	1.61
Tryptophan	%	0.44	0.48	0.35	0.48
Tyrosine	%	0.82	0.77	0.86	0.76
Valine	%	1.67	1.79	1.38	1.79
Met + Cys	%	1.45	1.44	1.00	1.44
Phe + Tyr	%	1.75	2.58	2.04	2.58

[1]Milk replacer with whey based protein
[2]Milk replacer with whey based protein plus a specific amino acid composition
[3]Milk replacer with 50 % of the protein from enzymatically modified plant proteins
[4]Milk replacer with 50 % of the protein from enzymatically modified plant proteins plus a specific amino acid composition

Mcal/kg $BW^{0.75}$ during the study and the amounts were adjusted weekly based on the achieved weight gain. Starting on d 28 of treatment, calf starter (22% CP, Cargill, Inc. Table 7.2) was offered to the calves on an ad libitum basis. On d 43, milk replacer was reduced to 50% of previous intake and fed once per d in the evening for 7 d, and then removed unless the calf was not consuming at least 1 kg of starter grain.

Body weights were measured at birth and then weekly for the duration of the study. Growth rates were determined by regression of individual calf body weights over the 10-week period. Calves were fed milk replacer twice daily at 0700 and 1800 h by bucket. Fresh water in buckets was available at all times. Calves were bedded on sawdust shavings.

Calves were monitored several times daily for general health. All feed intakes and refusals were recorded. Fecal scores were monitored and recorded. If it was determined that a calf was dehydrated, then electrolytes were offered.

Statistical Analysis

All data were analyzed using the MIXED procedures of SAS 9.2 (SAS Inst. Inc., Cary, NC). Linear regressions to calculate average daily gain (ADG) and change in hip height were made for each calf before analysis. Body weight, hip height milk powder intake and grain intake were analyzed as repeated measures using birth weight or hip height at birth as a covariant for body weights and hip height respectively. Other factors in the model included wk and the interaction of TRT by wk. The model used was: $Y_{ijk} = S_i + G_j + D_k + F_{jk} + E_{ijk}$

where; Y_{ijk} = is the dependent, continuous variable; S_i = the effect of initial weight or height; G_j = the effect of treatment; D_k = the effect of week; F_{jk} = the effect of the interaction of treatment by week; E_{ikj} = the residual error of the the i^{th} basal concentration, the j^{th} treatment and the k^{th} of week. Significance was declared at $P < 0.05$.

Table 7.2. Chemical composition of pelleted calf starter.

Nutrient	DM Conc.	As Fed Conc.
Dry matter, %	100.0	88.9
Fat, %	4.27	3.79
Unsaturated Fat, %	3.51	3.12
Adjusted protein, %	21.95	19.50
Non Fiber carbohydrate, %	30.91	27.45
Rumen soluble sugars, %	7.32	6.50
Adjusted total starch, %	14.14	12.56
Gelatinazed starch, %	8.65	7.68
Neutral Detergent Fiber, %	33.13	29.43
Calcium, %	1.55	1.38
Phosphorus, %	1.00	0.89
Sulfur, %	0.33	0.29
Magnesium, %	0.35	0.31
Potassium, %	1.12	0.99
Copper, mg/kg	35.01	31.10
Iodine, mg/kg	1.46	1.30
Zinc, mg/kg	151.99	135.00
Added selenium, mg/kg	0.56	0.50
Vitamin A, IU/g	43.07	38.26
Vitamin E, IU/kg	197.03	175.00
Monensin, g/ton	56.29	50.00
Sol Prot, % of CP, ratio	22.74	22.74

RESULTS AND DISCUSSION

Eighty three calves were assigned to TRT and data from 73 were used for analysis. Ten calves died pre-weaning for reasons independent of TRT (Table 7.3). No differences were observed among TRT for birth weight (43.2 ± 0.99 kg; $P = 0.55$; Table 7.4), hip height (80.4 ± 0.9 cm; $P = 0.58$) or plasma IgG levels at 24 h of life (3,351 ± 69 mg/dl; $P = 0.59$; Table 7.4) among TRT. At week 1 calves fed the MRP TRT had lower body weights than the rest of the TRT, by week 2 calves fed the MRP TRT and MRP+AA TRT had lower body weights than PCTRL and PCTRL+AA and this difference persisted through weaning at wk 7 (73.0, 72.01, 79.7 and 78.55 kg, respectively; $P < 0.02$). By the end of the study at wk 10, PCTRL calves were heavier (95.54 kg) than calves in MRP and MRP+AA TRT (87.56 and 88.89 kg respectively) and this weight difference is attributed to greater starter intake during the immediate post-weaning period, but no other significant difference was observed.

Average daily gain during the first four wk was highest for the PCTRL and PCTRL+AA than for the MRP and MRP+AA TRT (0.72 and 0.76 vs. 0.63 and 0.55 ± 0.03 kg respectively $P < 0.02$). These differences in ADG persisted through weaning

at wk 7 (0.81 and 0.81 vs. 0.72 and 0.68 ± 0.02 kg for PCTRL, PCTRL+AA, MRP and MRP+AA; Table 7.4). Kanjanapruthipong (1998) observed improved performance of calves on a milk replacer with soy protein and amino acids added. Also, Nabte-Solis (2009) observed no differences in ADG pre-weaning among calves fed milk replacer containing milk proteins or soy proteins with or without enzymes added. The performance of calves fed the alternative proteins was not similar to the whey protein based milk replacer even with the addition of the amino acids.

Average daily gain during the 3 wk post weaning was higher for MRP+AA calves compared to PCTRL+AA and MRP calves, PCRTL calves were intermediate (0.99, 0.85, 0.84 and 1.07 ± 0.08 kg for PCTRL, PCTR+AA, MRP and MRP+AA). The overall ADG from birth to 10 wk

Table 7.3. Total number of animals per treatment and animals removed from study for each treatment

TRT	n	Calves died	# calves use in the evaluation
PCTRL	21	1	20
PCTRL + AA	20	3	17
MRP	21	2	19
MRP + AA	21	4	17
Total	83	10	73

Table 7.4. Immunoglobulin G (mg/dl) 24 h post birth, body weight (kg) and average daily gain (ADG) (kg) at different time points during the study by treatment (TRT). Mean and standard errors are presented.

TRT	PCTRL	PCTRL+AA	MRP	MRP+AA	Std Error
IgG, mg/dl	3,437	3,184	3,341	3,442	162
Birth WT, kg	44.08	43.14	43.34	42.12	0.99
Weaning WT, kg	79.70[a]	78.55[a]	73.00[b]	72.01[b]	1.79
Final WT[1], kg	95.54[a]	91.67[ab]	87.56[b]	88.89[b]	2.37
ADG4[2], kg	0.72[a]	0.76[a]	0.63[b]	0.55[b]	0.03
ADG7[3], kg	0.81[a]	0.81[a]	0.72[b]	0.68[b]	0.02
ADG post[4], kg	0.99[ab]	0.85[ab]	0.84[a]	1.07[b]	0.08
ADG overall[5], kg	0.75[a]	0.71[ab]	0.68[b]	0.69[b]	0.02

Treatments with different superscripts differ $P < 0.05$
[1]Body weight at 10 wk of age
[2] Average daily gain during the first 4 wk of life
[3] Average daily gain up to weaning at 7 wk
[4] Average daily gain post weaning from wk 8 to wk 10
[5] Average daily gain from birth to 10 wk of age

were lower for MRP and MRP+AA TRT compared with PCTRL, PCTRL+AA was intermediate (0.75 vs. 0.68 and 0.69 ± 0.02 kg for PCTRL, MRP and MRP+AA $P < 0.05$; Table 7.4).

As previously mentioned, hip height at birth was not different among TRT (Table 7.5), by four wk of age, PCTRL and PCTRL+AA calves tended to have higher hip heights than MRP+AA calves ($P < 0.1$). When measured as daily change in hip height from birth to weaning, PCTRL and PCTRL+AA calves had higher hip height gains than MRP and MRP+AA calves (0.25 and 0.28 vs. 0.21 and 0.21 ± 0.01 cm/d; $P < 0.03$; Table 7.5). The average daily change in hip height during the 10 wk tended to be higher for PCTRL ($P = 0.07$) and was higher for PCTRL+AA ($P < 0.02$) than for MRP and MRP+AA but the final hip height at wk 10 was only significantly higher for PCTRL over MRP (95.3 vs. 93.3 ± 0.7 cm; Table 7.5) all others where intermediate.

Milk replacer intake over 7 wk was higher for PCTRL than for MRP and MRP+AA calves, it was also higher for PCTRL+AA than for MRP+AA calves and tended to be higher for PCRTL+AA than for MRP calves (57.6, 57.2, 54.9 and 54.0 ± 0.9 kg for PCTRL, PCTRL+AA, MRP and MRP+AA). The difference in intake among TRT was not observed by Nabte-Solis (2009) when comparing a whey based milk replacer with milk replacer containing soy proteins.

Total starter grain intake tended to be higher for PCTRL calves than MRP calves ($P = 0.06$), there was no other difference among TRT (53.0, 46.7, 45.6 and 51.3 ± 2.9 kg for PCTRL, PCTRL+AA, MRP and MRP+AA).

When analyzed by TRT, calves fed the PCTRL+AA milk replacer had their first case of diarrhea at an older age than calves in all other TRT (15 days old vs. 10 days old; $P = 0.02$). Calves in PCTRL+AA also had less cases of diarrhea than MRP and MRP+AA calves (0.47 cases per calf for PCTRL+AA vs. 1 case per calf for MRP and MRP+AA; $P = 0.03$); PCTRL calves were in between with 0.75 cases per calf. These findings are different to those observed by Nabte-Solis (2009) who did not observed differences in fecal consistency of calves fed milk replacers with milk or soy protein sources.

Table 7.5. Hip height (cm) and Average Daily Change (ADC) in hip height (HH) (cm) at different time points during the study by treatment (TRT). Mean and standard errors presented.

TRT	PCTRL	PCTRL+AA	MRP	MRP+AA	Std Error
Birth HH, cm	80.58	80.30	80.04	80.31	0.91
Weaning HH, cm	91.73^{ab}	92.32^{a}	89.99^{b}	90.61^{ab}	0.77
Final HH[1], cm	95.34^{a}	94.67^{ab}	93.32^{b}	94.13^{ab}	0.77
ADC4[2], cm	0.22	0.23	0.20	0.17	0.02
ADC7[3], cm	0.25^{a}	0.27^{a}	0.21^{b}	0.21^{b}	0.01
ADC post[4], cm	0.16	0.10	0.15	0.18	0.03
ADC total[5], cm	0.23^{ab}	0.23^{a}	0.21^{bc}	0.20^{c}	0.01

Treatments with different superscripts differ $P < 0.05$
[1] Hip height at 10 wk of age
[2] Average daily change in hip height from birth to wk 4
[3] Average daily change in hip height from birth to weaning at 7 wk
[4] Average daily change in hip height from wk 8 to wk 10
[5] Average daily change in hip height from birth to wk 10

In this study, alternative soy-based proteins, although enzymatically treated, were not effective at replacing whey protein in milk replacer. Given the formulation removing the differences in amino profile and intake, this data suggests that the enzyme modification step was not entirely efficient at removing the potential anti-nutritional factors or improving overall digestibility to be consistent with whey proteins. The differences in performance are consistent with previous studies evaluating alternative proteins. The difference in ADG among the PCTRL and MRP TRT was approximately 13%, consistent with the summary by Tannan (2005) which suggested that energy utilization for growth was reduced due to greater protein synthesis by the gastrointestinal tract in calves fed the alternative proteins.

CONCLUSIONS

Milk replacers made with whey based protein had better performance pre-weaning as assessed by body weight, ADG and hip height. Adjusting the amino acid profile of whey protein milk replacer did not improve performance of calves. Three weeks after weaning, calves that received the milk replacer with MRP protein source and amino acid profile, had similar body weights and hip heights than those calves receiving the whey based protein milk replacers. The ADG of these calves was not different by 10 wk than those from whey based protein sources.

ACKNOWLEDGMENTS

We gratefully acknowledge Milk Specialties Global for financial and other support of this work and the staff of the Teaching and Research Farm for their help and support of this work.

REFERENCES

Davis, C. L. and J. K. Drackley. 1998. The development, nutrition and management of the young calf. Iowa State University Press, Ames, IA.

Kanjanapruthipong J. 1998. Supplementation of milk replacers containing soy protein with threonine, methionine, and lysine in the diets of calves. J Dairy Sci 81:2912–2915

Lalles, J. P. 1993. Nutritional and antinutritional aspects of soyabean and field pea proteins used in veal calf production.: A review. Livestock Production Sci: 34:181-202.

Hill T. M., H. G. Bateman II, J. M. Aldrich, R. L. Schlotterbeck, K. G. Tanan. 2008. Optimal concentrations of lysine, methionine, and threonine in milk replacers for calves less than five weeks of age. J. Dairy Sci. 91:2433–2442.

Lalles, J. P. and R. Toullec. 1998. Soyabean products in milk replacers for farm animals: processing, digestion and adverse reactions. Recent Res. Devel. In Agricultural Foood Chem. 2:565-576.

Lalles, J. P. R. Toullec, P. Branco Pardal, J. W. Sissons. 1995. Hydrolyzed soya protein isolate sustains high nutritional performance in veal calves. J. Dairy Sci. 78:194-204.

Montagne, L., R. Toullec, and J.P. Lalles. 2001. Intestinal digestion and endogenous proteins along the small intestine of calves fed soyabean or potato. J. Animal Sci. 79:2719-2730.

Nabte-Solis, L. A. 2009. Effect of Beta-Mannanase enzyme addition to soy-containing milk replacers on growth and health of neonatal dairy calves. Cornell University Thesis.

Tanan, K.G. 2005. Nutrient sources for liquid feeding of calves. Pp 83-112 in Calf and heifer rearing, Principles of rearing the modern dairy heifer from calf to calving. Nottingham University Press, Nottingham, England.

CHAPTER VIII

SUMMARY

Replacement heifers have been traditionally considered a major expense of every dairy operation. However, recent data reveals that management of replacement calves represents an area of opportunity for the future profitability of every dairy farm. It has been widely accepted that environmental conditions can have a permanent effect on the phenotypic expression of many species, including a wide variety of mammals. Dairy cattle are no exception, and understanding the implications of environment on genetic expression is an area of active research.

The long-lasting and in some cases, heritable changes in gene expression brought about by the environment are known as epigenetic elements of the developmental program. Within this category of epigenetic changes, nutritional programming and maternal programming are of special interest in dairy cattle.

To better comprehend the relevance of metabolic programming, first the potential impacts of such mechanisms are to be understood. In Chapter II, a retrospective study was reported. The objective of that study was to investigate the relationship between nutrient intake from milk replacer and pre and post-weaning growth rate with lactation performance in two dairy herds. This study revealed a strong correlation between pre-weaning average daily gain (ADG) and first lactation milk yield. The correlation between ADG and first lactation milk yield was linear such that for every additional kg of ADG during the pre-weaning period, first lactation milk yield was increased by 850 kg and 1,113 kg for each of the two farms analyzed. The main difference between the two farms was their rolling herd average and this study revealed that even though the response to pre-weaning nutrition may vary among farms, it is positive in all cases. The farm with the higher average production resulted in higher response to pre-weaning ADG. Therefore, assumptions could be made that with higher genetic merit or better herd management, the response to early life nutrition is improved.

The variation in ADG among calves in both herds was traced to differences in maintenance requirements of the calves due to the environmental temperature at the time of birth. Neither of the two farms changed their nutritional management throughout the year; therefore, calves born within thermo-neutral temperatures had more energy available for growth than calves born during colder temperatures. This realization resulted in the regression of energy intake above maintenance with lactation performance, and a significant positive correlation was observed for energy intake above maintenance with first, third and life time milk production. There are other reports in the literature where increased milk production was associated with improved nutrient intake pre-weaning (Foldager and Krohn, 1994; Bar-Peled et al, 1997; Shamay et al., 2005; Terré et al., 2009; Moallem et al. 2010). A very important observation from this study was that 22% of the variation in first lactation milk production was explained by pre-weaning growth rate.

Once the relevance of early life nutrition had been established, the need to understand the biological mechanisms responsible for this effect was evident. A first step to describe such processes was to analyze the effects of pre-weaning nutrient intake in the mammary gland. Chapter IV describes a study geared to qualify the development of the mammary gland during the first months of life, heifer calves were fed either a restricted amount of milk replacer or were fed sufficient nutrients to achieve an ADG of 0.8 kg. Heifers were harvested at 54 ± 2 d. Results from this study contrasted with the traditional belief that the mammary gland has a period of isometric growth with the body during the first months of life (Sinha and Tucker, 1969). It was shown that during the liquid feed period, the mammary parenchymal tissue is responsive to nutrient intake and therefore, if sufficient nutrients are supplied, the mammary gland grows at an allometric rate from an earlier age. Other two reports in the literature have also reported the mammary parenchymal tissue to be nutrient responsive during the first months of life (Brown et al., 2005 and Meyer et al., 2006).

Pre-weaning nutrient intake is not the only early life management practice that has been observed to influence future performance. Colostrum intake, or in most cases, colostrum status has also been found to have long-lasting effects in various species. In cattle, increased feed

efficiency, increased ADG, and increased milk production have been reported for calves with better colostrum status (Robison et al., 1988; DeNise et al., 1989; Jones et al., 2004; Faber et al., 2005). The interaction of colostrum status and nutrient intake pre-weaning has been poorly explored. Chapter V describes an experiment designed to evaluate the effects of colostrum status and pre-weaning nutrient intake on feed intake and feed efficiency and showed that when calves are offered sufficient nutrients pre-weaning, calves that received more colostrum had better ADG through 80 d (0.78 vs. 0.66 kg; $P < 0.05$), better daily gains in hip height up to 80 d (0.21 vs. 0.18 cm; $P < 0.05$) and greater feed intake, both of milk replacer (44.4 vs. 40.9 kg DMI; $P < 0.05$) as well as starter grain post-weaning (2.89 vs. 2.58 kg/d; $P < 0.05$). When nutrient intake pre-weaning is restricted, growth rates and pre-weaning feed intakes are similar among calves with different colostrum intakes; however, DMI post-weaning is still greater for calves fed more colostrum at birth.

In this study, no differences were observed in feed efficiency; however, all treatments received maternal colostrum. Different observations have been made with calves receiving colostrum replacers (Jones et al., 2004), leading to the conclusion that colostrum influences metabolic programming through multiple mechanisms. In some cases, the presence of colostrum is sufficient to activate such mechanisms while in other instances, the response is dependent upon the quantity of colostrum administered.

Throughout the years, most of the benefits of colostrum have been attributed to the immunoglobulins transferred from the mother to the offspring through this secretion. However, the role of other non-nutritional elements within colostrum has been shown to be responsible for some of the metabolic programming occurring during the first days of life. For example, in sow colostrum, relaxin was shown to have profound effects upon the development of the uterus in the offspring (Bartol et al., 2009). Bovine colostrum is rich in many non-nutritional components such as insulin, growth hormone (GH), insulin-like growth factor-1 (IGF-1), leptin, and relaxin. Therefore, it is of particular interest to understand the effects that some of these growth factors or bioactive molecules may have in the developing calf. Chapter VI presents the analysis of plasma

samples collected at frequent intervals after colostrum administration in calves receiving either two or four L of colostrum at birth. These analyses revealed no differences in average hormone concentrations of insulin, GH, IGF-1 or leptin. However, insulin, IGF-1, GH and leptin were elevated 2.5 h post-colostrum ingestion in calves that received higher volumes of colostrum. The threshold concentration needed for biological significance has not been established and differences in concentration at critical developmental stages could have long-lasting implications.

The field of epigenetics is developing rapidly, but there is still much to understand of the triggers and the timing in regards to regulation. From the data presented here, protein accretion appears to have a profound effect on metabolic programming, and therefore, all of the factors contributing to increased protein accretion, such as growth factors in colostrum, increased feed intake and increased availability of nutrients pre-weaning, would stimulate positive changes in the epigenome.

Future research should aim to understand the effects of specific hormones present in colostrum, as well as the effects of colostrum on reproductive performance in dairy cattle. It is important to keep in mind that nutrient intake pre-weaning should be sufficient to promote growth during this stage of development to be able to maximize the metabolic programming brought about by colostrum status.

The mammary gland response differently to nutritional status during the first months of life than it does during the pre-pubertal stage. Specific cell populations are most likely responsible for this differential response to nutrient through development; so far, attempts to identify the cell population responsible for such response have been unsuccessful. Future work should attempt to classify the growth of mammary parenchymal tissue in response to nutrient intake by cell types to better understand the implications of early parenchymal growth.

Lastly, protein synthesis during the pre-weaning stage has been identified as a possible trigger for metabolic programming. Future work should aim to identify specific stages of growth or organ where protein synthesis might have the greatest influence in future performance.

REFERENCES

Bar-Peled, U., B. Robinzon, E. Maltz, H. Tagari, Y. Folman, I. Bruckental, H. Voet, H. Gacitua, and A. R. Lehrer. 1997. Increased weight gain and effects on production parameters of Holstein heifer calves that were allowed to suckle from birth to six weeks of age. J Dairy Sci. 80:2523-2528.

Bartol, F. F., Wiley, A. A., Bagnell, C. A. 2009. Epigenetic programming of porcine endometrial function and the lactocrine hypothesis. Rep. Domestic Anim. 43:273-279.

Brown E. G., M. J. VandeHaar, K. M. Daniels, J. S. Liesman, L. T. Chapin, J. W. Forrest, R. M. Akers, R. E. Pearson, M. S. Weber Nielsen. 2005. Effect of increasing energy and protein intake on mammary development in heifer calves. J. Dairy Sci. 88:595-603.

DeNise, S. K., J. D. Robison, G. H. Stott, and D. V. Armstrong. 1989. Effects of passive immunity on subsequent production in dairy heifers. J. Dairy Sci. 72:552-554.

Faber, S. N., N. E. Faber, T. C. McCauley, and R. L. Ax. 2005. Case Study: Effects of colostrum ingestion on lactational performance. Prof. Anim. Scientist 21:420.

Foldager, J. and C.C. Krohn. 1994. Heifer calves reared on very high or normal levels of whole milk from birth to 6-8 weeks of age and their subsequent milk production. Proc. Soc. Nutr. Physiol. 3:301

Jones C. M., R. E. James, D. D 3rd Quigley, M. L. McGilliard. 2004. Influence of pooled colostrum or colostrum replacement on IgG and evaluation of animal plasma in milk replacer. J Dairy Sci. 87:1806-14.

Meyer M. J., A. V. Capuco, D. A. Ross, L. M. Lintault, and M. E. Van Amburgh. 2006. Developmental and nutritional regulation of the prepubertal bovine mammary gland: II. Epithelial cell proliferation, parenchymal accretion rate, and allometric growth. J. Dairy Sci. 89:4298-4304

Moallem, U., D. Werner, H. Lehrer, M. Zachut, L. Livshitz, S. Yakoby, and A. Shamay. 2010. Long-term effects of ad libitum whole milk prior to weaning and prepubertal protein

supplementation on skeletal growth rate and first-lactation milk production. J. Dairy Sci. 93:2639-2650

Robison J. D., G. H. Stott, S. K. DeNise. 1988. Effects of Passive Immunity on Growth and Survival in the Dairy Heifer. J Dairy Sci 71:1283-1287.

Shamay, A., D. Werner, U. Moallem, H. Barash, and I. Bruckental. 2005. Effect of nursing management and skeletal size at weaning on puberty, skeletal growth rate, and milk production during first lactation of dairy heifers. J. Dairy Sci. 88:1460–1469.

Sinha Y. N., Tucker H. A. 1969. Mammary development and pituitary prolactin level of heifers from birth through puberty and during the estrous cycle. J Dairy Sci. 52:507-12.

Terré, M., C. Tejero, and A. Bach. 2009. Long-term effects on heifer performance of an enhanced growth feeding programme applied during the pre-weaning period. J. Dairy Res. 76:331-339.